Cancer Drug Discovery and Development

Vinata B. Lokeshwar
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Editors

Bladder Tumors

Molecular Aspects and Clinical Management

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Preface

Bladder cancer is a common cancer of the urinary tract. It is the fourth leading cause of cancer-related death among men and the seventh among women. Clinical management of bladder cancer is challenging because of the heterogeneity among bladder tumors with respect to invasion and metastasis and frequent occurrence of new tumors in the bladder among patients treated with bladder preservation treatments. Due to these factors it has been said that the cost per patient of bladder cancer from diagnosis to death is the highest of all cancers. In addition to it being a significant health problem, bladder cancer is an interesting cancer to study in many ways than one. For example, environmental factors such as cigarette smoking and other carcinogens play a major role in the development of transitional carcinoma of the bladder, whereas schistosomiasis, a protozoan infection, results in squamous cell carcinoma of the bladder. Different molecular pathways with distinct molecular signatures appear to be involved in the development of low-grade versus high-grade bladder tumors. Currently being monitored by an invasive endoscopic procedure, cystectomy, with urine cytology as an adjunct, bladder cancer is at the forefront of developing cancer biomarkers for noninvasive detection. Due to the differences in the invasive and metastatic potential of bladder tumors, treatment options differ depending upon the grade and stage of the tumor. New advances are being made in treatment options to improve the outcome and quality of life for patients with bladder cancer. Similarly, new molecular nomograms are being discovered to predict treatment outcome so that individualized treatment options can be offered to patients.

This new text book on bladder cancer is organized to give both the clinicians and laboratory investigators state-of-the art information on basic science and clinical aspects of bladder cancer. Organizing this book that includes both the molecular basis as well as clinical practices in the management of bladder cancer would not have been possible without the invaluable contributions of the authors of each chapter. These authors who are experts in various aspects of bladder cancer were assembled from institutions in different parts of the world. All of these authors were generous with their time and commitment for bringing the readers up-to-date information on current advances in each area of bladder cancer. In addition, these experts have provided critical evaluation of the material presented in each chapter. Therefore, as editors of this book it has been our privilege to work with each

contributor and we believe that this book will serve as a comprehensive reference on bladder cancer.

Although, the readers are encouraged to read the entire book, we would like to present the highlight of each chapter in order to guide the readers to select the material of interest. Chapters 1–9 focus on molecular basis of bladder cancer, translational research into the areas of tumor markers, and standard mode of bladder diagnosis and detection. Chapters 10–22 focus on clinical aspects of bladder cancer.

Smoking is well known; however, in Chap. 1 on epidemiology of bladder cancer, Dr. Ribal reminds us that other causes like occupational exposure, genetic predisposition, and infection are also linked to the development of bladder cancer. Bladder cancer is a carcinogenesis-driven cancer, with polycyclic aromatic hydrocarbons (PAH) and aromatic amines having causal links. Chapter 2 by Escudero, Shirodkar, and Lokeshwar focuses on xenobiotic metabolisms that convert PAH and aromatic amines into active carcinogens and on genetic polymorphisms that increase the risk for bladder cancer development. The chapter discusses theories of bladder cancer development (field cancerization versus clonal origin) and chromosomal aberrations associated with bladder cancer.

Chapter 3 by Dr. Arndt Hartmann is a guide to TMN classification versus WHO classification, various types of bladder tumors (TCC, adenocarcinoma, squamous carcinoma), tumor grade, and stage. Cytology and cystoscopy is the mainstay of bladder cancer diagnosis and detection. However, several newer diagnostic techniques are finding their way in the clinic. Dr. Fred Witjes discusses these newer detection techniques such as photodynamic detection, narrow band imaging, optical coherence tomography, computed tomography (CT), and magnetic resonance imaging (MRI) in Chap. 4. Although new tumor markers may be better in detecting bladder cancer, few have been able to replace cytology as a standard adjunct to cystoscopy. Dr. Eva Wojcek in Chap. 5 discusses the value of urine cytology as an adjunct to cystoscopy in the detection of bladder cancer and various factors affecting cytology. Other aspects discussed in the chapter are the value of fluorescence in situ hybridization in the detection of bladder cancer and DNA ploidy.

Due to the ease of obtaining voided urine specimens, bladder cancer is on the forefront of developing tumor markers. Drs. McNeil, Ekwenna, and Getzenberg take an in depth look at various tumor markers and molecular signatures of bladder cancer in Chap. 6. Although several new tumor markers for bladder cancer are discovered each year and are the subject of numerous review articles, only few reviews are written on the subject of healthcare cost associated with bladder cancer diagnosis, screening, and surveillance. Chapter 7 by Yair Lotan is devoted to the subject of cost associated with bladder cancer detection and surveillance in the general versus high-risk population and using noninvasive techniques such as hematuria detection and tumor markers.

Prognostic markers and molecular nomograms involving proteomics and genomics are highly researched and some of the new emerging areas in bladder cancer. In Chap. 8, Dr. Habuchi focuses on seven different classes of molecules ranging from cell adhesion molecules to genetic alterations, which have been investigated for predicting disease progression, response to treatment (local versus systemic control of

the disease), and survival. Chapter 9 by Smith and Theodorescu dwells on a novel idea of molecular nomograms for personalized medicine. While Chap. 8 includes information on individual markers, this chapter focuses on multiplexing of molecular biomarkers to predict response to therapy. Of note is COXEN or Co-expression Extrapolation) algorithm that compares microarray gene expression profiles between cell lines and patient tumors to generate signatures predictive of drug sensitivity or resistance.

Bladder cancer being a complex disease, a practical guide that provides the necessary facts at the fingertips is very useful and Chap. 10 by Drs. Levy and Jones provides just that for the management of nonmuscle invasive bladder cancer. Specifically the chapter provides a succinct description of epidemiology, etiology, pathophysiology, clinical and diagnostic evaluations, available molecular markers for disease, as well as the current American Urological Association Guidelines Panel Recommendations and therapies for nonmuscle invasive and recurrent bladder cancer.

Chapters 11–22 encompass clinical management of bladder cancer. Starting from the low-grade bladder cancer, Chap. 11 by Dr. William Oosterlink focuses on histology, risk factors, and diagnosis and detection of low-grade tumors in the bladder and the upper tract, whereas Chap. 12 by Allaparthi and Balaji covers the clinical management of low-grade tumors.

Intravesical chemotherapy or immunotherapy (Bacillus Calmette-Guérin [BCG]) are key adjuvant therapies for the control of high-grade nonmuscle invasive bladder cancer. In Chap. 13, Drs. Adiyat, Katkoori, and Soloway is a review of indications and practical aspects of administration of intravesical chemotherapy, properties, efficacy, and side effects of various intravesical agents, and newer methods improving the efficacy of the intravesical drugs. Although, many reviews have been written on intravesical BCG therapy, the review by Drs. Bishay, Park, and Hemstreet is unique because of the depth of discussion on the mechanism of action of BCG in animal versus cell culture models, and the involvement of the immune system and inflammatory cytokines/chemokines in mediating response to BCG.

For the practicing urologist it is often difficult to inform the patient on muscle invasive bladder cancer and the often need for radical surgery and some kind of urinary diversion to follow; however, it is even more elaborate to do so in case of a nonmuscle invasive tumor where the evidence calls for radical treatment. In Chap. 15, Waalkes, Merseburger, and Kuczyk present pathologies where a radical treatment is strongly advised.

In Chapters 16–18 focus various aspects of cystectomy. In Chap. 16, radical surgery of the bladder is discussed by Dr. Gschwend. The improvement in surgical techniques had led this formerly challenging procedure into a more standardized one. Chapter 17 includes urinary diversion by Drs. Richard and Stefan Hautmann. The ileal neobladder has become one of the worldwide chosen procedures for continent orthotopic urinary diversion. Chapter 18, laparoscopic cystectomy by Dr. John, is the latest evolvement in bladder surgery and covers innovative techniques as well as the well-established surgical routines in radical treatment of invasive bladder cancer.

In 2010, only 5% of all urologists are performing neoadjuvant chemotherapy in patients with muscle invasive bladder cancer, hence the 5% survival benefit in 5 years and possible down staging of the tumor. Dr. Sherif guides us along the current literature and discusses the pros and cons of the neoadjuvant chemotherapy. Diagnosis and treatment of upper tract tumors is challenging and Chap. 20 by Dr. Remzi discusses the basics as well as recent advances in this field. In Chap. 21, De Santis and Bachner focus on the development and optimal use of new regimens for systemic agents as well as standard treatment options for the treatment of metastatic urinary carcinoma in the areas of targeted drugs. Options for "unfit" patients and elderly as well as in second-line setting are discussed. In Chap. 22 non-TCC tumors: Diagnosis and treatment is discussed by Dr. Abol-Enein. He focuses mainly on the squamous cell and adenocarcinoma of the bladder.

We hope that this brief synopsis of the topics covered in each chapter will encourage the readers to use this book for a general read on bladder cancer and as a reference guide for specific molecular and clinical aspects of bladder cancer. We again thank the authors for contributing to this project. We thank our Mr. Michael Koy, production editor at Springer and Spi Editorial Department, India for helping us in the publication of this book. We would like to thank Brian Halm of Springer for helping us with the publication of this book.

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Chapter 1
Bladder Cancer Epidemiology

Maria J. Ribal

Abstract Bladder cancer (BC) is a worldwide health problem. In 2006 in Europe, there were an estimated 104,400 incident cases of BC diagnosed (82,800 in men and 21,600 in women) that represent a 6.6% of the total cancers in men and 2.1% in women.

Tobacco use is a major preventable cause of death, and especially involved with BC carcinogenesis. Tobacco smoking is the most well-established risk factor for BC, causing around 50%–65% of male cases and 20%–30% of female cases.

Occupational exposure has been considered the second most important risk factor for BC. Work related cases account for a 20%–25% of all BC cases in several series.

In addition, chronic urinary tract infection had been related to BC, particularly, with invasive squamous cell carcinoma. Bladder schistosomiasis has particularly-been considered by the international agency for research on cancer (IARC) as a definitive cause or urinary BC with an associated fivefold risk.

BC is a disease of the environment and age . Populations are increasing in number, and they are growing old as well., . Since more people are living longer, more are at potential risk. Furthermore, the changing environments in developed and developing countries are causing more carcinogen concentration than can be associated to genesis of BC. Several carcinogens have been correlated to BC carcinogenesis.

However, it has been proposed that other environmental factors could affect the incidence on urothelial tumors. In fact, as for many other cancers, molecular researchers try to establish genetic alterations linked to carcinogenesis that could justify genetic predisposition.

Cancer is a major public health problem. At the end of the twentieth century, more than 930,000 people died of cancer every year in 15 member countries of the European Union (EU) (Coleman et al. 2003). Using population projections, if the age-specific death rates remain constant, the absolute number of cancer deaths in 2015 will increase to 140,500 (Boyle and Ferlay 2005). BC is a worldwide health problem. In 2006 in Europe, there were an estimated 104,400 incident cases of BC

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diagnosed (82,800 in men and 21,600 in women) that represent a 6.6% of the total cancers in men and 2.1% in women. The estimated ratio by gender was 3.8:1, respectively. In men BC was the fourth most common cancer. Bladder cancer represents a 4.1% of total deaths for cancer in men and 1.8% of total deaths in women (Ferlay et al. 2007). In the EU overall (27 countries), BC mortality rates were stable up to early 1990s, and declined, thereafter, by 16% in men and 12% in women, to reach values of 6 and 1.3/100,000, respectively, in the early years of the present decade. The only countries without declining mortality are Croatia and Poland in both sexes, Romania in men, and Denmark in women. This documented and quantified reduction in BC mortality seems related to decrease in tobacco smoking, while its relationship with other risk factors remains controversial (Ferlay et al. 2008).

In the United States, it is estimated that about 1.4 million new cases of cancer was diagnosed in 2008. Cancers of the prostate and breast are the most frequently diagnosed cancers in men and women, respectively, followed by lung and colorectal cancers in both men and in women. The fourth most common among men is the urinary BC. The 5-year relative survival rate for BC is 81% among whites and 65% among African–Americans (AAs) (taking the normal life expectancy into consideration) with an absolute difference of 16%. The survival rates for BC combined with certain site-specific cancer have improved significantly since the 1970s—being 74% during 1975–1977, 78% during 1984–1986, and 81% during 1996–2003.

Contrary to this data, the prevalence of BC among Native Americans/Alaskan Natives (NA/AN) is generally considered to be low. Despite this low incidence, NA/AN men and women seem to be at relatively greater risk of dying from BC, once it has been diagnosed (Watson and Sidor 2008).

Tobacco use is a major preventable cause of death, and especially involved in BC carcinogenesis. The year 2004 marks the anniversary of the release of the first Surgeon General's report on Tobacco and Health, which initiated a decline in per capita cigarette consumption in the United States (Jemal et al. 2008).

In Egypt, where BC has always been related to bilharziasis, a significance decline of the relative frequency of BC was observed from 27.63% in the old series to 11.7% in the recent series. Bilharzias association dropped from 82.4% to 55.3% and there was a significant increase of transitional cell carcinoma from 16% to 65%, while squamous cell carcinoma was less frequent—from 76% to 28%. Intimately related to this, there was an increase in the median age of patients from 47 to 60 years. The decline in the frequency of BC is related to a decline in bilharzias egg positivity in the specimen, and this suggests a better control of the endemic disease in rural population. This trend of less association with bilharzias has changed the clinical and pathological characteristics of BC diagnosed, with significant predominance of transitional cell carcinoma and an increase in the age of patients, a pattern more similar to that in western series (Gouda et al. 2007).

The incidence and mortality rates associated with BC vary by country, ethnicity, gender, and age. For indeterminate causes, the AAs have only half the risk of white European Americans, but overall, the survival seems to be worse among the primer group. The higher incidence in European Americans is limited to superficial tumors, both groups having a similar risk of invasive tumor (Kirkali et al. 2005).

In most populations the incidence of BC is three to four times higher in men than in women (Pelucchi et al. 2006). The excess of BC in men is not fully explained by differences in smoking habits and occupation.

BC is a disease of the environment and age . Populations are increasing in number, but , they are growing older as well. Since more people are living longer, more are at potential risk. Furthermore, the changing environments in developed and developing countries are causing more carcinogen concentration than can be associated to genesis of BC.

Several risk factors have been proposed for BC.

1.1 Tobacco Smoking and BC

Epidemiological evidence of the association between cigarette smoking and cancer began to be considered from the 1920s, and in 1950s, its relationship with lung cancer was perfectly established (Gandini et al. 2008). Tobacco smoking is currently responsible for 30% of all cancer deaths in developed countries. If the current pattern of tobacco smoking continues, there will be more than one billion deaths attributable to tobacco in the twenty-first century compared with 100 million deaths in the twentieth century (Vineis et al. 2004). In the IARC Monographs of the Evaluation of Carcinogenic Risks to Humans, it is reported that there is sufficient evidence in humans that tobacco smoking causes cancer of lung, oral cavity, naso-, oro-, and hypopharynx, nasal cavity and paranasal sinuses, larynx, esophagus, stomach, pancreas, liver, kidney, ureter, urinary bladder, uterine cervix, and bone marrow (myeloid leukemia). (Sufficient evidence means that the Working group considers that a casual relationship has been established between exposure to the agent and cancer in studies in which chance, bias, and confounding could be ruled out with reasonable confidence) (International Agency for Research on Cancer 2002). Putative carcinogenic constituents of tobacco smoke include arylamines, in particular, the potent carcinogen 4-aminobiphenyl, polycyclic aromatic hydrocarbons (PAHs), *N*-nitroso compounds, heterocyclic amines, and various epoxides.

Tobacco smoking is the most well-established risk factor for BC, causing around 50%–65% of male cases and 20%–30% of female cases. The lower cases in women than in men is explained by the earlier stage of the tobacco-related epidemic among European women, and it is likely to increase in the future (Boffetta 2008). In addition, it has been estimated that smoking is responsible for about 34% of deaths from BC in males worldwide and for 13% of BC deaths in females. Time trends in BC incidence and mortality are consistent with those of other tobacco-related cancers, with mortality rates being highest in birth cohorts with the maximum exposure to tobacco (Maxwell 2008).

Recently, a metaanalysis of observational studies on cigarette smoking and cancer from 1961 to 2003 has been published. The authors extracted data from 254 reports published during that period of time and included them in the 2004 IARC Monograph on *Tobacco Smoke and Involuntary Smoking*. The analyses were

carried out on 216 studies with reported estimates for current and/or former smokers. The pooled risk estimates for BC demonstrated significant association for both current and former smokers. In an analysis of 21 studies, the overall relative risk calculated for current smokers was 2.77 [95% confidence interval (CI) 2.17, 3.54]; while from the analyses of 15 studies, the overall relative risk calculated for former smokers was 1.72 (95% CI 1.46, 2.04) (Gandini et al. 2008).

In a pooled analysis of 11 case–control studies regarding cigarette smoking and BC, the following three variables were analyzed: duration of smoking, average number of cigarettes smoked per day, and time since quitting smoking. The population consisted of 2600 cases and 5524 controls. An increasing risk of BC was observed with increasing duration of smoking, which appeared to be linear. The relative increase was approximately 100% after 20 years smoking and reaches to 400% after 50 years smoking. In addition, a relationship was observed between the number of cigarettes smoked per day and BC.

The OR increased to nearly threefold for those who smoked between 15 and 20 cigarettes per day, after which a plateau in the risk graph was observed. They concluded that the duration of smoking habit and not the amount of cigarettes smoked per day was the main determining factor for BC. An immediate decrease in risk of BC was observed for those who quit smoking. This reduction was about 40% within 1–4 years of quitting smoking and reaches 60% after 25 years of cessation. However, the risk does not reach the level of nonsmokers even after 25 years. This suggests that tobacco has a late effect in the carcinogenesis of BC, but the fact that this risk does not reach the levels of nonsmokers until 25 years after quitting smoking suggests that tobacco may also be involved in an early irreversible stage in the carcinogenesis process (Brennan et al. 2000).

Other issues as type of tobacco could be taken into account. Six studies have published a significant higher risk of BC for the blacks who are cigarette smokers compared to smokers of otherraces. Also, case–control studies suggest a strong evidence of a carcinogenic effect of cigars and pipe, which is comparable to that of cigarettes (Boffetta 2008). The mode of inhalation of tobacco smoke has been related to BC risk, as well. In a case–control study of smoking and BC from Spain that included 1219 cases and 1271 controls, they concluded that the former and current smokers experienced risks of BC three to seven times higher than nonsmokers, respectively.

In addition, they found that the risk was higher for subjects who inhaled into the throat or chest [OR 4.8 (95% CI 2.3–9.9)] compared with those who inhaled only into the mouth [OR 10.0 (95% CI 6.7–15.0)], at each level of duration (Samanic et al. 2006).

Taking into account that current smokers have higher risk of BC than nonsmokers, and that this risk decreases by 40% after 1–4 years of quitting smoking, the promotion of cessation of smoking would allow reducing the incidence of BC in men and women.

Internationally, there is a general agreement on the broad strategy needed to successfully combat the tobacco epidemic.

The WHO Framework Convention on Tobacco Control (WHO FCTC) is the first treaty negotiated under the auspices of the WHO. It was adopted by the World Health Assembly on May 21, 2003 and entered into force on February 27, 2005. It

has since become one of the most widely embraced treaties in the history of the UN and, as of today, already has 163 Parties. The WHO FCTC was developed in response to the globalization of the tobacco epidemic, and it is an evidence-based treaty that reaffirms the right of all people to the highest standard of health. The Convention represents a milestone for the promotion of public health and provides new legal dimensions for international health cooperation. The Convention points out some general obligations: to develop comprehensive multisectorial national tobacco control strategies, to establish and finance a national coordinating mechanism or focal point, and to protect public health policies from commercial and other vested interests of the tobacco industry (Boethius 2008).

1.2 Occupational Exposure and BC

Occupational exposure has been considered the second most important risk factor for BC. Work related cases account for a 20%–25% of all BC cases in several series. The cause relation between chemical exposure and BC was reported more than a century ago among workers employed in the manufacture of dyestuffs containing aromatic amines (Pelucchi et al. 2006). Rehn reported in 1895 and 1896 a relationship between chemical exposure and BC among workers involved in the manufacture of coal tar derived magenta and auramine dyes, and 47 years later Hueper and Wolf demonstrated that 2-naphthylamines was the substance responsible for BC risk associated with chemical exposure (Johansson and Cohen 1997).

The substances involved in chemical exposure had been benzene derivatives and arylamines (2-naphthylamine, 4-ABP, 4,4′-methylenedianiline and o-toluidine).

The IARC has classified 11 specific aromatic amines as Group 1 (definite), Group 2A (Probable), or Group 2B (possible) human carcinogens (Table 1.1) (Delclos and Lerner 2008). Professions related to this exposure had been those

Table 1.1 Specific aromatic amines considered as human carcinogens by International Agency for Research on Cancer (IARC)

Specific aromatic amines considered human carcinogens by IARC		
Group 1	Group 2A	Group 2B
4-Aminobyphenyl	4-Chloro-ortho-toluidine	4,4′-Methylene bis(2-methylaniline)
Arsenic		Perchloroethylene
Benzo[a]pyrene		Trichloroethylene
Benzidine		Tetrachloroethylene
β-Naphthylamine		
N,*N*-bis(2-chloroethyl)-2-naphthyllamine(chlornaphazine)		
4,4′-Methylene bis(2-chloroaniline) (MOCA)		
Ortho-toluidine		

related to dyes, rubbers, textiles, paints, leathers, and chemicals (Pashos et al. 2002). Because of strict regulations, these chemicals contribute minimally to the current incidence of BC in the Western countries (Kirkali et al. 2005).

One study of municipal distribution of BC in Spain detected 34,281 BC deaths registered between 1989 and 1998. They could observe that determinate zones exhibited a higher risk than others, these being provinces of Cadiz, Seville, Huelva, Barcelona, and Almeria. The municipal mortality patterns suggested that the industrial and mining activity in the Provinces of Seville and Huelva could be associated with higher BC mortality in these provinces. The mortality pattern assessed in two different areas of the Province of Barcelona, which is only observable in women, might be related to the textile industry traditionally situated in these areas (Lopez-Abente et al. 2006).

The trend to decrease BC due to occupational exposure was reported in a pooled analysis of 11 case–control studies on BC conducted in European countries between 1976 and 1996. This analysis included 3346 male cases and 6840 male controls. Thirty-one occupations showed increase risk for BC and these occupations were grouped as metal workers, textile workers, painters, miners, and transport operators. Higher odd ratios were observed on those people with duration of employment more than 25 years. However, the author concluded that the ratio of BCs caused by occupational exposure was lower than those identified one year ago and that the exposure to occupational carcinogens had been reduced in the European Union.

This would likely be due to the improvement in working conditions and the reduction of exposure, particularly, to aromatic amines in work. Currently, employments that relate more to BC risk are those in metal sector, machinists, transport operators, and miners (Kogevinas et al. 2003).

In addition to the analysis on men, a pooled analysis of 11 case–control studies in BC conducted in Western Europe showed that the rates of BC due to occupational exposure had been reduced in women, with only a 8% of BC in women attributable to occupational carcinogens (Mannetje et al. 1999). Although in developed countries strict regulatory controls may have contributed to a decreased burden of exposure to bladder carcinogens in the workplace, the situation is less apparent in developing countries.

As in BC, in general, occupational case is more frequent in men than in women, although, an increased risk among women has been documented in several studies, including those employed in the rubber industry and, more recently, in healthcare settings. In a case–control study conducted in Iowa, female teachers, domestic service employees, and workers in laundering and dry-cleaning business had elevated risk of BC. Other gender and racial differences had been documented in occupational BC. In this way, in a recent mortality study in the United States, the mortality ratios for AA men and women and Latino males in various occupations were found to be increased compared with workers of the same gender and ethnic–racial group (Delclos and Lerner 2008).

Other occupational carcinogens recently correlated to increase of BC risk had been the exhausts from diesel engine. In a metaanalysis of 29 reported studies (7 cohort studies, 16 case–control studies, and 6 retrospective studies based on

collected data) the reviewers could find a small increase in BC incidence among workers exposed to diesel exhausts with an overall relative risk of 1.1–1.3.

However, the heterogeneity of the studies included in the analysis could not allow the establishment of a significant causal relationship between diesel exhausts and BC (Boffetta and Silverman 2001).

A recently published metaanalysis examined 130 single studies, including 66 cohort studies and 64 case–control studies, most of them coming from Europe, Canada and the United States, New Zealand or Australia, and only four studies from Asia. For each occupation, a summary relative risk (SRR) was calculated by means of a random effects model. Significantly, increased risks with SRR >1.20 were identified for miners (SRR 1.31, 95% CI 1.09–1.57), bus drivers (SRR 1.29, 95% CI 1.08–1.53), rubber workers (SRR 1.29, 95% CI 1.06–1.58), motor mechanics (SRR 1.27, 95% CI 1.10–1.46), leather workers (SRR 1.27, 95% CI 1.07–1.49), blacksmiths (SRR 1.27, 95% CI 1.02–1.58),machine setters (SRR 1.24, 95% CI 1.09–1.42), hairdressers (SRR1.23, 95% CI 1.11–1.37), and mechanics (SRR 1.21, 95% CI 1.12–1.31). The study concluded that these nine occupations are related to a small but significant increased risk of BC (Reulen et al. 2008).

1.3 Genetic Predisposition and BC

Tobacco smoking and occupational exposure have been the two major factors related to BC risk; however, not all smokers develop BC and not all cases of BC occurred in smokers or patients with chemical exposure. It has been proposed that there could be factors other than environmental that could affect the incidence on urothelial tumors. In fact, as for many other cancers, molecular researchers are trying to establish genetic alterations linked to carcinogenesis that could justify genetic predisposition.

An important research has been conducted in patients with BC in relation to smoking and chemical exposure , trying to identify those patients with higher susceptibility of being affected by environmental carcinogens. Aromatic amines were established carcinogens for urothelium.

They could be inactivated by acetylation pathway, and it has been postulated that those patients with slow acetylation capability were more susceptible to BC than those that are rapid acetylators. NAT-1 and NAT-2 are *N*-acetyltransferase genes located on the short arm of human chromosome 8 and they are involved in amines inactivation. Reduction in NAT-2 activity has been suggested as mechanism for BC predisposition among patients exposed to environmental carcinogens such as aromatic amines.

A number of SNPs have been reported in NAT-2 coding exon, as well as over 35 NAT-2 haplotypes have been identified (Hein 2006). Several of these haplotypes corresponded to NAT-2 slow acetylator phenotype and NAT-2 slow acetylation genotype has been related to higher risk of BC.

The Spanish Bladder Cancer Study is a hospital-based case–control study on BC conducted in five different areas in Spain that included 1150 cases and 1149 controls. They evaluated in this great population the association of several polymorphisms in NAT and GST genes with BC risk and their interaction with cigarette smoking. In addition, they reported a metaanalysis of 29 studies of NAT-2 and BC including 5096 cases and 6519 controls. They demonstrated that NAT-2 slow acetylators had a 40% increase in BC risk compared to rapid/intermediate acetylators with an OR of 1.4 (95% CI, 1.2–1.7). They could also demonstrate a significant multiplication interaction between NAT-2 slow acetylation genotype and cigarette smoking, that is, NAT-2 slow acetylators were especially susceptible to the adverse effects of cigarette smoking on BC risk. On the other hand, the metaanalysis performed corroborated their own data, being the summary on relative risk for NAT-2 slow acetylators compared to rapid/intermediate acetylators of 1.4 (Garcia-Closas et al. 2005).

Other SNPs in different genes have been studied. Nucleotide excision repair (NER) pathway is a complex mechanism for repairing DNA damage and subsequently for preventing carcinogenesis. NER pathway included several genes, and different SNPs on those genes have been related to an increase in BC risk. Twenty-two SNPs on seven NER genes were evaluated in 1150 cases and 1149 controls included in The Spanish Bladder Cancer Study. Four of these 22 SNPs in NER genes could be significantly related to a small increase in BC risk and interestingly it could be demonstrated as a stronger association between BC and polymorphism in ERCC2 gene (ERCC2 *R156R*) for never-smokers compared with ever-smokers (Garcia-Closas et al. 2006).

Other study including 696 patients with BC and 629 controls evaluated the association with BC risk of a comprehensive panel of 44 SNPs in genes of NER pathway and genes involved in cell cycle control. They concluded that patients with higher numbers of variants in NER genes rather than single polymorphism are at increased risk for BC (Wu et al. 2006).

1.4 Infection and BC

Chronic urinary tract infection had been related to BC, particularly with invasive squamous cell carcinoma. , Bladder schistosomiasis has particularly been considered by the international agency for research on cancer (IARC) as a definitive cause or urinary BC with an associated fivefold risk. Schistosomiasis is the second most common parasitic infection after malaria and about 600 million people are exposed to infection in Africa, Asia, South America, and Caribbean (Khurana et al. 2005).

The first to report on bilharziasis association with BC was Ferguson in 1911 and later on reports of the NCI registry stated that frequency of BC in Egypt was elevated, being 27.6% of all cancers (Gouda et al. 2007).

Even though the mechanism by which schistosomiasis causes BC remains unknown, two hypotheses have been proposed. First, the chronic inflammatory proliferation that allows and promotes genetic alterations, which ultimately can

lead to higher cancer incidence. And on the other hand, it has been suggested that in urine of patients infected with schistosomiasis, there is a production of carcinogenic substances as nitrosamines, concretely *N*-butyl-*N*(4-hydroxybutyl) nitrosamine. Importantly associated to that inflammatory reaction, there is a conversion of the transitional urothelium toward squamous epithelium; this is the cause for 70% of patients developing cancer because of bilharziasis, present with squamous cell carcinoma, rather than transitional cell carcinoma, as is usual in other geographic sites (Abol-Enein 2008).

Although the relationship between squamous cell carcinoma of bladder and schistosoma infection is well established, currently the trends of BC in endemic zones, as Egypt, are changing. In fact, data from the largest tertiary cancer hospital in Egypt, NCI Cairo, were analyzed to verify the incidence of squamous cell carcinoma in the area. Data from 1980 to 2005 were obtained and data from 2778cases were available for analyses. The authors demonstrated a statically significant association between period of diagnoses and histopathological type. In this way, patients diagnosed in 2005 had a sixfold higher odds associated to transitional cell carcinoma compared to those patients diagnosed in 1980 (Felix et al. 2008). Bilharzias association dropped from 82.4% to 55.3% and there was a significant increase of transitional cell carcinoma from 16% to 65%, while squamous cell carcinoma was less frequent, from 76% to 28%. Intimately related to this, there was an increase in the median age of patients from 47 to 60 years. The decline in the frequency of BC is related to a decline in bilharzias egg positivity in the specimen and this suggests a better control of the endemic disease in rural population (Gouda et al. 2007).

Even though association between inflammation in schistosoma infection and squamous BC is well established, the role of inflammation due to other infections in the origin of BC, even transitional cell carcinoma, is less clear.

Of the epidemiologic studies regarding urinary tract infection (UTIs) and BC, including transitional cell carcinoma, with one exception (Kjaer et al.1989), all the retrospective observational studies have demonstrated a positive association between BC and UTIs. Relative risk in these studies range between 1.4 and 16 for any history of urinary infection versus none, and similar associations have been found in men and women. To date no prospective study has been conducted to clearly establish the role of infection in bladder carcinogenesis.

Therefore, it could be possible that those positive associations result from detection bias or differential recall between cases and controls. Prospective studies with large number of patients and controls are warranted to determine the role of inflammation in BC (Michaud 2007).

1.5 Radiation and BC

Extensive research has been performed on long-term effects of ionizing radiation in cancer development. The most well studied population is Japanese atomic bomb survivors and data obtained from them continue to demonstrate an increased

risk of BC. The most recent report stated that 222 BCs were diagnosed between 1950 and 1997. The excess relative risk was higher for women although not significant. Contrary to other cancers the risk was constant over age (Hall 2008). Similar findings have been shown in studies of secondary cancers in patients treated with high dose radiotherapy. For example, incidence of bladder cancer was compared among 243082 men who underwent therapy for prostate cancer between 1988 and 2003. The patient cohort was identified in the Surveillance, Epidemiology and End Results database (SEER) in United States. Of these 109,178 (45%) were submitted to radical prostatectomy, 93,059 (38%) to external beam radiotherapy (EBRT), 22,889 (9%) to brachytherapy, and 17,956 (7%) to combination of ERBT-BT. Median follow-up was 49 months. The relative risk of BC developing after EBRT, BT, and ERBT-BT compared to radical prostatectomy was 1.88, 1.52, and 1.85, respectively. Compared to the general US population, the standardized incidence ratio (SIR) for BC developing after RP, ERBT, BT, and ERBT-BT was 0.99, 1.42, 1.10, and 1.39, respectively. The increased risk of BC of patients submitted to ERBT, BT, or combination must be taken into account during follow-up of patients, and considering that BC requires longer to develop, patients treated with radiation at young age are at highest risk and should be followed closely (Nieder et al. 2008).

In the same way, the incidence of secondary malignancies using data from 104,760 1-year survivors of cervical cancer reported to 13 population-based cancer registries in Denmark, Finland, Norway, Sweden, and the United States has been published . Patients with cervical cancer treated with radiotherapy, but not those who did not receive radiotherapy, were at increased risk for all second cancers and cancers at heavily irradiated sites (colon, rectum/anus, urinary bladder, ovary, and genital sites), beyond 40 years of follow-up compared with women in the general population. The association of RT with second cancer was modified by age at cervical cancer diagnosis. The 40-year cumulative risk of any second cancer was higher among women diagnosed with cervical cancer before age 50 (22.2%, 95% CI = 21.5%–22.8%) than among women diagnosed after age 50 (16.4%, 95% CI = 16.1%–16.9%) (Chaturvedi et al. 2007).

1.6 Dietary Factors and BC

Several dietary factors had been related to BC, however the results of several studies were controversial and currently there is no sufficient evidence but a limited evidence of a cause relationship between BC and dietary factors. Despite this, several researchers had pointed out that a small part of BC cases would be influenced by dietary habits and that those factors must be considered.

The Netherland Cohort Study on diet and cancer (NLCS) was initiated in 1986 with 120,852 men and women aged 55–69 years. Based on regional cancer registries in the Netherlands and with the national pathology register (PALGA) the authors established the follow-up for BC incidents . Between 1986 and 1992, 619

new cases of BC were diagnosed. In their analysis, the authors did not find that total fluid intake was related to BC risk. However, they demonstrated a small correlation between alcohol consumption and BC in men, which was independent of the stage of tumor. That relationships was not confirmed in BC in women .On the other hand, they concluded that high consumption of total fruit was probably associated with small decrease in BC risk as well as they established a negative association between BC and selenium intake (Zeegers et al. 2004).

With opposite results, it was reported by The Health Professionals Follow-up Study, which is, as well as NLCS, a prospective cohort study. It started in 1986 with 51,529 male health professionals aged 40–75 years from all 50 states of the United States. Of these 47,909 men were eligible for follow-up, and the follow-up rate averaged at 94% per follow-up cycle during the five biennial cycles between 1986 and 1996. They diagnosed 252 new cases of BC, and age and smoking were the two major risk factors related to cancer. Interestingly, and contrary to NLCS, they found that daily water consumption had a protective effect in risk of BC. In that way, when fluid intake was considered as a continuous variable the risk of BC decreased by 7% for every increment of 240 mL in daily fluid intake.

Those patients in the highest quintile of fluid intake (>2531 mL/day) had a 49% lower incidence of BC than those in lowest quintile (<1290 mL/day). This relation between fluid intake and BC was observed among smokers' patients as well as among nonsmokers. This suggested to the authors that residual confounding by smoking is unlikely to be responsible for their results.

An explanation for the decrease in risk of BC with increase on fluid intake could be that high fluid intake could dilute the concentration of carcinogens in urine or reduce the contact of those carcinogens with urothelium by increasing the voiding frequency. However, it had been postulated that contrary to that supposed, high fluid intake could increase the risk of BC if that fluid contents contain contaminants that are bladder carcinogens. In that way, a pooled analysis of six case–control studies of BC with detailed information on fluid intake and water pollutants were reported. The pooled study included 2729 cases and 5150 controls from studies performed at the Untied States, Canada, Finland, France, and Italy between 1978 and 2000. They found an increased risk of BC for tap water consumption, and this was consistently found in the six studies analyzed. A total tap water intake more than 2.01 L/day increased the risk of BC in 50% compared to total tap water ingestion less than 0.5 L/day. The association of tap water ingestion but not with nontap water fluids suggested to the authors that the increased risk observed in tap water intake was related to carcinogens diluted in such type of fluids (Villanueva et al. 2006).

Due to controversial results obtained in different epidemiological studies, further studies will be necessary to establish the real influence of fluid intake in BC risk.

Other dietary factors had been related to BC. A prospective study of atomic bomb survivors showed that green–yellow-vegetable and fruit consumption were significantly associated with decreased relative risk for BC. The study included 39,824 survivors of atomic bomb from 93,000 who have been under continuous surveillance by the Radiation Effects Research Foundation since 1950. They could observe that a frequency in green–yellow vegetable more than five times per week

was associated with a RR 0.54 (95% CI 0.3–0.94) that was significantly lower that RR of those with an intake frequency of 0–1 per week (1.00). In addition, those people with a fruit frequency intake of more than 5 per week showed a lower RR for BC 0.62 (95% CI 0.39–0.99) than those with less fruit frequency intake. The authors suggested that carotenoids and vitamin C contained in fruit and vegetables could explain the protective anticarcinogenic effect (Nagano et al. 2000). A metaanalysis of 38 articles reporting data on diet and BC confirmed the relation and support the hypothesis that vegetable and fruit intake reduces the risk of BC (Steinmaus et al. 2000).

A recent report of World Cancer Research Fund/American Institute for Cancer Research in 2007 concludes that the evidence is too limited to correlate any aspect of food, nutrition, and physical activity directly with modified risk of BC. The authors performed a systematic literature review and analyzed 349 reports on nutrition factors and BC and finally, they only found a limited evidence suggesting that milk protects against BC and that arsenic in drinking water is one of the cause for BC. (WCRF www.dietandcancerreport.org). Similarly, other systematic literature review was published in 2008. In this case, the authors conclude that the strongest evidence for a protective effect against BC was associated with fruit. They also detected a more frequent and pronounced effect in case–control studies compared with prospective studies. In their review, they obtained that fruit and yellow-orange vegetables, particularly carrots and selenium, are probably associated with a moderately reduced risk of BC.

Citrus fruits and cruciferous vegetables were also identified as having a possible protective effect. Possible risk factors are salted and barbecued meat, pork, total fat, pickled vegetables, salt, soy products, spices, and artificial sweeteners (Brinkman and Zeegers 2008).

Nevertheless, due to inconclusive results even of the systematic reviews, future studies on BC should investigate the effect of food categorization, quantity consumed, and gender differences.

1.7 Gender Related Differences in BC

BC is less prevalent in women than in men; however, several reports suggest that women are diagnosed at more advanced stage of the disease and, in general, have poor survival than men. Also, women could be under effect of different exposure than men are and females could have different susceptibility to develop BC. Finally there has been an appreciable increase in BC occurrence in women.

In a retrospective study of patients submitted to radical cystectomy it could be demonstrated that women are more likely to be diagnosed with primary muscle invasive disease than men (85% vs 51%) (Vaidya et al. 2001). Other retrospective study included 31,009 cases of BC diagnosed between 1991 and 2001. The authors could observe that women were more likely to be diagnosed at older age than men; in fact, 22.9% of females were diagnosed at an age older than 80 years, while only

15.8% of males were diagnosed at that age. In a multivariate analysis, the significant risk factors for developing regional and distant disease were older age, AA ethnicity, and being female. In addition, women with regional spreading had worse survival than men (28.2 months vs 31.9 months, respectively). Interestingly, the poor survival in women could be demonstrated to be related to older age at diagnosis, since after adjusting for advance age at diagnosis women showed better survival than men.

Authors concluded that women are diagnosed later than men and this has a direct effect on their survival. They suggest that women are more suitable to be delayed in hematuria study because differential diagnosis of hematuria in women includes diseases more prevalent than BC (Cardenas-Turanzas et al. 2006).

Differences in gender prevalence of BC seem to be due to factors other than tobacco and chemical exposure. A large prospective cohort study that included data from 106,057 women aged 30–55 years, with 26 years of follow-up had been recently published. Between 1976 and 2002, 336 (prevalence 0.3%) new cases of BC were diagnosed in the cohort. Among women diagnosed with BC 39.5% were former smokers, 35% were current smokers, and 25% were never smokers. The authors could observe that postmenopausal status was associated to an increase in BC risk even after adjusting for smoking status. Among nonsmokers the OR for postmenopausal women compared with premenopausal was 1.87 (95% CI 0.6–5.4), among smokers the OR for postmenopausal women was 1.97 (95% CI 0.84–4.62) when compared with premenopausal. Earlier age at menopause less than 45 years was associated with a higher risk of BC when compared with later age menopause, more than 50 years. Authors suggested an hormonal influence in BC occurrence and proposed that differences in estrogen and androgen levels between men and women could justify some of the differences in gender prevalence of BC (McGrath et al. 1984).

Another set of two retrospective studies, one from the Untied States and other from Austria, confirm the sex and racial differences in BC. In 2008, a retrospective review of 1269 patients with BC from a single center was reported. The male-to-female BC ratio was 2.2:1. Significantly, tumors were diagnosed at younger age in men than in women (62 vs 67, $p < 0.001$), primary tumors were more aggressive in men and tumor recurrences were more invasive, and in a subset of muscle-invasive BC, women showed a worse overall survival rate than men ($p = 0.02$).

Authors suggested gender differences in BC beyond the higher incidence in males. They proposed a protective effect of female gender in the early stages of BC with less-invasive and less-aggressive tumors. However, once advanced stages of disease have occurred, the protective effect seems to disappear, resulting in a worse overall survival rate in female patients. This suggests that in addition to exogenous factors (e.g., exposure to carcinogens), endogenous factors (related to gender differences) need to be evaluated to understand the epidemiology and molecular basis of BC (Horstmann et al. 2008).

In the US data from 16 population-based registries, included in the Surveillance Epidemiology and End Results (SEER), limited-use database were used to define the study cohort of 101,249 patients. These patients represent a 88% of all black and white patients in the SEER who were diagnosed with BC between 1990 and

2003. On an average, women were older than men within each race at BC presentation ($p < 0.001$). For both races women were more likely to present with muscle-invasive disease compared with men, although this difference was much smaller for white patients (22% vs 25%) than for AA patients (30% vs 43%). Among white patients with nonmuscle invasive BC (NMIBC) , men were more likely to present with high-grade tumors than women ($p < 0.001$). Women and AA patients had a higher proportion on nonurothelial cell types at presentation. Taking into account the sex and racial differences in the distribution of prognostic factors, the authors investigated the effect of such distribution in BC mortality. They observed that a excess hazard of death from BC was present during the first 2–3 years of follow-up among women, but after adjustment for age and tumor characteristics, this hazard was reduced in 30% among white women. So, the authors proposed that it is not the significant differences alone in tumor characteristics and age at presentation that explain the excess hazard of death from BC among women. Other factors must also be investigated (Scosyrev et al. 2009).

1.8 Hereditary BC

Familial BC is an uncommon entity. In 1994, it was reported by Goldgar et al. that familial BC risk in first-degree young relatives of BC probands was 5.1 (95% CI, 1.0–12.5) (Kirkali et al. 2005).

Identification of familial subtype of BC could help in the research of molecular carcinogenesis of BC. However, identification of this subtype of BC is not always feasible, in most cases, the data regarding to family members of patients affected of BC are not available. Hemminkki et al. reported the results of 2002 update of the Swedish Family-Cancer Database that included 754,165 first invasive parenteral (diagnosed in 1961–2000) and 112,216 offspring (diagnosed in 1991–2000) cancers. Authors studied the familial risk of urological cancers. They detected 2987 BC in offsprings and according to the occurrence of BC in their families they could conclude that familial risk for BC was increased with a standardized incidence ratio (SIR) of 1.75. When they considered only siblings the ratio increased to 2.02 (Hemminki et al. 2004).

One of the most exhaustive works performed in the research of familial urothelial carcinoma has been conducted in the Netherlands. Aben et al. reported a case–control study that included 1193 newly diagnosed cases of urothelial cancer and 853 controls that consisted of the family of the probands' partner. The authors searched the urothelial cancer occurrence among the relatives of urothelial cancer probands. They could obtain information of 8014 first-degree case relatives and 5673 first-degree control relatives. Overall, 8% of the patients had a positive family history of urothelial cancer compared to 4% of controls. The authors conclude that urothelial carcinoma showed a pattern of familial aggregation and that the first-degree relatives of patients affected of urothelial carcinoma presented a 1.8-fold increase in the risk (Aben et al. 2002).

Thirty families were selected from those with at least one relative diagnosed of urothelial carcinoma, and blood sample was obtained from each proband. Authors performed classical cytogenetic analysis on metaphase spreads. Karyotyping for the detection of chromosomal alterations was negative in all 30 patients and a inherited genetic defect could not be assessed (Aben et al. 2001). Of the initial 95 patients with at least one first-degree relative affected of urothelial carcinoma, authors selected eight families suggestive of familial BC. They applied, this time, a high-throughput genomic analysis technique, array-CGH, which has 100 times higher resolution for detecting genomic copy number alterations than classical karyotyping. Also,lso, with classical approach, the authors could not find any candidate region for being considered responsive of hereditary BC (Kiemeney et al. 2006).

In a population-based study of 2982 BC patients and 5782 controls in ten geographic areas of the United States, which was designed to assess the role of environmental risk factors, information was also obtained on the history of urinary tract cancer in first-degree relatives. A family history of urinary tract cancer, significantly, elevated the risk of BC [relative risk (RR) = 1.45], with higher risks observed among patients under age 45. The risks of BC associated with positive family history were generally higher among persons with suspected environmental exposures, particularly, heavy cigarette smoking (RR = 10.7 among those who smoked three or more packs per day) (Kantor et al. 1985).

Another large case–control study was conducted in Spain. Information on family history was obtained for 1158 newly diagnosed BC cases and 1124 controls included in 18 hospitals between 1998 and 2001. The odds ratio for BC among subjects reporting a family history of cancer was 2.34 (95% CI, 0.95–5.77).

The OR of BC for those reporting family history of BC was 4.76 (95% CI, 1.25–18.09) among NAT-2–slow acetylators and 1.17 (95% CI, 0.17–7.86) among NAT-2–rapid/intermediate acetylators ($P = 0.6$) (Murta-Nascimento et al. 2007).

In a recent review, the authors analyzed details of nine case–control and four cohort studies in which family history of BC was quantitatively evaluated as risk factor. These studies differ widely in sample size, methodology, and analysis, inclusion criteria, confirmation of diagnosis, but surprisingly all of them resulted in similar risk ratios. The risk ratios ranged from 1.2 to 6.1 among male and female cases combined (Mueller et al. 2008).

The cause of familial clustering is still controversial, but most of evidences suggest an underlying genetic predisposition. These genetic factors may be infrequent but with a high penetrance, although not so high as in other cancers. An alternative approach to the study of this genetic factor causing clustering in families is genome-wide association (GWA) studies in case–control designs. High-resolution GWA studies, with extensive replications of positive findings in other case and control series, can map the susceptibility loci involved in familial cases. Currently, there are at least three independent GWA studies ongoing with results coming up nearly in 2–3 years (Kiemeney 2008; Wu et al. 2008).

1.9 Other Related Risk Factors: Cyclophosphamide, Phenacetin

1.9.1 Cyclophosphamide

Cyclophosphamide is an alkylating agent used for treatment of lymphoproliferative diseases and other nonneoplastic diseases. It has been correlated with the use of this treatment to the posterior development of BC, especially muscle-invasive BC with a period of latency of 6–13 years (Kirkali et al. 2005). It seems that acrolein, one of the metabolites of cyclophosphamide, is the one responsible for an increase in occurrence of BC, and also, the incidence of BC after cyclophosphamide treatment seems to be independent of the occurrence of hemorrhagic cystitis related to the same treatment (Johansson and Cohen 1997).

1.9.2 Phenacetin

Phenacetin was included as among the proven human carcinogens by the IARC in 1987, and some studies suggest that the intake of phenacetin was positively related to BC risk in a dose-dependent manner, although there is controversial data concerning its metabolite acetaminophen (Castelao et al. 2000).

1.10 Screening of BC

Muscle-invasive BC is an aggressive neoplastic that was associated with a mortality ratio of 50% at 5 years despite the aggressive treatment. Furthermore, those patients that die from BC undergo the treatment because of metastatic disease, and in 90% of the cases metastasis appears 2 years after muscle-invasive disease was diagnosed.

At the initial diagnosis of BC, 70% of cases are diagnosed as superficial diseases, and the remainder 30% as muscle-invasive disease. Of those patients submitted to radical cystectomy because of muscle-invasive disease, 57% has muscle invasion at presentation, while 32% has been initially diagnosed with non-muscle-invasive disease that progressed despite the treatment (Vaidya et al. 2001).

All these data suggest that early detection of BC would decrease the ratio of muscle-invasive disease since currently most of the patients with invasive disease show the condition at presentation. On the other hand, there are a percentage of patients with superficial disease who are about to die because of progression, and the identification of this group of patients prior to establishment of invasive disease, as well, might contribute to increase the survival of patients affected by BC.

The way to reduce mortality of BC include the diagnosis of the illness at early stages, the improvement of treatment of superficial BC to avoid progression, and the improvement of treatment of muscle-invasive disease to avoid metastatic spreading.

The diagnosis of BC at early stages could be achieved by a screening program; however, the role of BC screening remains controversial since no prospective, randomized studies have been conducted to demonstrate that screening reduces mortality in BC.

A screening program improves its results when prevalence of the disease increases. Low prevalence of BC in general population limits the application of a screening program, since it would raise the possibility of too many false-positive results and would not be cost-effective (Lokeshwar et al. 2005) However, screening could be applied for an early detection of BC among high-risk population, where prevalence of the disease is higher than in general population (Kirkali et al. 2005).

Despite there being no consensus for BC screening, several studies have been reported with adequate sample cases and follow-up.

Screening of general population does not seem to be cost-effective. In a retrospective study of 20,571, men aged >35 years and women aged >55 years with hematuria were detected by a single dipstick urinalysis in 876 cases (4.3%). However, urological cancers (two prostate, one bladder) developed in only three patients within the next 3 years. Among patients whose test results were negative for hematuria, cancer rates were found to be almost the same as the rate among patients with asymptomatic microhematuria. The authors concluded that their results were consistent with the lack of recommendations for screening for microhematuria among asymptomatic adults (Hiatt and Ordonez 1994).

In this way, several authors proposed that hematuria due to BC used to be intermittent and that a single urinalysis was not sensitive enough for being used as a marker in BC screening program. Comparison of BC outcome in asymptomatic men submitted to repetitive hematuria home screening versus those with BC detected by standard clinical presentation was reported. The screening sample included 1575 men aged 50 years who were asked to test their urine daily for 14 days and, if all tests were negative, to repeat 14 daily testing months later. The control unscreened sample consisted in 493 newly diagnosed BC reported to the Wisconsin Cancer Reporting System for 1988. Incidence of BC among population screened was 1.3% (21 cases). Of the 21 cases diagnosed in screened population, 4.8% were muscle-invasive (0.06% among total screened population), 42.9% high-risk superficial BC (0.5% among total screened population), and 52.4% low and intermediate risk superficial BC (0.6% among total screened population). Among the unscreened population ratio of muscle-invasive BC at presentation was 23.9%. In screened population after 30 month of follow-up, no death had been recorded in contrast to 16.4% of patients with BC diagnosed outside the screening program who died of BC. The authors concluded that the lower rate of muscle-invasive BC at presentation and reduced disease-related mortality in patients included in screening program suggested the convenience for conducting randomized trial to confirm the efficacy of BC screening in asymptomatic population (Messing et al. 1995).

However, the low incidence of life-threatening BC detected in asymptomatic population by dipstick urinalysis probably would not justify a screening program in general population.

In the same way, in community-based screening program 2356 men aged >60 years had a repetitive dipstick test for microscopic hematuria. Seventeen (0.7%) were found to have bladder tumors and none of them were muscle-invasive at diagnosis. At 7 years of follow-up, two patients with tumors having a potentially worse prognosis progressed to muscle-invasive disease (22% progression among patients with high-risk superficial BC). Among the initial screened population, incidence of BC was 0.7%, and ratio of life-threatening cancer was 0.08%, with a mortality rate of 0.1%. Authors concluded that diagnosis of those tumors in early stage could allow early aggressive treatment prior to progression to muscle invasive BC (Mayfield and Whelan 1998).

Taking account the number of high-grade invasive BC cases detected among asymptomatic population, the cost-effectiveness of the screening program based on dipstick urinalysis would probably not be acceptable.

Probably, the establishment of bladder screening in high-risk population would achieve better results than in general population.

Not only is the population that has to be screened controversial but the test which must be used in the screening program as well is . The screening of asymptomatic population for BC by testing for hematuria would lead to many falsepositive results that would generate further exams with a low rate detection of BC. In these way, several others markers have been valuated for being candidates for BC screening and follow-up.

An ideal tumor marker is that noninvasive, ease of assaying, with low intraassay and interassay variability and a high level of accuracy (Lokeshwar et al. 2005).

Several markers have been tested. BTA, BTA stat, BTA track, NMP22, telomerase, UroVysion, UBC, Survivin, Cytokeratins, CYFRA 21-1, ImmunocYt, DD23, microsatellite DNA test. In a recent metaanalysis reported on 42 articles suitable in the literature from urinary markers for BC, the authors concluded that cytology was found to have the best specificity and telomerase the best sensitivity; but the data were not consistent enough for recommending a specific test combination for BC screening (Glas et al. 2003).

In 2001, the American Urological Association's best practices policies indicates that there are insufficient data available to recommend the routine use of voided urinary markers in the evaluation of patients with microscopic hematuria. In January 2005, the UroVysion Bladder Cancer Kit was approved by the FDA for use in assisting in the detection of the initial diagnosis of bladder carcinoma in patients with hematuria or recurrence of BC.

The International Consensus Panel on bladder tumor markers reported in 2005 an exhaustive review of current urinary markers available for BC diagnosis and follow-up. The Panel concluded that currently, the "most practical use of noninvasive tests would be for monitoring bladder cancer recurrence and reducing the number of surveillance cystoscopies performed each year." The panel did not report any recommendation for use of urinary markers in BC screening since no results of

prospective, randomized studies are yet available. However, the panel remarked, "several bladder cancer markers are more accurate in detecting bladder cancer than prostate-specific antigen (PSA) is in detecting prostate cancer. Therefore, the use of bladder cancer markers will require the willingness of both urologists and clinicians to accept them" (Lokeshwar et al. 2005).

1.11 Chemoprevention of BC

The strategies for prevention of cancer include three levels. Primary prevention consists in avoiding the development of BC in healthy population. Latency between exposure and BC occurrence, the low incidence of the disease and the lack of strategy to prevent bladder carcinogenesis, make chemoprevention a less likely approach for urothelial carcinoma of the bladder.

Secondary prevention consists in preventing the progression of premalignant lesions onto neoplastic disease. Again, this prevention cannot be applicable to BC, since there is no well defined premalignant lesion. Finally, tertiary prevention consists in avoiding recurrence and progression of superficial BC to invasive disease (Grossman 2006).

Other prevention strategies different from smoking cessation have less strength of evidence or they are in a very preliminary phase of development. Some of these prevention strategies include, an increase in fluid, fruit and vegetable intake, vitamin supplementation, increase in folate intake and the use of antiinflammatory drugs (Castelao et al. 2000; Leppert et al. 2006; Schabath et al. 2005). However, all of the prevention studies are preliminary and there is no sufficient data to establish a prevention program in BC. The only program that has sufficient strength is smoking cessation, and so strategies to encourage people to quit smoking would directly affect the incidence of BC.

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Chapter 2
Bladder Carcinogenesis and Molecular Pathways

Diogo O. Escudero, Samir P. Shirodkar, and Vinata B. Lokeshwar

2.1 Introduction

Bladder cancer is primarily a "carcinogenesis driven" cancer. Exposure to carcinogens such as polycyclic aromatic hydrocarbons (PAH), aromatic amines (AA), and nitrosamines through cigarette smoking, occupational exposure, and hair dyes, among other substances, is strongly associated with increased risk for bladder cancer (DeMarini 2004; Murta-Nascimento et al. 2007). Many of these carcinogens form bulky DNA adducts, eventually causing mutations and chromosomal aberrations (Castaño-Vinyals et al. 2007; Veglia et al. 2008). Such molecular events take place when potential carcinogens are "activated" during the process of detoxification by metabolic enzymes that are involved in the metabolism of xenobiotic compounds. Genetic polymorphism in many of these genes is associated with risk for developing bladder cancer. In this first part of this review, we will discuss the relationship between chemical carcinogens and the development of bladder cancer. Furthermore, we will describe the role of the carcinogen-metabolic enzymes and the polymorphism in the genes encoding them in the development of bladder cancer. The exposure of the bladder urothelium to carcinogens that are excreted through urine has given rise to the theory of "field effect" and it is used to explain the multifocality and recurrent nature of bladder cancer. The heterogeneity of low- and high-grade bladder tumors to invade and metastasize has given rise to the concept of "divergent molecular pathways" for bladder cancer development. In the second part of this review, we will discuss the clonal origin of this multifocal disease and elaborate on the molecular pathways in the development of low- and high-grade bladder tumors.

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2.2 Bladder Carcinogenesis

2.2.1 Bladder Carcinogens and DNA Adduct Formation

The major risk factor for bladder cancer development is the exposure of the urothelium to chemical carcinogens. PAHs and aromatic amines (AA) are the most widely known and studied bladder carcinogens, as described in the literature. These carcinogens, when converted into active forms due to body's response to detoxify them, form DNA adducts in urothelial cells. DNA adducts are known to induce point mutations and such mutations in oncogenes (e.g., H-ras) or tumor suppressor genes (e.g., p53) cause cellular transformation. Additionally, point mutations in DNA repair enzymes can cause chromosomal instability leading to cellular transformation. Chromosomal instability is a major factor in the development of invasive bladder cancer (Florl and Schulz 2008).

2.2.2 Phase I and II Enzymes

The xenobiotic metabolism involves two different classes of enzymes. Phase I enzymes (e.g., cytochrome P450 dependent monooxygenases) are usually involved either in oxidation (to form *N*-hydroxy, phenol, or dihydrodiol intermediates) or reduction, which activates carcinogens into a reactive form (Talalay 1989). For example, AAs (e.g., 4-amino biphenyl) are converted into the *N*-hydroxy amine form by CYP-A2. The *N*-hydroxy arylamines can be transported through the blood, where it can form adducts with hemoglobin. These *N*-hydroxy AAs (electrophilic) pass through renal filtration into bladder lumen and can form adducts with DNA (nucleophilic) in urothelial cells (Kadlubar 1990; Kadlubar and Badawi 1995). In fact, urinary mutagenecity is correlated with the levels of 4-aminobiphenyl adducts in exfoliated urothelial cells from smokers (Talaska et al. 1991). Chemical fractionation of urine from smokers indicates that much of the mutagenic activity is due to PAH or AA (DeMarini 2004).

2.2.2.1 Cytochrome P450 (CYP) Monooxygenases

CYP450 enzymes are a superfamily of microsomal enzymes with more than 20 members. These enzymes are extremely polymorphic and the polymorphism in some of the enzymes is associated with higher risk for bladder cancer development. The CYP profile is altered in different types of cancers. For example, important CYPs in bladder urothelium that are reported are CYP1B1 and CYP4B1 (Roos and Bolt 2005). In a case–control study Grando et al. found that CYP1A1 (A2455 -> G) polymorphism significantly associated with risk for bladder cancer (Grando et al. 2009). However, Srivastava et al. found no association between the

CYP1A1*2A genotype and increased risk for bladder cancer among people from North India (Srivastava et al. 2008). Similarly, Fontana et al. found no association between CYP1A1 or CYP1B1 polymorphisms and bladder cancer risk (Fontana et al. 2009). Among the Japanese population, people carrying CYP4B1*1/*2 or *2/*2 genotypes were found to have increased risk for developing bladder cancer than individuals with the CYP4B1*1/*1 genotype (Sasaki et al. 2008). Extensive metabolizer genotype CYP2D6*1A was reported to be significantly associated with the development of transitional cell carcinoma of the bladder cancer, rather than squamous cell carcinoma of the bladder (Abdel-Rahman et al. 1997). Contrarily, carriers of at least one CYP2A6*4 allele were reported to have lower risk for developing bladder cancer than noncarriers (Song et al. 2009). However, Querhani reported no association between CYP2D6*4 allele and susceptibility to bladder cancer in Tunisian population (Ouerhani et al. 2008). Altayli et al. also found no association between polymorphism in CYP1A2 or CYP2D6 genes and risk for bladder cancer (Altayli et al. 2009).

Taken together, polymorphisms in CYP genes have different degree of association with the development of bladder cancer and this association may depend on ethnic origin, as well as, smoking history.

2.2.3 Phase II Enzymes

Phase II enzymes are mainly involved in detoxification of chemical carcinogens, such as AA and PAH. The inducers of phase II enzymes have an electrophilic olefin or related electron-deficient center that is susceptible to attack by nucleophiles. Thus, all inducers of phase II enzymes are "Michael reaction acceptors" characterized by olefinic or acetylenic linkages that become electrophilic by conjugation with electron withdrawing groups (Talalay 1989; Talalay et al. 1988). Examples of well-studied phase II enzymes in the context of bladder cancer are *N*-acetyl transferases (NAT) and glutathione-*S*-transferases (GSTs). Polymorphisms in these genes are associated with bladder cancer risk.

2.2.3.1 *N*-Acetyl Transferases (NAT)

NAT1 and NAT2 isoenzymes catalyze both N-acetylation (deactivation) and O-acetylation (activation) of aromatic and heterocyclic amines (Franekova et al. 2008). They catalyze the transfer of acetyl group from acetyl-CoA to AA and hydrazine substrates. AA and PAH are O-acetylated by both NATs. Although NAT1 and NAT2 share 87% nucleotide homology (only 55 out of 290 amino acids are different), NAT2 has three- to fourfold affinity to bladder urinary carcinogens such as 4-ABP than NAT1 (Hein 2006). The association between polymorphisms in NAT1 gene and bladder cancer risk is not well established. Fast acetylation status of NAT1 has been associated with risk for colorectal cancer, however, no such

correlation was found in metaanalysis of case–control and cohort studies (Sanderson et al. 2007). Certain alleles of NAT1 (NAT1*14, NAT1*15) do not have enzyme activity, however, their association with risk for bladder cancer has not been evaluated (Franekova et al. 2008).

A number of single nucleotide polymorphisms in the coding region result in the slow acetylator phenotype (i.e., NAT2) is unable to efficiently acetylate bladder carcionogens such as AA and PAH. The slow acetylator phenotype has been associated with risk for a variety of carcinomas, including bladder cancer. The proposed mechanism for the association between the slow acetylator phenotype and bladder cancer is that the slow acetylation of AA by NAT2 is competed out by the metabolic activation of these AAs by CYP enzymes (Hein 2006). Therefore, the slow acetylator phenotype combined with Fast CYP phenotype carries higher risk for developing bladder cancer than either phenotype alone or the reverse phenotype (i.e., fast NAT2 and slow CYP). NAT2 alleles that contain arginine64->glutamine; isoleucine114->threonine, arginine197->glutamine, or lysine268->arginine substitutions are associated with slow acetylator phenotype. The association between NAT2 polymorphism and the risk for bladder cancer has been summarized in a variety of excellent reviews (Hein 2006; Franekova et al. 2008; Sanderson et al. 2007). Recent studies show that the slow acetylator NAT2 phenotype increases risk for bladder cancer by about threefold in a variety of ethnic groups and the risk may be higher in females (Ouerhani et al. 2008; Fontana et al. 2009; Song et al. 2009). However, the slow acetylator NAT2 phenotype is not related to racial differences among blacks and whites regarding the risk for developing bladder cancer (Muscat et al. 2008). Smokers with slow acetylator phenotype may have up to 12-fold increase risk for developing bladder cancer (Rouissi et al. 2009) and this may explain the variation observed among smokers regarding bladder cancer development.

2.2.3.2 Glutathione-*S*-Transferase (GST)

In humans, eight distinct gene families encode different GSTs: alpha, mu, theta, pi, zeta, sigma, kappa and chi (or omega; Franekova et al. 2008). Among these, GSTM1, GSTT1, and to a lesser extent GSTP1 are the most well studied with respect to the risk for developing bladder cancer. As reviewed by Franekova et al., GSTT1 (theta group) detoxifies smaller reactive hydrocarbons, whereas, GSTM1 (mu group) detoxifies PAH (Franekova et al. 2008). Polymorphism in GSTM1, GSTT1, and GSTP1 has been well documented in bladder cancer. Earlier studies showed increased GSTP1 and GSTM1 activity in bladder tumor tissues when compared to normal bladder tissues. GSTT1–1 and GSTM1–1 are genetically deleted (nonfunctional alleles GSTT1*0 and GSTM1*0) in a high percentage of the human population, with major ethnic differences. For example, 20% of Caucasians are homozygous for a null allele GSTT1*0 (Franekova et al. 2008). Similarly, nearly 50% of the Caucasian population may have GSTM1 null phenotype. GSTM1 and GSTT1 null genotypes are associated with an increased risk of bladder cancer and

the risk increases further when GSTT1 null phenotype is combined with smoking or occupational exposure to AA (Salagovic et al. 1998; García-Closas et al. 2005; Moore et al. 2007; Yuan et al. 2008). Double negative individuals (GSTM1 and GST1 null) have even higher risk for developing bladder cancer (El Nouby et al. 2008; Song et al. 2009). Similarly, G/G genotype of the GSTP1 gene polymorphism may also be associated with risk for bladder cancer (Srivastava et al. 2005a, b). Interestingly, Rouissi et al. found that individuals with NAT2 slow acetylator and wild type GSTT1 and GSTM1 null phenotype have the highest risk for bladder cancer in Tunisian population (Rouissi et al. 2009).

The two electron reductase NADP(H) dehydrogenase quinine 1 (NQO1) can either activate or detoxify quinones from AA or PAH intermediates (Joseph et al. 1994). Similarly, sulfotransferases are a supergene family of enzymes that catalyze sulfonation of several xenobiotic compounds. Sulfonation of a nucleophilic group decreases its activity; however, may also generate electrophilic species, which can then form DNA adducts. There are three sulfotransferase families (SULT1, SULT2 and SULT3), each with more than ten members (Franekova et al. 2008). Polymorphisms in both NQO1 and SULT genes have been shown to be associated with risk for bladder cancer. For example, subjects carrying both the NQO1 C/T and T/T genotypes and the SULT1A1 G/G genotype have nearly fourfold increased risk of developing bladder cancer than noncarriers. The risk doubles if these individuals are either current or former smokers (Wang et al. 2008). C to T base change at position 609 of the human NQO1 cDNA (C609T) changes Proline 187 to Serine. Individuals with this change have trace amount of NQO1 protein and no NQO1 activity (Siegel et al. 1999). Variant allele carriers of the NQO1 (P187S) polymorphism may have a higher risk for high-stage bladder cancer than noncarriers at diagnosis. Furthermore, patients with NQO1 (R139W) variant allele carrier along with Ta/T1 high-grade bladder cancer may have shorter disease-free survival than noncarriers (Sanyal et al. 2007). Similar to the polymorphisms in NQO1 gene, the SULT1A1 gene possesses a G→A polymorphism that changes Arginine 213 to Histidine. The Histidine (213) allele has been shown to have low activity and low thermal stability. Zheng et al. found reduced risk for developing bladder cancer if the individuals are either homozygous or heterozygous for Histidine 213 (Zheng et al. 2003). Recently, Figuerora et al. in a case–control study involving over 2000 subjects reported that polymorphisms in an aldo-keto reductase gene AKR1C3 and in aryl hydrocarbon nuclear translocator gene significantly associate with risk for developing bladder cancer (Figueroa et al. 2008).

Taken together, the risk for bladder carcinogenesis is dependent on exposure to carcinogens, as well as, the relative enzyme activity of phase I and II enzymes. Individuals with fast acetylator phase I enzyme (i.e., faster conversion of PAH and AA into active electrophilic species), when combined with slow acetylator phenotype for NAT2 enzyme or the polymorphisms in GST enzymes, which render the phase II enzyme to have low activity (i.e., slower detoxification rate), have the highest risk for bladder cancer while individuals with opposite phenotype for phase I and II enzymes have the lowest risk.

2.3 Field Cancerization and Clonal Origin of Bladder Cancer

Synchronous and metachronous tumors, as well as frequent tumor recurrence, can be explained by the concept of "field cancerization," where the entire bladder urothelium is exposed to carcinogens and the entire urothelium is primed to undergo transformation (Braakhuis et al. 2003). This concept was initially introduced by Slaughter et al. (Slaughter et al. 1953) in 1953, when studying oral squamous cell carcinoma. It has now been applied to a variety of tumors, including bladder. When exposed to carcinogens, urothelial cells can accumulate independent point mutations, gene deletions, or duplications, some of which can cause cellular transformation. Thus, field cancerization should lead to independent transformation of many urothelial cells resulting in genetically unrelated tumors. With the discovery of cancer stem cells, the field cancerization concept has been modified. It is suggested that the resident urothelial stem cells within the urothelium, when exposed to carcinogens, undergo transformation into cancer stem cells, and the clonal expansion of different cancer stem cells results in multifocality and recurrent tumors. Additional accumulation of genetic alterations (mutations, loss of heterozygosity (LOH)) introduces more heterogeneity in bladder tumors (Habuchi 2005; Cheng et al. 2009).

The second concept is the clonal origin of multifocal and recurrent tumors. The clonogenic or single progenitor cell hypothesis states that genetic and phenotypic diversity, observed in multifocal bladder tumors, is the progressive accumulation of genetic alterations in clones of a single transformed cell. In addition to field cancerization and clonal expansion, epigenetic modifications and cellular microenvironment can further contribute to tumor heterogeneity (Duggan et al. 2004). Combining the concept of field cancerization, cancer stem cells, and clonal origin of bladder tumors Höglund expanded the concept of the field effect further, suggesting a new model "field first-tumor later" (Höglund 2007). In this model, aberrant cells (due to carcinogen exposure), with a stem cell or stem-cell like properties spread in the urothelium by cellular displacement, creating fields of premalignant cells. Tumor development at multiple sites then depends on the frequency and timing of critical genetic events in individual cells in such a field. Hence, recurring tumors originate from the field of same premalignant tumors, and not from previous overt tumors. Therefore, the "field" remains even after the removal of the primary tumor(s) (Braakhuis et al. 2003). This concept can also explain clinical observations that tumor recurrence is actually the occurrence of a new tumor in the bladder.

Genetic analyses such as evaluation of LOH using microstatellite markers, X-chromosome inactivation assays, comparative genomic hybridization (CGH), and fluorescence in situ hybridization (FISH) have been used in support of either the field cancerization effect or the clonal origin of bladder cancer. LOH is the most frequent alteration in bladder cancer. Among all genetic alterations, LOH has about 75% frequency followed by chromosome length alterations (25%; Berger et al. 2002; Fadl-Elmula 2005). LOH in chromosome 9 has been used in support of the clonal origin concept. Junker et al. reported that in their study, 80% of the cases of recurrent tumors from the same patient had the same LOH in chromosome 9

(Junker et al. 2005). Contrarily, Dahse et al. used p53 mutational analysis to conclude that recurrences may occur through genetically unrelated primary tumor sites. Furthermore, synchronous and metachronous tumors may have polyclonal origin due to field cancerization effect (Dahse et al. 2003). In addition of the chromosome 9 LOH, using CGH analyses, Prat et al. found gains of chromosome 1q, 2p, and 17q loci and loss of 4q locus in multicentric tumors. They concluded that accumulation of chromosomal alterations is a form of clonal evolution from a single progenitor cell. Furthermore, heterogeneity present in the same tumor is the result of genetic evolution of a clonally expanded progenitor cell, which also, probably, occurs in the synchronous tumors in a same patient (Prat et al. 2008).

The epithelial and mesenchymal components in sarcomatoid urothelial carcinoma may also arise from a single progenitor cell. Armstrong et al. reported that in sarcomatoid urothelial cell carcinoma, both epithelial and mesenchymal components have identical p53 mutations, and, therefore, have the same clonal origin (Armstrong et al. 2009). Similarly, Sung et al. found identical pattern of LOH with six polymorphic microsatellite markers and X-chromosome inactivation in both the carinomatous and sarcomatoid components (Sung et al. 2007). Small cell carcinoma of the bladder is often mixed with transitional cell carcinoma. Using microstatellite markers on chromosomes 3p25–26, 9p21, 9q32–33, and the TP53 locus and an X-chromosome inactivation assay, Cheng et al. reported that both the coexisting tumor components originate from the same cells in the urothelium (Cheng et al. 2005). Lymph node metastases and primary tumors can also be traced to a common clonal origin. Using LOH in chromosome 9 and 17p13 (p53 locus) and X-chromosome inactivation analysis, Jones et al. showed that metastasis often arises from only a single clonal population in the primary tumor. Additional gene variations then arise during clonal evolution of urothelial carcinoma (Jones et al. 1993, 2005a). Contrarily, using the same allelic markers, the same authors reported that each of the coexisting tumors in multifocal urothelial carcinoma has a unique clonal origin. These tumors arise from independently transformed urothelial progenitor cells as a result of the field cancerization effect (Jones et al. 2005b).

A review of the study cited above and the published literature suggest that multifocal and recurrent tumors are a result of both clonal origin and field cancerization effect. However, these two are not necessarily mutually exclusive events. Changes in the urothelial progenitor cells can trigger clonal expansion, but accumulation of different genetic alterations in different clones (due to field cancerization effect) results in tumor heterogeneity. Depending upon the microsatellite marker analyses, one may find evidence for one of these concepts.

2.4 Chromosomal Aberrations in Bladder Cancer

Rearrangements and/or chromosomal aberrations are most common in chromosome 9 and occur in more than 50% of all bladder tumors. These alterations are present in both low-grade nonmuscle-invasive tumors and in high-grade muscle-invasive tumors. Thus, the loss of chromosome 9 is considered an early event in bladder

cancer and the regions most often are the 9p21 locus and three or more regions in the long arm of chromosome 9 (pq22, 9q32–33, and 9q34) (Fadl-Elmula 2005). The candidate tumor suppressor genes in these loci are CDKN2A/ARF (p16/p14ARF; 9p21), CDKN2B (p15; 9p21), PTCH (Gorlin Syndrome gene; 9q22), DBC1 (deleted in bladder cancer 1 locus; 9q32–33), and TSC-1 (tuberous sclerosis syndrome gene; 9q34) (Knowles 2006, 2008). Gain of chromosome 7 (chromosome 7 trisomy) is a common finding in bladder cancer and it is one of the three chromosome gains that are examined to make an inference on the UroVysion test (a FISH test for bladder cancer). A known consequence of this chromosome gain is the increased number of alleles for epidermal growth factor receptor (Knowles 2006).

Other chromosomal aberrations that are detected at a higher frequency in bladder cancer include rearrangements in chromosome 1, 8, and 11. For example, amplification of the chromosome 1Q32 has been reported in bladder cancer tissues using CGH array on a whole genome BAC/PAC cosmid. Mouse double minute 4 (MDM4) homologue is the amplified gene in this locus and the amplification occurs in tissues that express wild type p53 (Veerakumarasivam et al. 2008). Allelic imbalance at chromosome 1q36 locus is associated with poor survival among patients who receive chemoradiation therapy following cystectomy for muscle-invasive bladder cancer (Matsumoto et al. 2004). Most frequently, chromosomal aberrations associated with bladder cancer are the deletion of the 8p locus and gain of 8q locus. CMYC is the candidate oncogene on 8q24 and alternations in CMYC gene, including copy number changes, are associated with bladder cancer (Zaharieva et al. 2005). Similarly, in a genome-wide SNP association study involving over 4000 bladder cancer casesand over 37,000 controls, polymorphism in the chromosome 8q24 locus (allele T of rs9642880) was found to confer susceptibility to nine smoking-related cancer cases, including bladder cancer (Park et al. 2008). Contrarily, tumor suppressor genes such as human beta defensin-1 and MTUS1 have been found on chromosome 8p23 and 8p22 loci, respectively, which may explain why the loss of chromosome 8q is associated with bladder cancer (Di Benedetto et al. 2006; Sun et al. 2006).

Aberrations in chromosome 11 (polysomy) may be found in up to 70% of bladder tumors (Watters et al. 2002; Panani et al. 2004). Amplification of the 11q13 locus has been reported in bladder cancer. Putative candidate oncogenes located in this region are CCND1 (cyclin D1: PRAD1, bcl-1), EMS1, FGF3 (Int-2), and FGF4 (hst1, hstf1). Zaharieva et al. evaluated the involvement of these genes in a FISH study involving over 2000 bladder specimens. The frequency of gains and amplification of all four genes was observed in about 70% of the specimens and correlated with tumor grade and stage. In addition, the amplification correlated with poor survival and the progression of T1 tumors (Zaharieva et al. 2003). Similarly, Shao et al. observed CCND1 translocation and amplification only in bladder cancer patients (Shao et al. 2004). Contrary to the 11q13 locus, loss of the 11p locus is observed frequently in patients with bladder cancer (Brunner et al. 2008).

In addition, chromosomal imbalances have been observed a variety of loci, including 1q, 2q, 4q, 10p, 10q, 11p, 11q, 12q, 13q, 15q, 17p, and 19q. By CGH array, common chromosomal alterations included gain of 1p, 1q, 12q, 16p, 17q, and

19p as well as loss of 4q and 9p, in most of the cases (Brunner et al. 2008; Chan et al. 2009). As reviewed recently by Knowles, low-grade and high-grade tumors show different allelic imbalance. For example, as discussed below, FGFR3 mutations are associated with low-grade tumors, whereas, the LOH of 10q is frequent in muscle-invasive tumors. This LOH site harbors the PTEN gene, which is a negative regulator of Akt signaling (Knowles 2008).

TP53 mutation is an event in bladder tumors and its relation with tumor progression and the molecular pathways of bladder cancer development has been extensively examined. The p53 gene is located in the chromosome 17p13 locus. The expression of mutated P53 is highly elevated particularly in invasive bladder cancer. The wild type P53 protein has a half-life of 15–30 min, however, mutated P53 has a longer half-life (Nishiyama et al. 2008). Therefore, nuclear accumulation of mutated P53 can be detected by immunohistochemistry, although in one study accumulation of wild type P53 protein has been reported (Abdel-Fattah et al. 1998). About 75% of P53 mutations are missense substitutions, while the remaining include frame shift and deletion mutations (Nishiyama et al. 2008). The missense mutations are often in the DNA binding domain and, hence, the mutated protein loses its transactivation activity (73). The rate of P53 mutations in infiltrating tumors is about 60% and P53 pathway is inactive in T1G3 tumor (López-Knowles 2006; Nishiyama et al. 2008). The progression-free survival is often significantly shorter in patients with tumors expressing mutant TP53 (Ecke et al. 2008). Mutated P53 is also an independent predictor of death among patients with muscle-invasive bladder cancer and of cancer specific mortality, following radical cystectomy (Salinas-Sánchez et al. 2008; Shariat et al. 2009). However, wild type P53 can also accumulate in the nucleus and have prognostic significance. Datar et al. reported that not only the presence of mutation but mutation site in the P53 gene is also associated with disease outcome (i.e., time to first recurrence). For example, they reported that the mutation in exon 5 have similar outcome as the wild type P53 gene. Mutations in exon 8 had intermediate outcome, and the mutated P53 gene with mutations in several exons is associated with the worst outcome (George et al. 2007). With over 500 studies conducted on P53 mutations and its relation to bladder cancer, alterations in P53 remain one of the heavily investigated areas in bladder cancer.

Inhibition or alteration in the retinoblastoma (Rb) pathway occurs commonly in high-grade invasive tumors. However, LOH at or around the Rb locus has not been well studied. Miyamoto reported that LOH at the Rb locus occurs in 80% of invasive tumors but only in 20% of the low-grade tumors (Miyamoto et al. 1996). Wada et al. have reported that LOH found around (at 13q11–12.1) or at the Rb locus (13q14.3) is found in about 20%–30% of cases. Furthermore, the LOH at the Rb locus significantly correlates with tumor grade and stage (Wada et al. 2000).

Recently, using whole-organ histologic and genetic mapping six chromosomal regions, critical for clonal expansion of in situ neoplasia were identified; these include 3q22–24, 5q22–31, 9q21–22, 10q26, 13q14, and 17p13 (Lee et al. 2007; Majewski et al. 2008). LOH at these sites was found to persist through the entire sequence of neoplasia, from morphologically normal regions to invasive carcinoma. Some of the target genes identified in these regions have been termed as “forerunner

genes" and these genes are thought to be relevant for the development of bladder cancer. The concept of forerunner genes proposes three waves of genetic hits. First wave encompasses clonal expansion of phenotypically normal-appearing urothelial cells over large portions of bladder mucosa. The second wave is associated with subregions of clonally expanded cells showing some features of dysplasia. The last wave is associated with full transformation. The two of the six chromosomal regions include the P53 locus (17p13) and the Rb locus (13q14). As an evidence for the forerunner gene concept two new genes ITM2B and P2RY5 were identified. These genes, when silenced by methylation, contribute to the development of neoplasia (Crawford 2008; Majewski et al. 2008). Additional candidate genes include GPR38, CAB39L, RCBTB1, and ARL11. The forerunner gene concept is highly attractive, however, the identity and the functional utility of many of the genes remains unknown. Furthermore, it remains unexplored whether and how the forerunner gene concept can explain the tumor heterogeneity, polymorphism in phase I and II enzymes, and divergent pathways of bladder cancer development.

2.5 Molecular Pathways for Bladder Cancer Development

Unlike prostate cancer, bladder cancer is rarely an incidental finding. Clinical and pathological evaluations identify three different phenotypes in bladder tumors. Low-grade tumors are hyperproliferative lesions. Neoplastic cells continue to proliferate, induce neovascularization and develop into nonmuscle-invasive tumors. These tumors can extend into the bladder lumen, but rarely invade the basement membrane and penetrate into bladder wall. Low-grade tumors account for nearly 75%–80% of bladder tumors. Low-grade tumors do not "progress" to become high-grade tumors, and therefore, must have a distinct molecular pathway of development. The second phenotype in bladder tumors is high-grade tumors. These tumors are made up of highly proliferative neoplastic cells, which also have the ability to invade lamina propria and beyond. In molecular terms, a Ta tumor is not necessarily similar to a T1 tumor because the latter already has established invasive activity (Lee and Droller 2000). The third pathway of development is carcinoma in situ (CIS). These are hyperproliferative, but highly invasive bladder cancer cells that spread horizontally, maintaining a flat appearance. In 2%–30% of cases, CIS can penetrate the basement membrane and lamina propria, with ultimate progression rate about 30%–50% (Lee and Droller 2000).

Based on these clinical observations, Droller was the first to suggest divergent pathways for the development of low-grade and high-grade tumors and CIS (Droller 1981). In 1993, Jones and Droller suggested that divergent, yet somewhat interconnected molecular pathways, may be involved in the development of the three distinct types of tumors (Jones and Droller 1993). Around the same, based on the molecular signatures available at that time, Spruck et al. proposed 2-pathway model for bladder cancer: one arm encompassing low-grade tumors, which display LOH in chromosome 9, and the other arm representing high-grade tumors characterized by p53 mutations (Spruck et al. 1994). They made an observation

that LOH in chromosome 9 is present at a higher frequency (34%) than in CIS and dysplasia. In contrast, they found that only 3% of the Ta tumors had mutated p53, while in CIS and dysplasia lesions, 65% of p53 was mutated. CIS and high-grade tumors from the patient had different mutations, supporting three divergent pathways of bladder cancer development (Spruck et al. 1994). As discussed above, the LOH in chromosome 9 occurs in >50% of all bladder tumors, regardless of tumor grade and stage. In addition, both synchronous and metachronous lesions show identical LOH at chromosome 9 loci, suggesting that the LOH in chromosome 9 is a very early event, prior to the molecular divergence of low-grade and high-grade tumors (Knowles 2006).

Mutations in FGFR3 and p53 have been suggested as the molecular signatures for the divergent pathways of low- and high-grade bladder tumor development. Mutations in fibroblast growth receptor (FGFR) 3 occur at high frequency (60%–80%) in noninvasive low-grade tumors, whereas, p53 mutations are common in high-grade invasive tumors and CIS. FGR3 is a member of the FGF-receptor family that binds more than one FGF family member (Eswarakumar et al. 2005). Binding of an FGF ligand to FGFR3 induces receptor dimerization, which then activates the tyrosine kinase activity of the receptor. FGFR3 mutations are associated with high expression of the mutated FGFR3 protein. For example, Tomlinson et al. reported that 85% of the tumors with mutated FGFR3 showed high expression of FGFR3 protein versus 42% of the tumors with high FGFR3 protein levels expressed wild type FGFR3 (Tomlinson et al. 2007). FGFR3 mutations constitutively activate receptor tyrosine kinase, which leads to downregulation of Akt, cell cycle-regulators, and activation of the MAP kinase pathway. Germline FGFR3 mutations that are identical to those found in bladder cancer induce achondroplasia, hypochodroplasia, and neonatal lethal forms of thanatophoric dysplasia (TD I and II) (Eswarakumar et al. 2005).

Using CGH analysis, Junker et al. recently reported a negative correlation between FGFR3 mutations to chromosomal aberrations (Junker et al. 2008). Furthermore, they observed a negative correlation between tumor stage and FGFR3 mutation frequency (Ta: 69%, T1, 38%, ≥ T2, 0%). Similar correlation was also observed between tumor grade and FGFR3 mutations (G1, 72%, G2, 56%, G3, 4%). Earlier studies also had made similar observations regarding the high frequency of FGFR3 mutations in low-grade, low-stage tumors (Bakkar et al. 2003; Rieger-Christ et al. 2003; van Rhijn et al. 2004). FGFR3 mutations are also found in benign urothelial papilloma and flat urothelial hyperplasia, but no mutations are found in the normal urothelium from either healthy controls or from patients with bladder cancer (Otto et al. 2009). Similarly, Lotto et al. reported that 45% of the inverted urothelial papillomas have FGFR3 mutations and the majority of the mutations are found in exon 7 (Lott et al. 2009). Kompier et al. in a recent study followed 118 patients for 8.8 years and reported that FGFR3 mutations are prevalent in both primary and recurrent tumors (63%). Patients were found to have different mutations in different tumors. However, in 81% of the recurrent tumors, the same mutation that was present in the primary tumor was found. Furthermore, patients with mutated FGFR3 had low-grade and low-stage tumors than patients with wild type FGFR3 (Kompier et al. 2009).

A direct comparison of FGFR3 and TP53 mutations in bladder tumors has revealed an inverse correlation between mutated FGFR3 and mutated TP53, i.e., TP53 was expressed at a higher frequency (>50%) in high-grade tumors, whereas, FGFR3 mutations were found in low-grade tumors (Bakkar et al. 2003; Rieger-Christ et al. 2003; van Rhijn et al. 2004). Furthermore, the occurrence of FGFR3 and TP53 mutations may be mutually exclusive (van Rhijn et al. 2004), suggesting that FGFR3 and TP53 mutations represent molecular signatures of the two divergent molecular pathways of bladder cancer development.

Consistent with the idea that FGFR3 and TP53 mutations represent two divergent pathways of bladder cancer development, Lamy et al. found a higher frequency of FGFR3 mutations in low-grade superficial tumors and a higher percentage of TP53 mutations in high-grade invasive tumors (Lamy et al. 2006). However, they observed higher frequency (85%) of FGFR3 mutations and a lower frequency (3%) of TP53 mutations in G2 tumors when compared to G1 tumors (FGFR3 mutations: 54%; TP53 mutations: 23%). The dichotomy of FGFR3 and P53 mutations in G1 and G3 tumors may not exist when comparing G1 and G2 tumors. In this regard, in a study of 119 patients with T1G3 disease, Hernandez et al. found neither a mutually exclusive occurrence of FGFR3 and TP53 mutations, nor any correlation between TP53/FGFR3 mutation status and tumor recurrence (Hernández et al. 2005). The authors suggested that since T1G3 tumors have undergone more molecular changes than low-grade tumors, the "good prognosis" associated with FGFR3 mutations is no longer observed in T1G3 tumors.

These studies suggest that although the "two divergent pathway" model for bladder cancer development and progression is attractive, there is overlap and/or crossover between these two pathways. Based on the current molecular understanding, the two divergent pathways for the development of low- and high-grade bladder tumors are summarized in Fig. 2.1. Since clinically, low-grade/low-stage tumors can progress to high-grade while the latter tumors do not regress to low-grade, it is most likely that the crossover occurs when low-grade tumors, with mutated FGFR3 representing good prognosis, become invasive by acquiring TP53 mutations and/or other alterations such as chromosome 9 LOH, including p16 loss, downregulation of pRb, p21, $p27^{kip-1}$, overexpression of MIB-1and TSC-1 LOH (Mhawech-Fauceglia et al. 2006). It is noteworthy that van Rhijn et al. found that a combination of FGFR3 and MIB-1, but not FGFR3 and TP53, is an independent predictor of prognosis (van Rhijn et al. 2004). Therefore, molecular signatures of low- and high-grade tumors may include MIB-1 or TSC-1 status, in addition to FGFR3 and P53 mutations (Lokeshwar, 2006).

2.6 Summary

The etiology of bladder cancer has been extensively investigated. Our current knowledge is that exposure of the bladder urothelium to carcinogens such as PAH and AAs, through smoking or occupational exposure, is the main cause of bladder cancer.

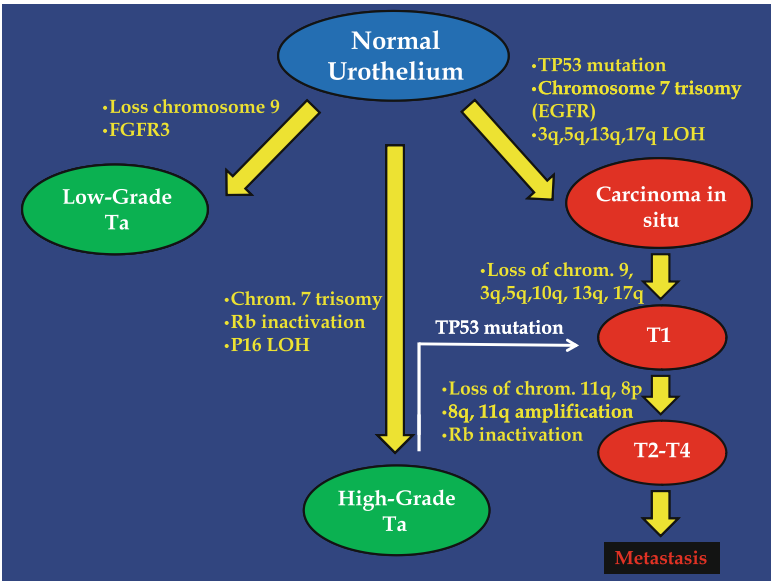

Fig. 2.1 Putative molecular signatures of bladder cancer development and progression

Low-grade tumors, accounting for 80% of all diagnosed cases, frequently recur even after the removal of the primary site. The prognostic for patients with metastatic high-grade tumors is often poor. Multifocal and frequent tumor recurrence has been suggested to be the result of both clonal origin and field cancerization effect. Additionally, ethinic and gender related polymorphism observed in genes encoding for phase I and phase II xenobiotic detoxifying enzymes have been associated with increased risk of developing bladder cancer for individuals of fast phase I acetylator and slow phase II acetylators phenotypes. Moreover, a number of chromosomal aberrations, such as loss of chromosome 9, amplification of chromosome 7 and 11q13, and genetic mutations (i.e., pRb, FGFR3, and TP53), have been associated with both risk for developing bladder cancer, as well as, tumor progression. Recently, MIB-1 and TSC-1 status have been suggested as possible molecular signature markers of low- and high-grade divergent pathways, in addition to FGFR3 and TP53 status.

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Chapter 3
Histopathology and Molecular Pathology of Bladder Cancer

Arndt Hartmann and Simone Bertz

Abstract The frequency of bladder carcinoma has increased continuously during the last few years. Therapeutic strategies adjusted to tumor stages have become an enormous challenge for the urologist. Unequivocal histopathological and cytological diagnoses are needed to support the decision on the most appropriate therapeutic management in bladder carcinoma. Difficulties in the risk stratification of papillary tumors, especially according to their risk of progression, have led to huge efforts in identifying prognostic molecular markers. The increasing knowledge on the molecular changes in bladder cancer led to the modification of the bladder cancer classification in the 2004 WHO classification. The stratification of papillary bladder tumors into benign (papilloma and papillary urothelial neoplasia of unknown malignant potential) and malignant with a two-tier grading system (low-grade and high-grade) is a step in the right direction to subclassify noninvasive papillary urothelial tumors in low-risk (e.g., benign) and high-risk (e.g., malignant) lesions. Other advantages of the 2004 WHO classification are the introduction of two reproducible categories (dysplasia and carcinoma in situ) in flat urothelial lesions and the clear distinction between noninvasive and invasive bladder cancer, omitting the term "superficial carcinoma." The combination of the updated histopathological classifications and new molecular markers will result, hopefully, in a better stratification of tumors and consequently more individualized therapies, in the future.

3.1 Introduction

With a worldwide incidence of about 350,000 cases, bladder carcinoma is ranked on positions 7 for men and 17 for women regarding the incidence rates of all malignant tumors (Murta-Nascimento et al. 2007). In the United States, new cases and

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deaths from bladder cancer in 2008 were estimated to be 68,810 and 14,100, respectively. The high frequency of malignant urothelial tumors seems to be the result of a continuous exposure of the urothelium to potential carcinogens, which results from its physiological function as part of the filtration system between blood circulation and urinary tract.

The majority of urothelial carcinomas are localized in the urinary bladder, which contains 90% of the urothelial covered surface of the urinary tract. Particularly in the urinary bladder, endoscopic methods make it quite easy to obtain tissue specimens not only for diagnostic reasons but also for studies on tumorigenesis and tumor progression, based on histopathological, molecular, and clinical analyses.

Typical clinical features of bladder carcinomas, like high recurrence rates and multifocality, need to be considered in therapeutic decisions especially regarding the frequency of cystoscopic examinations, instillation treatment with chemotherapeutic agents, and also surgical intervention, including cystectomy. Particularly, the high recurrence rates in bladder cancer afford a lifelong follow-up resulting in the highest expenses compared to all other tumors (Murta-Nascimento et al. 2007). Consequently, there is a need for improvement of diagnostic methods, therapies, and aftercare.

Numerous studies on molecular genetics of bladder carcinomas have led to better understanding of their clinical features and morphology and also to the development of new aspects of tumor classification of urothelial tumors. Whether these results will be of importance in the future will depend on their correlation with histopathological features and clinically relevant factors, like prognosis or response to chemotherapy. This new developments led to the change of the WHO classification of urothelial tumors, which was used consistently since 1973. The new WHO classification of 2004 integrates new aspects of molecular genetics and tries to define separate morphological, molecular, and prognostic groups of bladder cancers. The aim of this chapter is to review the new histopathological classification of bladder cancer and correlate it to molecular changes detected in these tumors.

3.2 New Aspects of the WHO Classification of Bladder Carcinoma

The 2004 WHO classification (Eble et al. 2004) is the first to differentiate between noninvasive and invasive bladder cancer, and the term “superficial bladder cancer” has been omitted. This classification is based on molecular analyses, which found identical molecular alterations in muscle-invasive tumors and “superficially invasive” (pT1) bladder cancers (Sauter and Mihatsch 1998). The tumor grading system, representing the differentiation grade of tumor cells, has been changed from a three-stage system with grades 1–3 to a two-stage system differentiating between low-grade and high-grade tumors. Low-grade tumors show only little cytologic atypia, few mitoses, and have a well-preserved histologic architecture (Fig. 3.1a). In contrast, in high-grade tumors the urothelium shows strong cytologic atypia with

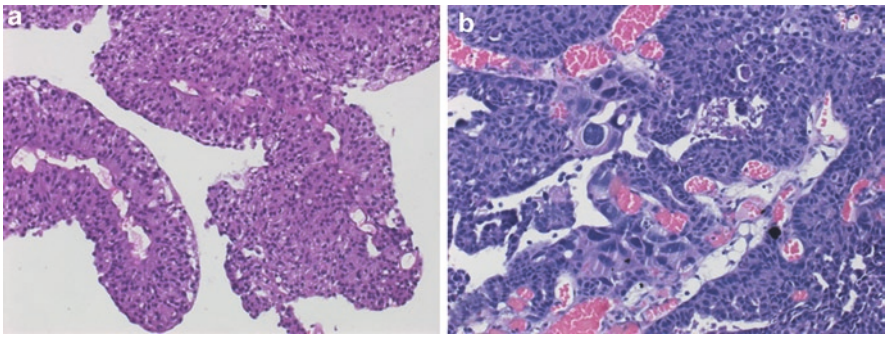

Fig. 3.1 Noninvasive papillary bladder cancer: (**a**) low-grade and (**b**) high-grade

numerous mitoses, highly pleomorphic nuclei with enlarged nucleoli, and a disturbed histologic architecture with loss of stratification (Fig. 3.1b). In addition, the 2004 WHO classification simplifies the classification of urothelial dysplasia and separates reactive changes from urothelial dysplasia and carcinoma in situ (CIS). This led to an increase in the diagnostic accuracy of the histopathological diagnosis of flat urothelial lesions.

In the following sections, we will discuss the most important diagnostic entities in the 2004 WHO classification of urothelial tumors.

3.3 Benign Urothelial Changes

3.3.1 Reactive Atypia in the Urothelium and Atypia of Unknown Significance

Reactive atypia (RA) in the urothelium is a benign urothelial change and characterized by distinct cellular atypies and changes in the architecture of the urothelium in combination with a chronic lymphocytic or active granulocytic inflammation. Frequent causes are urocystitis, or a history of stones, instrumentation, or intravesical therapy. For the pathologist, the distinction from urothelial neoplasia is often very difficult. Immunohistochemical stainings demonstrating a normal CK20 expression pattern in umbrella cells only, a relatively low proliferation activity mostly in the basal cell layer, and a negative staining for CD44 and p53 can help to distinguish RA from dysplasia. If the diagnosis is still uncertain the lesion is diagnosed as urothelial atypia with unknown significance (AUS) and a rebiopsy should indicate the presence or absence of dysplastic intraurothelial changes.

It is important to realize that AUS is not a diagnostic entity but merely a descriptive term used in diagnostically difficult cases. In a recent study, (Lakshmi et al. 2005) evaluated the efficacy of CK20 and Ki-67 to further differentiate AUS from dysplastic urothelium. Around 47% of the patients initially diagnosed as AUS demonstrated

abnormal CK20 and increased Ki-67 expression, suggestive of urethral discharge (UD), 29% were negative with both markers, suggestive of RA, and the remaining 24% of cases could not be resolved. Further follow-up of 10 cases revealed CIS in 7 cases and RA was confirmed in 3 patients. In contrast, Cheng et al. (2000) found that none of the 35 patients with AUS, included in their study, developed urothelial carcinoma during a mean follow-up of 3.9 years. Reviewing these findings, evidence supporting a premalignant nature in AUS is controversial.

3.3.2 Urothelial Hyperplasia

Urothelial hyperplasia (UH) is defined as increase in the thickness of the urothelium without cytological atypia. It may be seen in the flat mucosa adjacent to low-grade papillary urothelial tumors but is also rarely found as single finding after cystoscopy. The lesion consists of urothelium with increased number of cell layers but few or very low cytological abnormalities. When seen by itself there is no evidence suggesting that it has any premalignant potential. Therefore, UH is not an obligate precancerous lesion, according to the 2004 WHO classification. However, molecular analyses have shown that at least part of the lesions in patients with bladder cancer may be clonally related to the papillary tumors. These UH are frequently detected during fluorescence cystoscopy as "false positive" and show typical molecular changes found in papillary bladder cancer, like deletions at chromosome 9, FGFR3 mutations, and other molecular changes (Hartmann et al. 1999; Obermann et al. 2003; van Oers et al. 2006). Therefore, in patients with bladder cancer, UH could be the earliest histopathological change indicating a recurrence of the disease. Recent studies (Hartmann et al. 1999; Zaak et al. 2002) could show that photodynamic diagnosis enables the detection and excision of bladder lesions, including UH, which might have been missed in white light cystoscopy. If UH is diagnosed de novo, its neoplastic potential is controversial and no recommendations for clinical follow-up can be given as reliable data on the natural history of these lesions are absent. If papillary UH is found in a patient with a history of bladder cancer, this finding is often associated with recurrent tumor growth, and therefore, a close follow-up is recommended (Taylor et al. 1996; Swierczynski and Epstein 2002).

3.3.3 Urothelial Papilloma

Urothelial papilloma (UP) is a papillary urothelial lesion composed of a delicate fibrovascular core covered by urothelium, indistinguishable from the normal urothelium. The incidence of UP is low and is usually reported with 1%–2% of all bladder tumors. It can occur in younger patients and is also seen in children. The lesion is characterized by discrete papillary fronds with occasional branching, but

without fusion. The urothelium lacks atypia and superficial cells are often prominent. Mitosis are either absent or rare and, if present, only found in the basal cell layer. The main difference between the 1973 WHO definition of UP and the 2004 WHO classification is that the number of cell layers need not be counted in the recent classification as was previously required. According to Oosterhuis et al. (2002), some of the initially diagnosed noninvasive well-differentiated papillary carcinomas (pTaG1) were reclassified as UP when using the less stringent 2004 WHO criteria, which raised the total number of papillomas diagnosed in this study. The biological potential of UP is uncertain as there are only limited studies published on this issue using the criteria of the 2004 WHO classification. Whereas, Harnden et al. (1999) and Samaratunga et al. (2002) found neither recurrence nor progression of UP during a median follow-up of 18–50 months; publications by Cheng et al. (1999c), McKenney et al. (2003), and Magi-Galuzzi and Epstein (2004) reported recurrence rates of 7.1%–8.8% and progression in 1.9%–8.8%, during a mean follow-up of up to 9.8 years. Patients with progression developed noninvasive tumors and none demonstrated an invasive carcinoma. Immunohistochemical studies on cell differentiation and proliferative activity examining the expression of CK20, CD44, TP53, and Ki-67 in UP could not discriminate normal urothelium from UP using these markers. Mutations of the fibroblast growth factor receptor 3 (FGFR3), which are among the earliest events in urothelial carcinogenesis, were found in low-grade papillary urothelial tumors in 85%–88% and could also be detected in 75% of the investigated UPs (van Rhijn et al. 2002; Montironi et al. 2003). The results of the latter studies might serve as an explanation for the progression of UPs in rare cases. According to the definition of the 2004 WHO classification, UP is a heterogeneous neoplasm with different risk patterns of recurrence and progression. Molecular data favor the assumption that IP is the most differentiated tumor type in a spectrum of the genetically stable well-differentiated papillary urothelial neoplasms.

3.3.4 Inverted Urothelial Papillomas

Inverted papilloma (IP) is a benign urothelial tumor that has an inverted growth pattern with none or minimal cytologic atypia of the neoplastic cells. The lesion has a relatively smooth surface covered by a histologically and cytologically normal urothelium. Cords and nests of urothelial cells invaginate extensively from the surface urothelium into the subadjacent lamina propria but not into the muscular bladder wall. These anastomosing islands and cords have a uniform width of papillary lesions, which have invaginated into the lamina propia. The central proportions of the cords contain urothelial cells and the periphery contains palisades of the basal lamina, stromal cells, and vessels. Squamous metaplasia and pseudoglandular or glandular differentiation can sometimes occur. There are by definition no exophytic papillary components. As for UP, a maximum of six-cell layers is no longer a restrictive histopathological criterion for the diagnosis of IP, according to the

2004 WHO classification. In the past, the diagnosis of IP was associated with urothelial carcinoma occurring either simultaneously or subsequently (Witjes et al. 1997). However, recent studies comparing DNA ploidy, MIB-1 proliferative activity, and expression of p53 and CK20 in IP with and without cellular atypia and in IP of patients with and without a history of urothelial carcinoma could not detect any significant differences in IPs between either of the investigated groups (Cheville et al. 2000; Broussard et al. 2004). Furthermore, an extensive molecular study investigating inverted papillomas showed neither FGFR3 mutations nor chromosome 9 deletions or other molecular alterations, frequently found in papillary bladder cancer, favoring a benign reactive nature of these lesions (Eiber et al. 2007). Extensive follow-up data on patients with IP in these studies demonstrated no recurrences and this lesion does not seem to be a precursor for urothelial carcinoma. However, papillary urothelial carcinomas with dominant inverted growth pattern as well as invasive urothelial carcinomas have to be excluded in these patients (Amin et al. 1997).

3.3.5 Papillary Urothelial Neoplasia of Low Malignant Potential (PUNLMP)

In an attempt to distinguish papillary urothelial tumors with minimal cytological atypia and low risk of recurrence and progression, the WHO classification of 2004 included the new entity of papillary urothelial neoplasm of low malignant potential (PUNLMP). In this case, the term "neoplasia" instead of "carcinoma" emphasizes the lack of invasive growth, minimal risk of progression, and the lack of capability to metastasize and also avoids labeling the patients with this low-risk tumors with the diagnosis of cancer. These tumors resemble urothelial papillomas but shows increased cellular proliferation exceeding the thickness of normal urothelium. Morphologically, the papillary structures of PUNLMP are discrete, slender, and lined by urothelium with preserved polarity and minimal or absent cytological atypia. The basal layers show prominent palisading of the nuclei and the umbrella cells are often preserved. The diagnostic helpful immunohistochemical markers for the diagnosis are CK20 with a normal expression pattern with positivity of the umbrella cells and ki-67 with few proliferating cells in the basal layer only. Immunohistochemical studies on the expression of CK20, CD 44, and 34betaE12 as sensitive markers of urothelial differentiation reported a strong correlation of nonrecurrent disease with a normal expression pattern of these markers in PUNLMPs and low-grade papillary urothelial carcinomas (Harnden et al. 1999; Desai et al. 2000; Alsheikh et al. 2001). Further studies evaluated mitotic count and MIB-1 either alone or in association with the expression of p53, c-erbB-2, and bcl-2 in predicting the recurrence of PUNLMPs. In a multivariate analysis, only MIB-1 immunopositivity as a marker of proliferative activity retained prognostic significance (Pich et al. 2002). Molecular genetic analysis revealed FGFR3 mutations and chromosome 9 deletions in 30%–50% of cases.

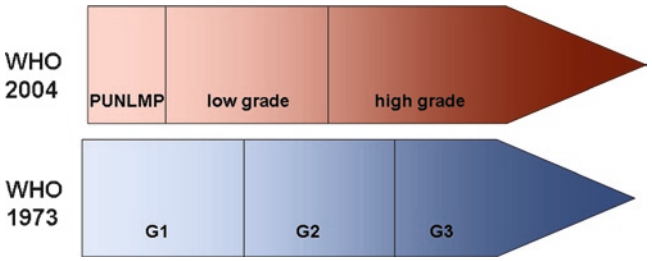

Fig. 3.2 Comparison of the classification of non-invasive papillary bladder tumors according to the 2004 and 1973 WHO classifications

The prognosis for patients with PUNLMP is excellent. Recurrences occur at a significantly lower frequency than in noninvasive papillary carcinomas (35%). The risk of progression or development of a second invasive bladder cancer is between 1% and 5% (Samaratunga et al. 2002; Campbell et al. 2004). Creating this new entity has lead to the separation of tumors with minimal risk of progression and mortality within the group of well-differentiated papillary tumors. According to the former WHO classification from 1973, PUNLMP lesions were classified among noninvasive highly differentiated tumors (pTaG1). However, it is inaccurate to merely equate the 1973 WHO pTaG1 urothelial bladder tumors with PUNLMP lesions (see Fig. 3.2). Recent publications (Samaratunga et al. 2002; Holmäng et al. 2001; Campbell et al. 2004) reported that after histopathological regrading, using the 1998 WHO/ISUP or 1999 WHO classification, 19%–44.8% of PUNLMPs were originally diagnosed as pTaG1-2 or G2 tumors, according to the 1973 WHO classification. With a mean recurrence rate, stage progression rate, and a tumor related mortality in approximately 37.5%, 3.3%, and 1.3%. respectively, in 11 available studies, PUNLMP lesions demonstrated a low malignant potential. Pathologists use the diagnosis of PUNLMP carefully as the existence of a malignant subclone needs to be excluded on biopsy specimens of possibly very large tumors. Until recently, proliferation rate is the only parameter of prognostic relevance in PUNLMP, and further molecular characterization will be required to find additional prognostic markers in this entity.

3.4 Malignant Urothelial Lesions

3.4.1 Urothelial Dysplasia (UD—Synonym: Intraurothelial Neoplasia, Low-Grade)

The incidence of UD in patients with nonmuscle-invasive bladder cancer varies from 22% to 86% and approaches 100% in patients with muscle-invasive carcinoma (Lopez-Beltran et al. 2002). The 2004 WHO classification simplifies the diagnosis of these lesions by the elimination of the poorly reproducible subgrading

of dysplasia. UD is now diagnosed in all lesions with clearly visible atypia, which do not fulfill the diagnostic criteria of CIS. Since dysplasia may be mimicked by reactive inflammatory atypia and even by normal urothelium, a wide spectrum of atypical changes in the urothelium are now included in the diagnosis of dysplasia. Histopathologically, UD is characterized by both cytological and architectural changes with loss of polarity and nuclear crowding. The cells have an increase in the nuclear–cytoplasmic ratio and the nuclei have irregular nuclear borders with mildly hyperchromatic chromatin and usually inconspicuous nucleoli. Proliferating cells are located in the basal part of the dysplastic urothelium and not in the entire thickness of the urothelium.

The diagnosis of UD is most relevant in patients with noninvasive papillary neoplasms, where it indicates an urothelial genetic instability. The presence of UD, either adjacent to a concominant urothelial carcinoma or as primary UD, has been correlated to the risk of tumor recurrence and progression in several studies. In two recent series (Cheng et al. 1999a; Cheng et al. 1999a), progression to either CIS or muscle-invasive disease was found in 19% versus 15% of patients with primary UD during a mean follow-up of 8.2 versus 3.9 years. The interval from initial diagnosis of UD to progression to muscle-invasive cancer was 0.7–10.0 years and for progression to CIS, the corresponding time range was 0.6–3.0 years. Furthermore, the study by Cheng et al. (1999) revealed that urothelial carcinomas did not arise in the region of UD in six of seven cases. These results indicate that UD appears to be a marker of a "field defect" with elevated cancer risk. In line with these clinical findings, molecular studies showed that approximately 60% of UD showed deletions and mutations of p53 and chromosome 9 deletions, arguing that many of these lesions could be precursors of CIS and solid invasive bladder cancer (Hartmann et al. 2002).

3.4.2 Carcinoma In Situ (CIS—Synonym: Intraurothelial Neoplasia, High-Grade)

Primary CIS accounts for less than 1%–3% of urothelial carcinomas. Concurrent CIS can be found in 45%–65% of muscle-invasive and in 5%–19% of noninvasive urothelial tumors (van der Meijden et al. 2005). CIS is an obligate precursor of invasive urothelial carcinoma. Histologically, it is characterized by flat urothelium with high cytological atypia with large hyperchromatic nuclei and frequent mitosis. In contrast to the 1973 WHO classification, the diagnostic criteria of CIS in the 2004 WHO classification were refined to emphasize that cytological changes including nucleomegaly, hyperchromasia, pleomorphism, and mitotic activity in the mid and upper urothelium were key markers for the diagnosis. However, the infiltration of the entire thickness of the urothelium by these cells are no longer required for the diagnosis of CIS. With the introduction of the two-tier system of flat urothelial lesions by the new classification (dysplasia and CIS), the frequency of CIS will certainly increase.

In both noninvasive and invasive urothelial carcinoma patients, concomitant CIS is associated with high-risk of tumor progression and even metastasis (Cheng et al. 1999b; Hassan et al. 2004; van der Meijden et al. 2005). From a clinical perspective, extent of disease (focal/multifocal), coexistent invasive carcinoma and recurrence were the principal determinants of clinical outcome in recent studies on CIS (Witjes 2004).

On a molecular level, the combined overexpression of p53 and p21, the loss of E-cadherin expression, and deletions of chromosome 9 were associated with progression and cancer specific survival in CIS patients. Recent molecular data with frequent p53 mutations, lack of FGFR3 mutations, high level of genomic instability, and specific global gene expression alterations in CIS (Hartmann et al. 2002, van Rhijn et al. 2004; Dyrskjot et al. 2004; Zieger et al. 2009) strongly suggest that CIS is the noninvasive precursor of potentially life-threatening solid bladder cancer.

3.4.3 Noninvasive Papillary Urothelial Carcinoma, Low-Grade and High-Grade

The noninvasive papillary carcinomas are now separated in two groups with different risk of recurrence and progression to invasive disease. The WHO classification of 2004 separates noninvasive papillary carcinomas low-grade (NIUC-LG) from high-grade tumors (NIUC-HG). This new classification is now used instead of the WHO classification of 1973, which distinguished G1, G2, and G3 tumors. Histologically, NIUC-LGs show relatively mild changes in cytology and architecture. The recurrence rate is high with 50%–70%, but only few patients progress to invasive disease (10%) with very low mortality due to bladder cancer (5%). Approximately 20%–25% of all noninvasive papillary tumors are regarded high-grade according to the WHO classification of 2004. This includes tumors with considerable cytological atypia and distinct changes in the architecture of the urothelium. These tumors have frequently high mitotic index and a loss of cohesion of the urothelial cells. NIUC-HGs also show, besides the high recurrence rate (70%–75%), a much higher progression (15%–40%) and mortality risk (20%—Holmang and Johansson 2002). NIUC-HGs include the former pTaG3 tumors of the 1973 WHO classification. In addition, a considerable fraction of former G2 tumors (15%–30%) have to be reclassified as high-grade papillary urothelial carcinoma. It is of clinical importance that according to the new WHO grading system 20%–25% of all noninvasive urothelial tumors are now high-grade (Fig. 3.2). It remains unclear whether or not high-grade tumors need the same therapeutic management as the former group of pTaG3 tumors. Prospective studies on this subject are urgently needed. Due to the controversies on the classifications systems of WHO 1973 and 2004, several authors propose tumor grading according to both systems in diagnostic reports until the 2004 WHO system has been fully validated (Lopez-Beltran et al. 2002).

Data on the predictive importance of molecular and genetic characteristics in NIUC-HG suggest a strong similarity between these tumors and invasive bladder cancer. PUNLMP and NIUC-LG are genetically stable in contrast to NIUC-HG, which show numerous chromosomal aberrations and a high frequency of p53, Her2 or EGFR, mutation, and the loss of p21 or p27 comparable to that seen in invasive cancers. Overall, no thoroughly evaluated molecular marker with sufficient predictive power to be used in clinical routine for NIUC-HG is available at present.

3.4.4 Invasive Bladder Cancer

Invasive (or infiltrating) urothelial carcinoma is defined as a urothelial tumor that invades beyond the basement membrane. The most common type of bladder cancer in developed countries is urothelial carcinoma, derived from the uroepithelium (more than 90% of the bladder cancer cases in the United States or in Europe). However, in other regions (e.g., Northern Africa) the incidence of urothelial carcinoma is lower, and there, the squamous cell carcinomas are the most frequent bladder tumors. It is estimated that 20%–30% of patients with newly diagnosed bladder cancer present with invasive disease. The 2004 WHO classification does not make any difference between superficially invasive bladder cancer (pT1, infiltration in subepithelial connective tissue, but not the lamina muscularis propria) and muscle-invasive disease. Numerous molecular-genetic studies showed that both types of tumor show similar genetic alterations with a high degree of genetic instability. Nevertheless, most of the pT1 bladder cancers are papillary, low-, or high–grade, whereas most pT2-4 carcinomas are nonpapillary and high-grade. These carcinomas are graded as low- and high–grade, depending upon the degree of nuclear anaplasia and architectural abnormalities.

The histology of invasive urothelial carcinoma is variable. In the WHO classification of 2004, a considerable list of histologic variants are introduced for the first time. Although only few studies exist, there is growing evidence that some of these variants show a different clinical course, mostly with adverse prognosis. Some examples for histological variants of invasive urothelial bladder with poor outcome are given in Table 3.1 and Fig. 3.3.

In pT1 tumors invasion is limited to the lamina propria. For this group, there is no generally recommended therapeutic scheme as it is still unclear which patients will benefit from early cystectomy. Several approaches to find a way to differentiate between aggressive and less aggressive tumors have been made. Studies were carried out on substaging systems according to the infiltration related to the lamina propria separate between substages pT1a–pT1c (pT1a: infiltration above, pT1b within, pT1c beyond the lamina muscularis mucosae). Difficulties in this method result from lack of orientation, especially in TUR specimens and partly severe artificial changes, e.g., due to thermal alteration. Another important problem of this

Table 3.1 TNM classification of bladder cancer 2010 (AJCC 2010)

Primary tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Ta	Noninvasive papillary carcinoma
Tis	Carcinoma in situ
T1	Tumor invades subepithelial connective tissue
T2	Tumor invades muscularis propria
pT2a	Tumor invades superficial muscularis propria (inner half)
pT2b	Tumor invades deep muscularis propria (outer half)
T3	Tumor invades perivesical tissue
pT3a	Microscopically
pT3b	Macroscopically (extravesical mass)
T4	Tumor invades any of the following: prostatic stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall
T4a	Tumor invades prostatic stroma, uterus, vagina
T4b	Tumor invades pelvic wall, abdominal wall
Regional lymph nodes (N)	
NX	Lymph nodes cannot be assessed
N0	No lymph node metastasis
N1	Single regional lymph node metastasis in the true pelvis (hypogastric, obturator, external iliac, or presacral lymph node)
N2	Multiple regional lymph node metastasis in the true pelvis (hypogastric, obturator, external iliac, or presacral lymph node)
N3	Lymph node metastasis to the common iliac lymph nodes
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis

substaging method is the incontinuity of the lamina muscularis mucosae, which is found in 15%–83% of TUR specimens. An improvement of applicability is given by another substaging system based on direct measurement of the invasive portion of the tumor. According to this system an association between bad prognosis and extent of invasion >0.5 mm was found (van der Aa et al. 2005). This new system has not yet been generally accepted and results need to be validated in further independent studies.

Like in noninvasive papillary carcinomas the grading of invasive tumors has also been changed to the two-tier system separating high-grade and low-grade tumors. In this case, the new grading system is questionable as it results in more than 90% of invasive carcinomas being classified as high-grade tumors. The three-tier tumor grading is one of six approved basic parameters of the EORTC prognostic scoring system (Sylvester et al. 2006) and the new grading system seems to devaluate its meaning as an important prognostic parameter.

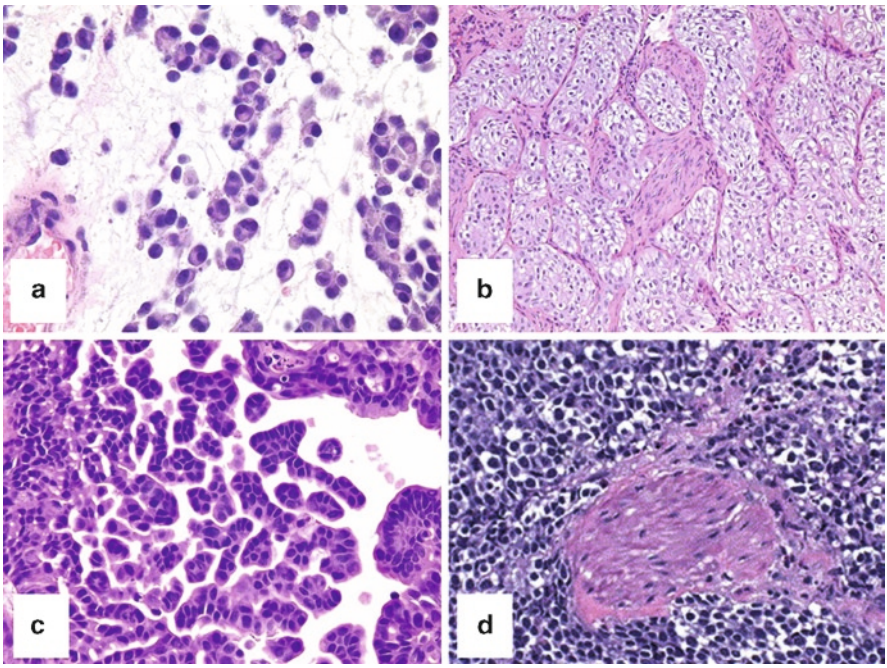

Fig. 3.3 Histological variants of bladder urothelial carcinoma with poor prognosis. (**a**) Plasmocytoid variant, (**b**) nested variant, (**c**) micropapillary variant, and (**d**) small cell neuroendocrine carcinoma

Table 3.2 Variants of invasive urothelial bladder cancer with poor outcome

• Urothelial carcinoma with squamous differentiation
• Nested-type urothelial carcinoma
• Micropapillary urothelial carcinoma
• Lymphoepithelioma-like urothelial carcinoma
• Plasmocytoid urothelial carcinoma
• Sarcomatoid urothelial carcinoma

3.4.5 TNM Classification 2010

The TNM classification (see Table 3.2, AJCC 2010) is an important parameter to determine the prognosis for a patient with bladder cancer and stratify the therapy. The exact histopathological examination of resected tumor tissue from biopsies, transurethral resections, or cystectomy specimens is crucial to define the tumor stage. In biopsies or transurethral resection specimens, determination of the exact tumor stage is often not possible. For instance, the stage given in a specimen with clear invasion but only limited amount of muscularis propria without tumor infiltration would be "at least pT1." In case of biopsy or transurethral resection, additional specimens from the adjacent urothelium and the base of the tumor are very helpful to reliably classify the tumor.

For determination of the nodal status, at least eight lymph nodes have to be investigated. Recent studies demonstrated that the survival of patients is significantly better if more than 15 lymph nodes are sectioned. Therefore, a resection of at least 15 lymph nodes should be done, whenever possible (Leissner et al. 2000).

3.5 Molecular Pathways in Bladder Cancer Pathogenesis

Patients with bladder cancer show a wide range of clinical courses, which are associated with the histopathological diagnosis. Until now two different pathways of bladder cancer pathogenesis have been described and a third pathway is currently being discussed (for review see Knowles 2006).

The first pathway (relevant for 70%–80% of tumors) leads to the development of mostly noninvasive papillary tumors with a usually favorable prognosis. The possible precursor of these tumors are urothelial hyperplasias. The second pathway (relevant for 20%–30% of tumors) could originate from UD and CIS and is biologically very aggressive. In those cases, tumors often show progression to muscle-invasive tumor stages.

Molecular studies have detected different molecular alterations associated with each of those pathways (summarized in Fig. 3.4). The most frequent molecular alterations (found in >50% of all tumors, regardless of tumor stage and grade) are LOH (loss of heterozygosity) and deletions on both arms of chromosome 9.

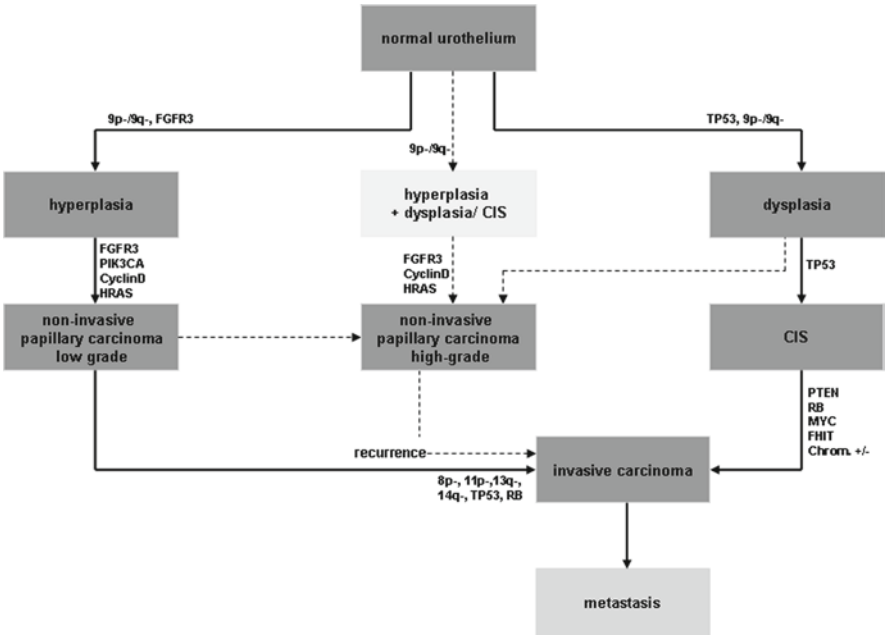

Fig. 3.4 Histopathological and molecular development of bladder cancer

The same alterations have been found in preneoplastic urothelial lesions (e.g., hyperplasia or dysplasia) and also in normal urothelium of tumor patients (Obermann et al. 2003; Stoehr et al. 2005). Additional microsatellite analyses on chromosome 9 detected four frequently deleted areas. They contain important key genes—among them cell-cycle-regulators—which probably play an essential role in tumorigenesis: 9p21 – *CDKN2A/ARF* (p16INK4a/p14ARF), *CDKN2B* (p15); 9q22 – *PTCH*; 9q32-33 – *DBC1*; 9q34 – TSC1 (Simoneau et al. 2000; Knowles 2006). Interestingly, in bladder carcinoma, there are also separate mutations in those genes without corresponding deletions (e.g., *TSC1*). According to these findings a haploinsufficiency of genes on chromosome 9p/q could be responsible for initiation of urothelial cancer (Knowles et al. 2003).

The most frequent oncogene mutation in urothelial carcinoma is a mutation of the fibroblast growth factor receptor 3 gene (*FGFR3*) on chromosome 4p16.3, which encodes a classical tyrosine kinase receptor (Cappellen et al. 1999). Activating germline mutations leading to a constitutive activity of the kinase subunit of *FGFR3* are already known for some time. They lead to congenital skeletal aberrations with different degrees of severity (Achondroplasia, SADDAN, Crouzon-Syndrome). In bladder tumors, somatic mutations within the hotspots of exon 7, 10, and 15 of the *FGFR3* gene were found predominantly in noninvasive papillary low-grade tumors (up to 80%) and in papillomas (van Rhijn et al. 2002; van Rhijn et al. 2003; Burger et al. 2007). Identical mutations were found in urothelial hyperplasia (van Oers et al. 2006), which underlines the role of this lesion as a precursor of papillary tumors. In contrast, *FGFR3* mutations are rarely found in CIS and invasive tumors. Additionally, p53 mutations, known as the most important alterations in initiation of aggressive CIS and invasive solid urothelial cancer, are never found together with *FGFR3* mutations (van Rhijn et al. 2004), which is another proof for the existence of two different pathways in the development of bladder carcinoma. Further mutations have recently been found in the *PIK3CA* gene, a member of the phosphatidyl-inositol-3-kinase family (Lopez-Knowles et al. 2006). They were frequently detected in noninvasive bladder tumors and rarely in invasive tumors. *PIK3CA* mutations occur simultaneously with *FGFR3* mutations and are also associated with a favorable prognosis.

The third, relatively new hypothesis of tumorigenesis in the bladder, is based on the analysis of dysplasias, which were located next to papillary high-grade tumors. Those dysplasias showed chromosome 9 alterations but no p53 alterations (Knowles 2006). These molecular findings suggest the existence of a third pathway in tumorigenesis via UH and UD/CIS to noninvasive papillary high-grade tumors.

3.5.1 Are New Findings in Molecular Analysis Clinically Relevant?

The identification of *FGFR3* mutations in bladder tumors, resulted in a large series of studies trying to define their clinical relevance. As described before, a

significant association of *FGFR3* mutations with noninvasive papillary low-grade tumors was found as well as a significantly better prognosis as a consequence of a very low progression rate in those tumors (van Rhijn et al. 2003). Those features cannot be found in early invasive pT1 tumors (Hernandez et al. 2005). Interestingly, tumors with high proliferation rate and p53 mutations, which would usually correlate with an unfavorable prognosis, have a low-risk of progression if there is an additional *FGFR3* mutation. A prospective multicenter study introduced a molecular grading system, based on the Ki-67 proliferation index and *FGFR3* mutation status, which showed a higher reliability in predicting risks of progression, recurrence, and tumor-associated death than the classical histopathologic grading system (van Rhijn et al. 2003). Another study proposed the combination of immunohistochemically analyzed normal cytokeratin 20 expression pattern and *FGFR3* mutation status as reliable markers of papillary low-grade tumors with low-risk of progression (van Oers et al. 2007). Both Ki-67 and cytokeratin 20 have been established as a routine immunohistochemical markers for assessment of the differentiation and proliferation of urothelial tumors (Fig. 3.5).

In summary, at present in bladder cancer, *FGFR3* mutation status is the first reproducible molecular parameter suitable to support and complement assessment of prognosis, based on histopathologic (and clinical) parameters.

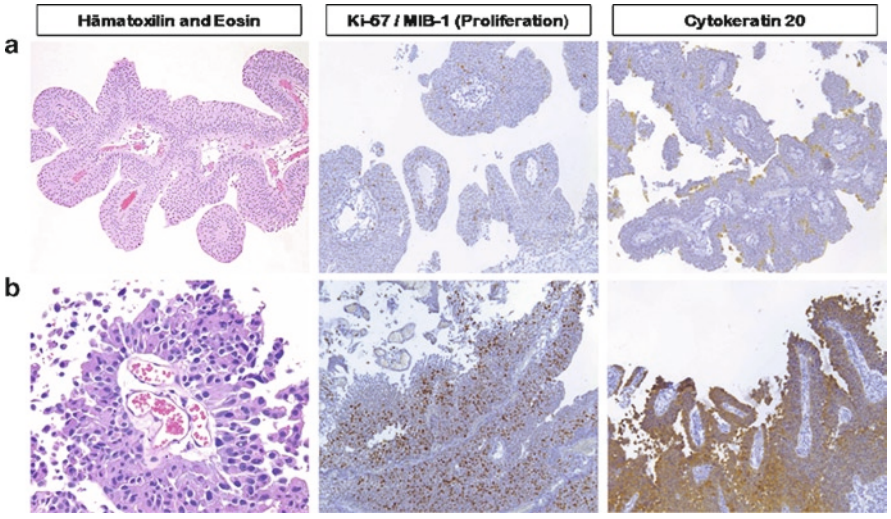

Fig. 3.5 Histopathology and immunohistochemical staining of prognostic markers in non-invasive papillary bladder cancer. (**a**) Low-grade tumor with limited atypia, low proliferation index in the Ki-67 staining and normal expression of cytokeratin 20 and (**b**) high-grade tumor with extensive cytological atypia, high proliferation index in the Ki-67 staining and dedifferentiation with abnormal cytokeratin 20 expression

3.5.2 *Microarrays: The Future in Bladder Pathology?*

Microarrays allow an analysis of gene expression of thousands of genes simultaneously. Studies of gene expression based on m-RNA analysis as well as on protein-expression analysis (antibody arrays) revealed new gene expression signatures and differentially expressed genes. Some were directly associated with the clinical course and showed great promise for predicting tumor progression of both noninvasive tumors and invasive tumors (Dyrskjot et al. 2005; Wild et al. 2005; Sanchez-Carbayo et al. 2006). In one of those studies, analysis of the expression status of a few genes led to an assessment of survival and identification of lymph node metastases. One of the signatures described above have been validated and confirmed by a multicenter study (Dyrskjot et al. 2007). A current study on patients with advanced bladder carcinoma after adjuvant chemotherapy with cisplatin described a signature associated with survival of patients. In this study, the expression of Emmprin and Survivin predicted independently the success of chemotherapy (Als et al. 2007). According to those results, the decision on a toxic adjuvant chemotherapy in bladder carcinoma will probably be facilitated by the use of molecular markers in the future. However, none of the gene signatures was validated in prospective studies. Hence, it will take some time until data will influence routine diagnostic and therapeutic procedures. After validation of data in retrospective studies performed at different centers, prospective studies will be needed for testing their clinical relevance.

3.6 Conclusions

The current WHO classification introduced in 2004 forms a solid basis for histopathological diagnosis. An important advantage was the introduction of different categories of noninvasive urothelial tumors with definition of detailed histological criteria for each entity and adaptation of categories to the clinical course. The exact definition of flat urothelial neoplasias and the classification in only two groups, i.e., dysplasia and CIS, will lead to an improvement of diagnostic reproducibility. Standardized definitions of pathological categories will permit a direct comparison of therapeutic results and data of clinical and molecular studies in different centers. Additionally, molecular analyses have lead to the identification and validation of markers, especially *FGFR3,* which support the prognostic evaluation of bladder tumors, which until recently, was based on histopathological and clinical criteria only. It is most likely that in future molecular markers will play an important role in routine diagnostic and therapeutic strategies, complementing classical histopathological diagnosis.

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Chapter 4
Bladder Cancer Diagnosis and Detection: Current Status

M. Ploeg and J. A. Witjes

Abstract The cornerstones in the detection of urinary bladder cancer is the combination of urinary cytology and urethrocystoscopy (UCS). Limitations concerning these tests are a low sensitivity of cytology and a problem in detection of small papillary lesions and flat carcinoma in situ (CIS) with standard UCS. These limitations have led to newer techniques, like photodynamic diagnosis (PDD) and narrow band imaging (NBI) to improve the visibility by UCS. Once a bladder tumor has been detected it should be resected by transurethral resection (TURBT) to obtain adequate tissue for pathological assessment. PDD-assisted resection is a promising technique with better tumor detection, more complete resection and a better recurrence free survival. Imaging techniques like CT and (enhanced-)MRI play an important role in accurate staging of UBC and in the detection of recurrences of MIBC during follow-up.

4.1 Urinary Cytology (A Brief Reference)

If the diagnosis urinary bladder cancer (UBC) is suspected, the initial assessment consists of voided urine cytology. Bladder tumors are in direct contact with urine and tumors usually have less cell–cell interaction, which causes tumor cells to leak into the urine (Lee et al. 2008). Cytology is a highly specific test, especially for high-grade urothelial carcinoma (UC) and carcinoma in situ (CIS). This means that a positive cytology may indicate the presence of UC anywhere in the urinary tract. On the other hand, however, cytology has a low sensitivity in low-grade cancer (Brown 2000; Koss LG 1997). This means that a negative cytology does not exclude the presence of a low-grade tumor. Inflammatory conditions of the bladder, like infection or intravesical instillation, can confound the results of cytology (Talwar et al. 2007).

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In a review, which summarized 18 studies about cytology, the authors came to an overall median sensitivity for cytology of 34% and an overall median specificity of 99% (Lotan and Roehrborn 2003). In addition, for the interpretation of a urine specimen intra- and interobserver reproducibility is poor (Sherman et al. 1984). Due to the mentioned disadvantages, especially the low sensitivity, several new tests (urinary markers) have been developed. Their value is yet to be established and cytology still remains the current standard bladder tumor marker.

4.2 Cystoscopy

In 1878, Maximilian Carl-Friedrich Nitze (1848–1906), a German urologist, presented the first working cystoscope in Vienna, which he had created together with instrument maker Joseph Leiter (Rainer 2007). Since then, urethrocystoscopy (UCS) has become the mainstay in diagnosis and follow-up of UBC. UCS is used to confirm the presence, location, and number of tumors in initial diagnosis, detection of recurrences during follow-up, and guiding the urologist during transurethral resection of the bladder tumor (TURBT).

4.2.1 Limitations

Although UCS is the current gold standard in diagnosis and follow-up of bladder cancer, it certainly has some limitations. Flat lesions, like CIS, are difficult to detect and easily missed. Also papillary lesions (Ta and T1) are often missed. Brausi et al. assessed the recurrence rate at first follow-up after TURBT in 2410 patients with Ta and T1 tumors. They reported that the lesions seen at first follow-up were, for a substantial part, missed tumors during initial TURBT. Of the patients with a single tumor 3.5%–20.6% had a "recurrence" at first follow-up. For patients with multiple tumors the range was 7.5%–45.8% (Brausi et al. 2002). For CIS, this problem in detection is even bigger. Studies showed that only 38.1% of CIS lesions were detected by standard (white light) UCS and no more than 71.4% of patients with CIS were diagnosed with standard (white light) UCS (Jocham et al. 2005; Schmidbauer et al. 2004).

Another limitation of UCS is the invasive character of the procedure and, thus, it is bothersome for the patient. However, when patients are asked about a cutoff level at which they would prefer a noninvasive urinary test instead of UCS, 98% of them choose UCS if the sensitivity of the urinary test is lower than 90%–95%. This means that the patient with bladder cancer prefers certainty in favor of less discomfort during an investigation (Vriesema et al. 2000; Yossepowitch et al. 2007).

A third limitation is the costs incurred. Bladder cancer has the highest lifetime treatment costs per patient of all cancers. Invasive monitoring by UCS, which is essential in view of the high recurrence rate in UBC, generates significant costs (Sievert et al. 2009).

4.2.2 Photodynamic Diagnosis

Limitations concerning the detectability of small papillary or flat lesions have led to newer techniques to improve the visibility by cystoscopy. One of these techniques is photodynamic diagnosis (PDD), also referred to as fluorescence cystoscopy, which uses a photoactive solution (photosensitizer) that accumulates up to ten times more in neoplastic tissue compared to normal tissue. This in turn enhances the visual difference between normal and tumor tissue after illumination at the appropriate wavelength (blue light). Cystoscopes with specially developed light sources and filters are used, and one can easily switch from standard white-light cystoscopy (WLC) to PDD. By illuminating the bladder wall with blue light, the lesion appears fluorescent pink on a blue background (Fig. 4.1). Intravesical photosensitizers that are used in current clinical practice are 5-aminolevulinic acid (5-ALA) and its hexyl ester hexylaminolevulinate (HAL or HEXVIX®). The 5-ALA is a photoactive protoporphyrin IX, which is part of haem biosynthesis. When illuminating with violet light, protoporphyrin IX returns to a lower energy level and emits a fluorescence light. Tumor detection rates will increase by 20%–90% (Witjes and Douglass 2007). HAL is a lipophilic derivate of 5-ALA and shows a better penetration of cell membranes and interstitial spaces. HAL produces at least twice the fluorescence in half of the time and with a 20 times lower concentration compared to 5-ALA (Marti et al. 1999; Lange et al. 1999). Several clinical trials have shown that PDD is superior to standard WLC in the detection of UBC. The reported sensitivities varied between 82% and 97% for PDD compared to 62%–84% for WLC, which means an increase of 20% (Jocham et al. 2005; Schmidbauer et al. 2004; Fradet et al. 2007; Grossman et al. 2007; Jocham et al. 2008). In the detection of CIS, PDD also performs much better, with detection rates of 92%–97% compared to 56%–68% for WLC (Schmidbauer et al. 2004; Fradet et al. 2007; Hungerhuber et al. 2007).

Positive fluorescence is not limited to tumors only. Due to inflammation, scarring after recent TURBT (≤ 6 weeks), prior intravesical instillation (≤ 3–6 months), and tangential illumination of the mucosa, PDD has a higher false-positive rate compared to WLC (Filbeck et al. 1999; Grimbergen et al. 2003). However, the benefits of the improved detection of bladder tumors outweigh this higher false-positive rate (Grimbergen et al. 2003). Despite the additional costs of equipment and photosensitizer, long-term cost benefits can be achieved through reduced recurrence rates and potentially reduced progression rates. (Sievert et al. 2009; Burger et al. 2007; Dindyal et al. 2008). Since PDD seems better and cost-effective, its implementation on a regular basis in daily practice is rapidly increasing.

4.2.3 Narrow Band Imaging

A second new technique to improve visualization of bladder tumors is the narrow band imaging (NBI), which is an endoscopic technique to enhance the contrast between mucosal surface and tissue microvasculature without special staining

(Hirata et al. 2007; Matsumoto et al. 2007). NBI depends on the depth of light penetration into the mucosa, which increases with increasing wavelength. The bladder surface is illuminated with light in the blue (415 nm) and green (540 nm) spectrum. These specific wavelengths are absorbed by hemoglobin, which results in dark brown and green appearance of the vascular structures against a white mucosa. NBI has already shown its clinical efficacy for diagnosis of superficial malignant lesions in the lung, esophagus, stomach, and colon (Lee et al. 2008). Two studies have shown additional value of NBI in the detection of bladder cancer. Bryan et al. used flexible WLC combined with NBI in 29 patients with recurrent NMIB. Fifteen additional tumors in 12 patients were found using NBI (Bryan et al. 2008). Herr et al. also found additional tumors with NBI. They reported an overall sensitivity of 87% for standard WLC compared to 100% for NBI cystoscopy and an overall specificity of 85% and 82%, respectively (Herr and Donat 2008). These studies suggest a possible role for NBI in the detection of NMIBC.

4.2.4 Optical Coherence Tomography

Optical Coherence Tomography (OCT) is an optical signal technique that provides high-resolution cross-sectional images of the bladder wall. This technique is similar to B-mode ultrasound imaging except that it measures reflected infrared light instead of acoustical waves. The amplitude of the backscattered light from structures within the tissue is displayed as a function of depth. Although the imaging depth of OCT is limited to a few millimeters (2–3 mm), the histological resolution is approximately ten times higher than high-frequency ultrasound. OCT can be easily incorporated into conventional endoscopes like a cystoscope (Tearney et al. 1997). The amount of backscattering is mainly determined by the nucleus of the cell. Malignant cells, which have increased nuclear/cytoplasm ratio, show increased backscattering and can be differentiated from normal tissue. Some studies have assessed the diagnostic accuracy of OCT for UBC and reported a sensitivity of 84%–100% and a specificity of 78%–97% (Goh et al. 2008; Hermes et al. 2008; Manyak et al. 2005). The current limitations of OCT are the costs, time required for the procedure (Lee et al. 2008), the inability to measure muscle-invasion due to the insufficient imaging depth, and virtually the inability to screen the whole bladder because the field of investigation is very small (Cauberg et al. 2009).

4.3 Transurethral Resection of Bladder Tumor

All patients with suspicious findings at (outpatient) UCS should undergo TURBT. The TURBT has three important goals: (1) removing all visible tumor; (2) assessment of size, location, aspect, and multiplicity of the tumor; and (3) obtaining

adequate tissue for pathological assessment to establishing tumor type, stage, and grade. Small tumors (< 1 cm) can be resected en bloc and larger tumors should be resected separately in fractions; first the exophytic part and second resection of the underlying bladder wall (Babjuk et al. 2009). A good TURBT specimen should include detrusor muscle for adequate assessment of the extent of invasion. Without underlying detrusor muscle in the specimen, the pathologist will not be able to differentiate between T1 and T2 tumors. In the past, bladder biopsies were taken on a routine basis for evaluation of bladder mucosa. Nowadays, this approach is abandoned in normal looking mucosa. Random biopsies are only recommended when abnormal areas of urothelium are seen or when cytology is positive but no lesions are seen in the bladder.

Early recurrence and progression are the most important problems in NMIBC. Of all Ta/T1 tumors, around 70% will recur and 15%–30% (especially Grade 3 tumors) will progress to MIBC. Other risk factors for progression are size of the tumor (>3.0 cm), concomitant CIS, multiplicity, and presence of tumor at first follow-up (3 months) after treatment (Michiel Sedelaar and Alfred Witjes 2007). Patients with progressive cancer, developing from NMIBC to MIBC have a worse prognosis compared to patients with primary MIBC (Schrier et al. 2004). This highlights the importance of an adequate diagnosis by TURBT. Another problem is the highly variable quality of TURBT with a significant risk of residual tumor (Brausi et al. 2002). Initial TURBT is often incomplete in a significant number of cases, with persistent disease found in 33%–53% of patients (Brauers et al. 2001; Divrik et al. 2006a; Grimm et al. 2003; Jakse et al. 2004; Miladi et al. 2003; Schips et al. 2002). For these reasons a re-TURBT is recommended to achieve a more complete tumor resection and to identify patients who are at risk for early tumor progression (Divrik et al. 2006a, b; Herr and Donat 2006; Schwaibold et al. 2006). The EAU guidelines recommend a re-TURBT (of the primary tumor site) when the initial resection was incomplete, when the specimen did not contain detrusor muscle, and when a high-grade NMIBC or a T1 was diagnosed. Most authors recommend a resection at 2–6 weeks after initial TURBT.

4.3.1 New Techniques

As mentioned in Sect. 4.2.2, PDD can improve both the detection of CIS and papillary tumors that may be missed during standard WLC. This improvement of tumor detection results in better transurethral tumor resection with more complete resection and consequently lower recurrence rates by approximately 20% (Jocham et al. 2005; Babjuk et al. 2005; Denzinger et al. 2007). The improved resection rate was confirmed in several studies comparing the residual tumor rate at repeat TURBT 6 weeks after the first TURBT, with either white light or PDD. All studies showed a significant decrease in residual tumor, if the resection was PDD assisted. Residual tumor rates varied from 25% to 53% for standard resection with white light and from 5% to 33% for PDD. The reported decrease in recurrence rate varies

between 31% and 43% when using PDD assisted resection instead of standard resection (Cauberg et al. 2009). PDD-assisted resection is promising and may well become a standard technique (Wilby et al. 2009).

4.4 Bimanual Palpation

Bimanual palpation (BP), rectal or vaginal, before and after TURBT is an integral part of routine pretreatment evaluation in order to obtain information about mobility of the tumor. With bimanual examination, NMIBC can be distinguished from MIBC. No mass felt on BP indicates a nonmuscle-invasive tumor. An immobile mass, on the other hand, suggests a T4 tumor. Mobile masses felt on BP only before TURBT indicates a T2 tumor and mobile masses felt before and after TURBT indicates a T3 tumor (or T2 when the TURBT was not radical). Table 4.1 shows all possible combinations of pre- and postoperative outcomes and the corresponding T-stages. Although BP is a standard care in patients with bladder cancer, especially when MIBC is suspected, the exact correlation between T-stage based on BP and the final pathological T-stage (after cystectomy) is not known yet. Data of an analysis by Ploeg et al. showed a discrepancy between T-stage based on BP and the final pathological T-stage in 42% of the patients. A total of 287 patients who underwent BP as well as cystectomy were included. Clinical overstaging based on BP was observed in around 11% of patients and clinical understaging in 31%.

4.5 Staging of Bladder Cancer

Bladder tumors are classified using the TNM staging system. The currently used TNM classification was approved in 2002 (Table 4.2) by the Union International Contre le Cancer (UICC). The TNM classification defines the extension of invasion of the tumor (T), the metastatic spread into local or regional lymph nodes (N), and the presence of distant metastasis (M). Clinical staging is referred to cTNM, and after histopathological confirmation, the stage is referred to as pTNM.

Table 4.1 All possible findings and matching T-stages after BP

After TURBT before TURBT	Negative	Mass mobile	Mass not mobile	Mass NOS	Unknown
Negative	T0/T1/CIS	–	–	–	T0/T1/CIS
Mass mobile	T2	T3a	–	T3a	T2 or T3
Mass not mobile	–	–	T4	T4	T4
Mass NOS	T2	T3a	T4	≥T3a	–
Unknown	≤T2	T3a	T4	≥T3a	Tx

[a]Or T2 if TURBT was not radical

Table 4.2 TNM classification of the urinary bladder, UICC 2002

T-primary tumour	Disease extend
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Ta	Non-invasive papillary carcinoma
Tis	Carcinoma in situ: "flat tumor"
T1	Tumor invades subepithelial connective tissue
T2	Tumor invades detrusor muscle
T2a	Superficial muscle (inner half)
T2b	Deep muscle (outer half)
T3	Tumor invades perivesical tissue
T3a	Microscopically
T3b	Macroscopically
T4a	Tumor invades prostate, uterus, vagina, pelvic, or abdominal wall Prostate, uterus, or vagina
T4b	Pelvic or abdominal wall
N-lymph nodes	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single lymph node ≤ 2 cm in greatest dimension
N2	Metastasis in a single lymph node >2 cm but ≤ 5 cm in greatest dimension, or multiple lymph nodes ≤ 5 cm in greatest dimension
N3	Metastasis in a lymph node >5 cm in greatest dimension
M-distant metastasis	
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

4.5.1 T-Category

A complete and correct TURBT is essential to determine the extension of invasion into the different layers of the bladder wall. Normal bladder epithelium consists of a transitional cell lining, called the urothelium. Just beneath the urothelium is the subepithelial connective tissue (lamina propria or submucosae), which contains some smooth muscle fibers. Adjacent to the lamina propria is the detrusor muscle (muscularis propria), which is surrounded by perivesical fat tissue. The most superficial tumor, limited to the bladder urothelium, is classified as pTa (noninvasive). A tumor invading the lamina propria is classified as pT1 (superficially invasive). CIS or pTis is a flat high-grade tumor confined to the urothelium (Fig. 4.2). In CIS, the mucosa appears erythematous. These pTa, pT1, and pTis tumors are termed nonmuscle-invasive bladder cancer (NMIBC). Tumors infiltrating the detrusor muscle are classified as pT2. The extent of a muscle-invasive tumor cannot be determined by TURBT, but only in the cystectomy specimens. Superficial extension of the tumor in the detrusor muscle (inner half of the detrusor) are termed pT2a and deep extension into the second half of the detrusor are called pT2b. pT3 tumors invade microscopically (pT3a) or macroscopically (pT3b) into the perivesical fat

tissue. If the tumor invades the adjacent organs like the prostate, uterus, and vagina, it is termed pT4a and if it invades the pelvic or abdominal wall it is called pT4b. Tumors ≥ pT2 are termed muscle-invasive bladder cancer (MIBC).

4.5.2 N-Category

The N-stage can be determined by the assessment of the lymph nodes (LN) by imaging techniques such as CT and MRI (see Sect. 4.6.2) or by pathological assessment

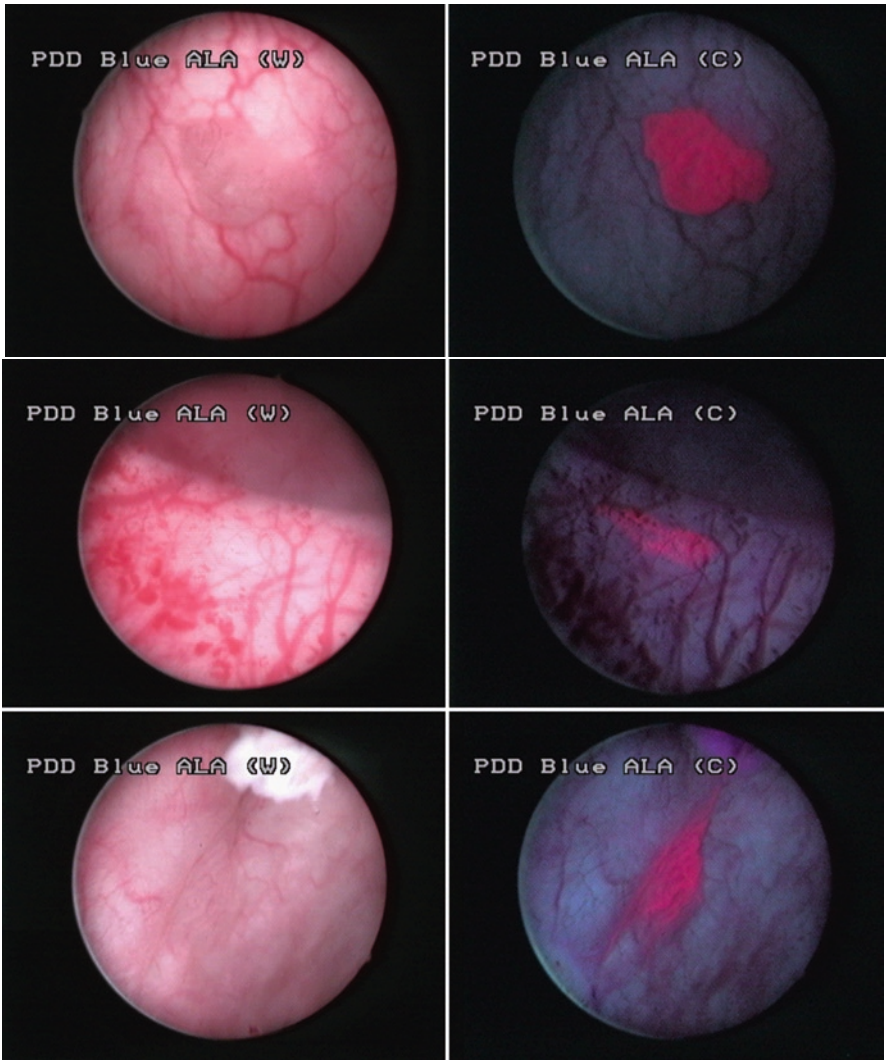

Fig. 4.1 Photodynamic diagnosis (*PDD*): detection of carcinoma in situ with standard white light (on the *left*) and blue light (on the *right*) cystoscopy

of the LNs obtained by pelvic lymph node dissection (PLND), which is a standard procedure when performing a radical cystectomy. Lymphatic spread occurs most of the time, first into the anterior and lateral pelvic LNs followed by the hypogastric, obturator, and internal and external iliac nodes. Eventually, the spread to the common iliac and paraaortic LNs is noted (Vikram et al. 2009; Kundra and Silverman 2003).

4.5.3 M-Category

Most common locations of blood borne metastases of bladder cancer are the lungs, liver, and bone. The M-stage can be determined by imaging, e.g., X-ray or CT of the thorax, ultrasound, CT or MRI of the liver, and skeletal scintigraphy. Around 50% of patients with MIBC already have occult distant metastases at time of diagnosis.

4.6 Role of Imaging

Imaging techniques like CT and MRI play an important role in accurate staging of bladder cancer, especially in muscle-invasive disease.

4.6.1 Local Staging

The primary tumor in the bladder may be detected on cross-sectional imaging (CT or MRI) either as a thickening of the bladder wall or as an intraluminal mass, which may appear plaque-like or papillary (Kundra and Silverman 2003). Calcifications may also be seen, in around 5% of UC and 50% of adenocarcinomas (Moon et al. 1992). Small bladder tumors can be difficult to assess on CT and MRI; underdistension of the bladder causes the appearance of bladder wall thickening, which can be falsely considered as a tumor and overdistension of the bladder can flatten some small bladder tumors (Vikram et al. 2009).

On CT, the tumor can be visualized in either the early and late scanning phase. In the early phase, before IV contrast materials reaches the bladder, the enhancing tumor is seen against a background of low-attenuation urine in the bladder. On delayed scanning the tumor is visualized against a background of high-attenuation contrast material in the bladder. In addition, the mass may invade the ureteral orifice, resulting in hydroureteronephrosis (Kundra and Silverman 2003). CT shows a sensitivity in detecting bladder cancer of 79%–89.7% and a specificity of 91%–94% (Knox et al. 2008; Sadow et al. 2008).

On MRI, the tumor is slightly hyperintense relative to muscle on T2-weighted sequence and isointense on T1-weighted sequence (Vikram et al. 2009). For staging with MRI, similar results have been found as with CT. The accuracy with MRI

ranges from 72% to 96%. UBC enhances after gadolinium injection with improvement of staging. However, there is no evidence yet in literature that MRI is superior to CT (Kundra and Silverman 2003). Better availability makes CT still the primary imaging modality for cancer of the urinary bladder.

A serious limitation of current CT and MRI in local tumor-staging is the inability to resolve the various layers of the bladder wall. This means that distinguishing between T1, T2, and T3 tumors is difficult. However, some signs seen on CT and MRI can be helpful to distinguish between the different T-stages. Retraction of the outer bladder wall is indirect evidence of MIBC (Vikram et al. 2009). T2a and T2b tumors can occasionally be differentiated on MRI; the hypointense band of muscle is preserved in T2a tumors. Microscopic fat invasion cannot be detected (T3a) and macroscopic extravesical extension (T3b) is seen on CT and MRI as an irregular, ill defined outer bladder wall (Vikram et al. 2009).

Another important limitation of CT and MRI is the inability to distinguish tumor spread from fat stranding from TURBT (Vikram et al. 2009). Therefore, imaging for local staging can best be performed before TURBT (Levy and Grossman 1996). Other confounders for CT and MRI include inflammation, history of radiation therapy, or intravesical instillations with chemo or *Bacillus Calmette-Guérin* (BCG), which can mimic tumor by circumferential wall thickening (Kundra and Silverman 2003).

Conventional ultrasound is a commonly used technique to screen the diagnosis of UBC due to its availability, low costs, noninvasiveness, and as there is no need for contrast agents. The diagnosis relies on the detection of bladder wall thickening or the presence of focal masses protruding into the bladder lumen (Nicolau et al. 2010). Accurate T-staging is not possible when using ultrasound because of the limited resolution of the different layers of the bladder wall. Intravesical sonography, although reported to be superior to transabdominal ultrasound, provides no added value over cystoscopy and is, therefore, abandoned (Vikram et al. 2009). The ultrasound technology has improved in recent years. Transabdominal ultrasound with newer sonography machines showed a good diagnostic accuracy of 88.2% for focal lesions of the bladder wall found on cystoscopy (Francica et al. 2008). Newer techniques such as 3D, virtual sonography, and contrast enhanced ultrasound may become useful tools in the diagnosis and staging of UBC (Nicolau et al. 2010).

4.6.2 Lymph Node Involvement

The incidence of nodal metastases is around 30% for tumors limited to the bladder wall and around 60% for tumors with extravesical extension (MacVicar 2000). CT and MRI rely on size of the LNs for detecting nodal involvement. LNs > 1 cm in size in the short axis are considered suspicious (Vinnicombe et al. 1995). However, this is not a reliable indicator since small LNs can contain tumor and enlarged LNs can be reactive without the presence of tumor inside. Sometimes the shape of the LN can be helpful—round nodes are more likely to be metastatic compared to ovoid nodes (Jager et al. 1996). Confounders are benign hyperplasia and infection

resulting in LN enlargement. The accuracy for nodal staging of standard CT and MRI is equal, ranging from 70% to 97% and 73% to 98%, respectively (Hofer et al. 2001; Husband 1995). Gadolinium-enhanced MRI, useful for local staging, is not helpful in detecting nodal metastases due to similar enhancement shown by normal sized metastatic LNs as well as nonmetastatic LNs (Deserno et al. 2004). A technique that does detect metastases in normal-sized LNs is "Ferumoxtran-10-enhanced MRI." This technique uses ultrasmall superparamagnetic particles of iron oxide (USPIO) (ferumoxtran-10) as a contrast agent for MRI to obtain different signal intensities between normal LNs and affected LNs containing metastases. The uptake of USPIO by macrophages in normal LNs gives a decrease in signal on MRI. This difference allows detection of metastases even in normal sized-nodes, which results in increased sensitivity from 76% to 96% in comparison with nonenhanced MRI (Deserno et al. 2004).

4.6.3 Distant Metastasis

As mentioned before, most common sites of hematogenously spread are liver, bones, and lungs. Chest radiography is most of the times sufficient for detecting lung metastases. Bone scintigraphy is only indicated in patients with symptoms and signs of bone metastases, like pain or elevated alkaline phosphatase.

4.6.4 Upper Tract Imaging (Brief Reference)

Bladder cancer is characterized by a high cumulative incidence of recurrence. They involve any part of the urothelium, which suggests that the entire mucosa is primed to become malignant ("field defect"). The reported rate of upper tract tumors (UTT) is 0.8%–7% (Sanderson et al. 2007; Wright et al. 2009; Palou et al. 2005). Palou et al. found in a group of 1529 patients with primary NMIBC, 28 (1.8%) patients with synchronous UTT. Of these UTI, 46% were invasive and >80% were grade 2 or 3. This implies that once a urothelial tumor is diagnosed in the bladder, evaluation and surveillance of the upper tract urothelium is essential. Risk factors for upper tract tumors are stage, high–grade, and location near ureteral orifice, bladder neck, or trigone at the time of primary bladder cancer diagnosis. Patients with tumor in the trigone are at almost sixfold higher risk for a synchronous UTT (Palou et al. 2005). Other risk factors are CIS and urethral UC (Sanderson et al. 2007; Wright et al. 2009). Traditionally, intravenous urography (IVU) is used for the diagnosis of UTT. More recently, CT urography (CTU) has emerged as a technique for screening of the upper urinary tract. CTU has a higher sensitivity and specificity compared to IVU (Gray Sears et al. 2002; Lang et al. 2003, 2004).

4.7 Summary

The gold standard in the diagnosis of UBC is the combination of urinary cytology combined with UCS and followed by TURBT for removing the tumor and obtaining tissue for pathological assessment. New techniques for the improvement of tumor visibility during UCS and TURBT are PDD, NBI, and OCT. These new tools are promising, and since the use of PDD is expanding rapidly, PDD-assisted resection may well become a standard technique in the near future. For local staging of UBC bimanual palpation, TURBT, and imaging techniques play an important role. However, the accuracy of BP in this staging process is still questionable. CT, MRI, and transabdominal ultrasound are the techniques most commonly used for local staging; however, serious limitations occur. For LN involvement, CT and MRI rely on size of the LNs only, which is not a reliable indicator, since there is a considerable overlap in size between benign and malignant LNs. USPIO-enhanced MRI solves this problem by detecting metastases even in normal sized-nodes, which results in an increase in sensitivity of 20% compared with nonenhanced MRI. Enhancement of current techniques and development of new techniques is still needed for further improvement of the detection of primary and recurrent bladder tumors.

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Chapter 5
Urine Cytology, DNA Ploidy and Morphometry

Eva M. Wojcik

Abstract Urothelial cancer is a challenging condition to be detected by urine cytology. The main challenge is to recognize low-grade lesions. By definition, the nuclear differences between low-grade urothelial carcinoma and normal urothelium are subtle and very subjective. Additionally, reactive processes, due to treatment, litiasis of the urinary tract and viral infections are difficult to distinguish from neoplastic processes.

A sensitive, noninvasive test to detect bladder cancer remains an elusive but desirable goal. Although urine cytology is highly sensitive for detection of high-grade urothelial cancer, its sensitivity for low-grade cancer remains unacceptably low. Consequently, invasive cystoscopy continues to be used to detect bladder urothelial carcinoma and to monitor patients with high-risk for recurrence and progression. Therefore, there is an obvious need for a development of additional more sensitive tests that could detect urothelial neoplasia. In this context, a number of techniques, including DNA ploidy, morphometry, immunohistochemistry, cytogenetics, urine chemical assays, and lately, molecular techniques have been proposed to be used either in conjunction with cytologic examination or even to completely replace cytology.

5.1 Introduction

Urine cytology is one of the most common type of nongynecological cytology pathologists encounter in their daily practice. At the same time, it is one of the most difficult and frustrating type of specimen they deal with. The main challenge is to detect low-grade lesions and recognize numerous pitfalls, including cellular changes induced by instrumentation, treatment, lithiasis, and viruses.

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Despite its inherent limitations, urinary cytology in conjunction with cystoscopy remains a mainstay technique for detecting urothelial malignancies. Although there are numerous noninvasive tests and markers for bladder cancer, these tests are still adjuvant to urine cytology. Until now, cytology has the highest specificity for detection of urothelial cell carcinoma. However, in conjunction with additional tests, high sensitivity and specificity can be achieved.

Successful urine cytology depends upon many factors. One of the most important is the availability of clinical information such as sex, age, type of specimen, symptoms, cytoscopic and radiographic findings, and the patient's previous history.

5.2 Cellular and Noncellular Components of Normal Urine Specimens

A specialized type of epithelium (transitional epithelium or, currently recommended name, urothelium) is lining a lower collecting system, which includes bladder and urethra and upper collecting system comprising of renal pelvis, calyceal system, and ureters. Urothelium is a multilayer epithelium, composed of six to seven layers of cells. The main role of the urothelium is to form a blood/urine barrier.

Cells normally occurring in urine are mainly urothelial cells. These include basal cells, intermediate cells, and superficial (umbrella) cells. The superficial cells form the top layer of the urothelium. These cells are large with abundant cytoplasm, characteristic scalloped borders and are often bi- or multinucleated. Superficial cells can range markedly in size, however, this feature should not be interpreted as a neoplastic process. Intermediate cells are much smaller and they are often arranged in loosely cohesive clusters seen particularly in instrumented urines (Fig. 5.1). In addition to urothelial cells, urine specimens often contain squamous cells. The most common origin of squamous cells, especially in women, is contamination from the genital track. However, squamous cells can also originate from the bladder trigone or from squamous metaplasia.

Other cells, less commonly occurring in urine, include inflammatory cells, such as leukocytes and lymphocytes. Occasionally, glandular cells can be encountered in urine specimens and they may originate from prostatic, endometrial, or paraurethral glands, as well as from cystitis glandularis. The presence of renal tubular cells indicates renal parenchymal disease and should be reported to prompt further workup. Sporadically, degenerated seminal vesicle cells can be seen in urine specimens, particularly from older patients. Seminal vesicle cells in urine specimens often have a bizarre appearance, with greatly enlarged nuclei and foamy, fragmented cytoplasm. Often, spermatozoa accompany seminal vesicle cells. These cells commonly have an abnormal DNA content (Wojcik et al. 1999).

A variety of noncellular elements can also be seen in urine specimens. These include crystals, renal casts, spermatozoa, corpora amylacea, lubricant, mucus, fibrin, and pollen.

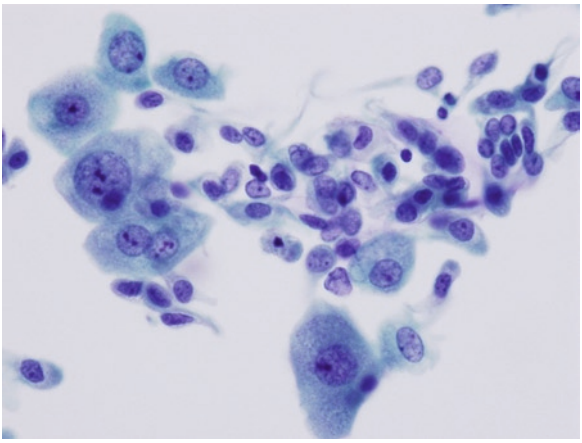

Fig. 5.1 Loosely cohesive cluster of urothelial cells. Notice large umbrella cells at the periphery. Variation in nuclear size and binucleation are NOT sign of malignancy

5.3 Types of Urinary Specimens

Voided urine is the most convenient and easily obtained urine specimen. Fresh, randomly voided urine is the simplest and the most appropriate specimen obtained in patients who do not require catheterization. These specimens usually contain few urothelial cells. However, they commonly contain contamination from external genitalia, particularly in women. In addition, urothelial cells in voided specimens often show marked degeneration. In contrast, catheterized urine lacks contamination from external genitalia, the specimens are much more cellular and often contain pseudopapillary fragments. Bladder washings are generally cellular and show better preservation. In these specimens numerous superficial cells and intermediate cells are arranged in monolayered sheets and loose clusters. In addition, there are also pseudopapillary fragments and single cells. Similar morphologic findings are seen in patients with low-grade urothelial carcinoma. In patients who underwent cystectomies, urine specimens obtained from ileal conduit/pouch/neobladder are usually hypercellular and contain numerous degenerated cells of ileal origin in a dirty background of mucus and bacteria. In these specimens, the urothelial cells originating from the upper urinary tract often show marked degeneration.

5.4 Cytology of Urothelial Carcinoma

The main purpose of performing cytologic evaluation of urinary specimens is to detect bladder cancer. Most cases of high-grade urothelial carcinoma can be diagnosed accurately, and there is a good interobserver agreement among

pathologists. The overall sensitivity and specificity for high-grade urothelial carcinoma are very high and approaching 95%–100% (Bastacky et al. 1999). However, with the recent trend to classify former TCC grade II as high-grade urothelial carcinoma the sensitivity in recent years appear to be lower (Curry and Wojcik 2002).

In general, the sensitivity of urine cytology depends upon tumor differentiation, and as previously stated, it is high for high-grade lesions. However, for low-grade tumors, the sensitivity is much lower and show great variation in published literature, ranging from 0% to 50% (Raab et al. 2007).

5.4.1 Cytology of High-Grade Urothelial Carcinoma

Specimens obtained from patients with high-grade urothelial carcinomas are usually very cellular and composed of loose clusters and single cells, showing moderate to marked pleomorphism. The nuclear to cytoplasmic (N/C) ratio is increased, nuclei are eccentrically located, and show irregular nuclear membrane and coarse chromatin (Fig. 5.2). In addition, high-grade urothelial carcinoma may show squamous differentiation and, less commonly, glandular differentiation.

In general, the same morphologic features are seen in both invasive and noninvasive high-grade papillary urothelial carcinomas as well as in carcinoma in situ (CIS). Therefore, urine cytology cannot be used to stage the tumor.

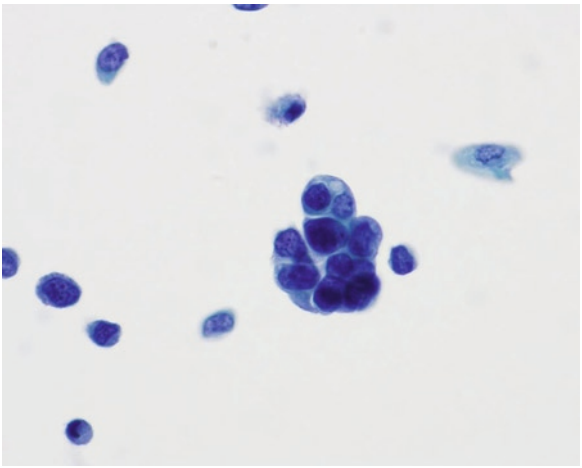

Fig. 5.2 Cluster and single malignant urothelial cells. Notice high nuclear/cytoplasmic ration, coarse chromatin, and irregularity of nuclear membrane

5.4.2 *Cytology of Low-Grade Urothelial Carcinoma*

Instrumented urine specimens obtained from patients with low-grade urothelial carcinomas also show increased cellularity and presence of papillary, cohesive clusters. Cells demonstrate only mild to moderate pleomorphism, mildly increased N/C ratio and eccentric placement of nuclei. The nuclear membrane is slightly irregular and chromatin is even and finely granular. The overall morphologic changes are minimal and similar findings can be seen in any instrumented urine. Therefore, the diagnosis of low-grade urothelial carcinoma is very challenging and most of the time impossible, particularly in instrumented urine specimens. Occasionally, a positive identification can be made if a significant number of tumor cells shed from a large bladder tumor are present (Fig. 5.3). In addition, there are numerous reactive processes that can further confound the ability to make an appropriate diagnosis. Since instrumentation, inflammation, infection, recent surgical manipulation, radio/chemotherapy, and presence of calculi can affect the cellularity and cytomorphology of the specimen, it is difficult, even for trained cytologists, to reliably discriminate malignant cells. Many of these cases are, therefore, categorized as atypical. However, reporting the presence of atypical cells in urine cytology, although common occurrence in a daily practice, does not provide meaningful information for the clinician. Therefore, it is important to limit this category to relatively few cases. The reported rate of atypia ranges from 1.9% to over 23.2% (Brimo et al. 2009). In our institution the rate of atypia is in the range of 6%, and in our recent study, we have shown that the significance of atypical cells in voided urine is much higher than in instrumented ones (Kapur et al. 2008).

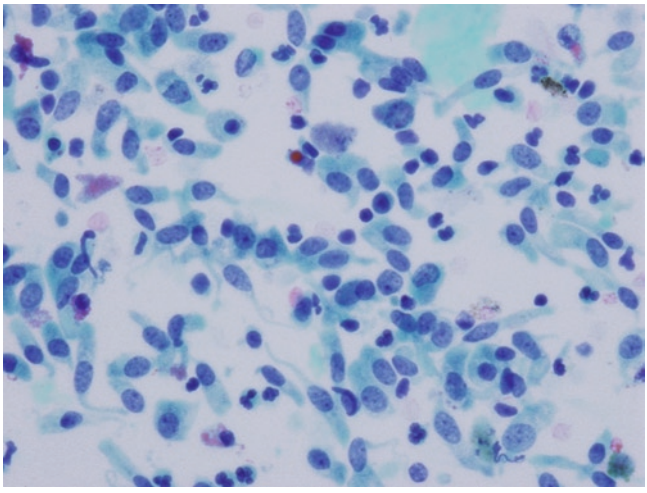

Fig. 5.3 Numerous cells originating from a large low-grade urothelial carcinoma. Notice minimal pleomorphism of individual cells

5.5 Cause of Atypical Urine Cytology

5.5.1 Urinary Tract Calculi (Nephrolithiasis)

Nephrolithiasis remains to be a number one pitfall in urinary cytology. Patients usually present with hematuria and/or filling defects. Cytology specimens may be cellular and three dimentional (3D) fragments composed of cells exhibiting significant pleomorphism may be seen. Inflammation, blood, and cellular debris may be present in the background. Clinical history is crucial to avoid a false positive diagnosis.

5.5.2 Therapeutic Effects

Radiation treatment and many chemotherapeutic agents can induce marked cytologic changes in urothelial cells. Particularly, the agents that concentrate in the urine or the agents that are used for intravesical treatment often produce morphologic changes that may be easily mistaken for malignancy.

One of the most common agents used to treat urothelial carcinoma is Bacillus Calmete-Guérin (BCG) vaccine. Intravesical BCG immunotherapy is one of the most widely used approaches to manage superficial bladder cancer. In cytologic specimens, we can find free histiocytes, histiocytic aggregates—granulomas (Fig. 5.4), and multinucleated histocytic giant cells.

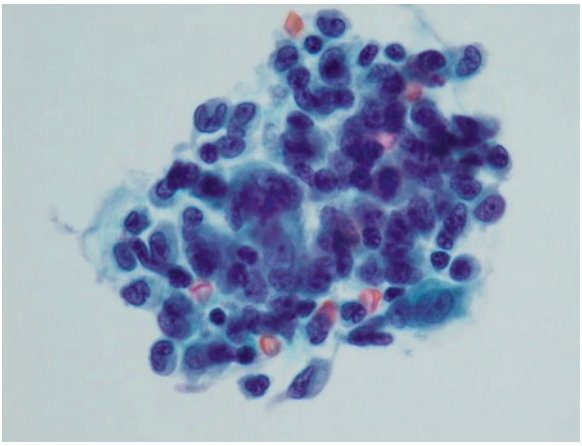

Fig. 5.4 Collection of epithelioid histiocytes and lymphocytes (granuloma) obtained from a patient treated with intravesicle BCG. These unexpected finding in urine cytology may be interpret as low-grade malignancy

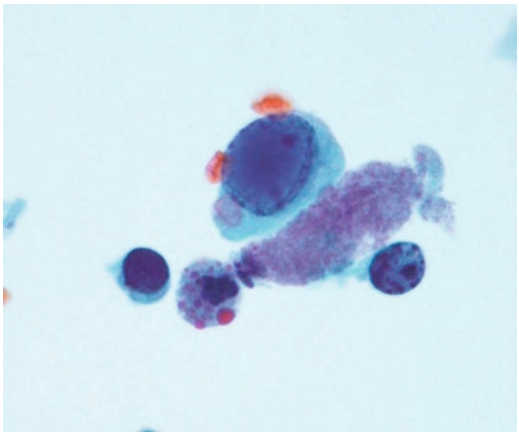

Fig. 5.5 Large cell at the center shows polyoma virus changes. Notice large intranuclear inclusion

5.5.3 Human Polyoma Virus

Human polyoma viruses are small, nonenveloped, double-stranded DNA viruses that are classified into two main strains, BK and JC. Classically, the JC strain of the virus is associated with progressive multifocal leukoencephalopathy. The BK strain of the virus affects the kidney and can be detected in the urine. Infection occurs during childhood and is usually subclinical. Over 90% of adults are seropositive. The virus generally remains latent in the kidney, but intermittent viruria is demonstrable in 0.3% of healthy adults. The infection is reactivated in individuals with various degrees of immunological deficiencies. In 2%–5% of renal transplant recipients, polyoma virus can cause polyonephrapathy. Patients who develop polyoma virus nephrapathy are in significant risk of losing graft. In these patients, the immunosuppression should be significantly lowered (Cimbaluk et al. 2009). In cytology specimens, there are usually single cells with large homogenous, basophilic inclusions occupying most of the enlarged nuclear area, so called "decoy cells" (Fig. 5.5). Occasionally, significant numbers of urothelial cells can be affected by the virus. It has been demonstrated that urothelial cells affected by the virus have an abnormal DNA content (Wojcik et al. 1997a).

5.6 DNA Ploidy

Cytometric assessments of the nuclear DNA content in urothelial carcinoma cells either by flow cytometry (FCM), static image analysis, or laser scanning cytometry have been extensively performed for more than 30 years (Tribukait and Esposti 1978, Koss et al. 1989). These techniques measure a total amount of DNA and are only able to detect a

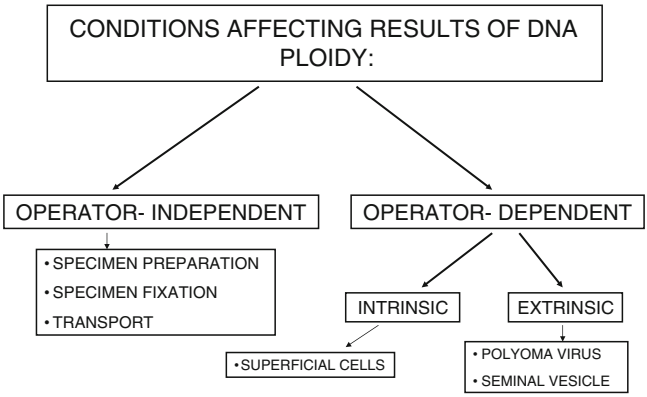

Fig. 5.6 Operator-dependent and independent factors that can affect the results of DNA ploidy

significant and numerous chromosomal changes. DNA ploidy has been used for both diagnostic and prognostic purposes. However, an abnormal DNA ploidy is not automatically associated with malignancy since low-grade urothelial carcinomas are diploid and only high-grade tumors are aneuploid (Wojcik et al. 1997b). As a result, aneuploidy is a strong indicator of high-grade malignancy as well as CIS. Also aneuploidy in conjunction with suspicious cytology is highly predictive of tumor recurrence. In addition, it has been shown that DNA ploidy analysis provides independent prognostic information. However, there are numerous operator-dependent and independent factors that can affect the results (Fig. 5.6). We have shown that superficial cells (Wojcik et al. 2000a), seminal vesicle cells (Wojcik et al. 1999), and cells infected with polyoma virus (Wojcik et al. 1997a) may cause false positive results. In addition, specimen fixation, preparation, and condition during transport may affect results (Wojcik et al. 2000b).

5.6.1 DNA Ploidy by Flow Cytometry

Flow cytometry (FCM) was the first method applied to study bladder washings from patients with urinary carcinomas (White De Vere and Deitch 1985). Numerous early studies reported the usefulness of this technique in the management of bladder cancer. However, FCM requires a large tissue sample for analysis (Bakhos et al. 2000), therefore, only bladder washes are suitable urinary specimens. In addition, in this technique there is no option to visually assess what cells are being measured. The advantages of FCM, however, are its speed, accuracy of quantitation, and automation.

5.6.2 DNA Ploidy by Static Image Analysis

The alternative technology to evaluate a DNA ploidy in urine specimens is image analysis (IA). IA is a broad term, encompassing morphometry, densitometry, and

even neural networks. Typically, the term is used to describe an integrated, interactive computer-based system in which measurements of specific cellular features, including the amount of DNA, are analyzed. In this method, cells (100–200) are visually selected by operator. All urine specimens are suitable for analysis. Nuclear DNA ploidy is evaluated on Feulgen-stained slides. In the DNA staining method, developed by Feulgen and Rossenbeck (1924), hydrochloric acid is used to hydrolyze the ribose-purine bonds in the DNA to give sugar aldehyde residues, and a dye is then coupled stoichometrically to the sugar aldehyde. The staining intensity becomes a measure of the DNA content in the nucleus. The absorption spectrum maximum for the Azure A stain is 620 nm. The IOD is assumed to be equivalent to the amount of DNA present in the nuclei. This technique, because it allows for the visual selection of morphologically abnormal cells by a trained operator, has the ability to detect small aneuploid cell populations. The disadvantages of IA include the potential for sampling error, since selection of cells depends on the operator's experience (Wojcik et al. 2000a) and because a relatively small number of cells are being analyzed. The analysis, in general, is slower and labor-intensive.

5.6.3 DNA Ploidy by Laser Scanning Cytometry

The laser scanning cytometer (LSC) is highly suited for DNA ploidy analysis. The LSC combines features of both flow and image cytometry and is capable of measuring multicolor fluorescence, light scatter, and location of cells fixed to a microscopic slide. It is capable of rapid and automatic measurements of a large number of cells. In addition, cell location is recorded so the cells of interest can be relocated for a morphologic examination and classification.

For DNA analysis, the cells are stained with propidium iodide (PI) and cytokeratin conjugated with fluorescein isothiocyanate (FITC). PI stoichiometrically intercalates with the nucleic acids on the DNA strand. The fluorescence signal from a cell is proportional to its DNA content. PI-stained nuclei emit fluorescence light at wavelengths between 580 and 650 nm. The emission color is red. FITC emits light at wavelengths between 488 and 525 nm. The emission color is green.

For many years we used LSC to evaluate DNA ploidy and we have found LSC to be a suitable instrument for a rapid and reliable evaluation of DNA ploidy in routine urine specimens (Wojcik et al. 2001).

5.7 Fluorescence In situ Hybridization

Fluorescence in situ hybridization (FISH) has been demonstrated to be a viable method for determination of chromosome specific anomalies in cells obtained from urine specimens. A multicolor FISH Probe Mixture designed for interphase cell analysis for detection and quantification of chromosome 3, 7, 17, and the 9p21 region

has been commercially available (UroVysion Bladder Cancer Kit, Abbott Molecular, Inc., Des Plaines, IL). In the initial study performed by Sokolova et al. (2000), a number of potential probes were studied. The four probes with the greatest sensitivity for urothelial carcinoma detection were selected. In addition, the definition of a positive FISH result has been proposed in the Sokolova's study. Accordingly, a specimen was considered FISH positive for bladder cancer if at least one of the following criteria were met: 1/5 or more cells with gain of more than one chromosome, 2/10 or more cells with gain of a single chromosome or 3/10 or more cells with homozygous loss of the 9p21 locus (both copies lost). The following study by Bubendorf et al. (2001) proposed another cut off criteria. According to these authors, a FISH positive specimen is a specimen containing more than two abnormal cells. Abnormal cells were defined as nontetrasomic cells showing at least three copies of any of the signals for chromosomes 3, 7, 17, and the 9p21 locus, or if there was heterozygous or homozygous loss of 9p21 (one copy or both copies lost). The change in the definition was based on the presence of a significant number of cases containing tetraploid cells in a negative control group. Similarly, we documented that umbrella cells can be associated with abnormal signals (Wojcik et al. 2002). Based on our study, we recommended to correlate the results of FISH with cytologic findings and, in rare cases, with numerous reactive umbrella cells, the results should be reevaluated.

Since the FDA approval, the UroVision FISH test has gained significant popularity and is being used by numerous laboratories. Also, by now there is an impressive body of literature validating this test. It has been shown that 95% of high-grade urothelial cell tumors show gain of at least two of these three chromosomes. Skacel et al. (2003) demonstrated, in a retrospective study, that cases that were cytology negative or atypical but biopsy positive for urothelial carcinoma could be positively diagnosed by UroVysion FISH in the majority of cases. However, at this time it is not entirely clear if a positive FISH may indicate frank neoplastic urothelial transformation or is an indicator of unstable urothelium capable of, or primed for, malignant transformation, thus detecting patients at significant risk.

5.8 Morphometry

Morphometry has been defined as the "quantitative description of a structure." In practice, this term is usually applied to quantitative techniques that measure features of size, shape, and texture in two dimensions and/or spatial relationships from cells or other tissue structures (Marchevsky and Erler 1999). The need for measurement comes from the recognition that interobserver and intraobserver diagnostic decisions are poorly reproducible. Morphometry has several advantages over conventional visual assessment: objectivity, reproducibility, and the ability to detect changes too subtle to be visually appreciated in individual cells. Therefore, morphologic diagnostic accuracy and precision can be improved by applying this technique.

Application of nuclear morphometry to distinguish normal urothelial cells from the malignant ones has a long history. Kern and his coworkers (1967) were the first to apply morphometric features in UC. Their original studies were based on the visual

description of the nuclear shape, size, number of nucleoli, nuclear density, and chromatin structure. Even using early, unsophisticated methods, authors found significant quantitative differences between normal urothelial cells and cells from moderately and poorly differentiated carcinomas. At the same time Koss et al. (1975) demonstrated the usefulness of computer assistance in the discrimination of normal and malignant urothelial cells. Since that time numerous studies demonstrating diagnostic and prognostic applications of morphometric analyses have been published.

A practical application of morphometry has been utilized by a group from the Netherlands that developed a quantitative karyometric cytology system. The QUANTICYT is a system based on evaluation of DNA content and nuclear shaped features and it determines a scoring spectrum—low-risk, intermediate-risk, and high-risk for urothelial carcinoma. By using this approach, the sensitivity for detection of tumor of 95.2% and specificity of 65% were achieved (van der Poel et al. 1996).

We evaluated the application of computer-assisted quantitative nuclear grading in differentiation between normal urothelium, reactive atypical changes, low-, and high-grade urothelial carcinoma. In each cytologic category, 38 nuclear morphometric descriptors were analyzed. Backward stepwise logistic regression analysis was applied to assess which of the descriptors contributed to statistical models that differentiated between these categories. Receiver operating characteristics (ROC) and area under the curves (AUC) were determined to define these differences. We found that there are significant morphometric differences between all four cytologic groups. We concluded that quantitative nuclear grading can be especially useful to differentiate inconclusive atypical cases from low-grade urothelial carcinoma (Wojcik et al. 1998).

5.9 Conclusion

As presented, urothelial cancer continues to be a challenging condition to be detected by urine cytology. The main challenge is to identify low-grade lesions. Morphologic changes in these tumors are so minimal that practically preclude their visual detection by a human eye. However, these changes can be detected on a molecular level as well as by morphometric techniques. Urine cytology, in conjunction with new adjuvant tests, continues to be a very powerful test to detect and monitor patients with bladder malignancies.

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Chapter 6
Molecular Signatures of Bladder Cancer

Brian K. McNeil, Obi O. Ekwenna, and Robert H. Getzenberg

Abstract Molecular signatures can be defined in many ways but are basically the characteristic features of the molecular composition of a cell or its surroundings. Crucial differences exist between normal and cancer cells that stem from discreet changes in specific genes that control tissue proliferation, apoptosis, invasion, as well as homeostasis (Harris 1996). There are a multitude of cancer related genes that have been discovered and implicated in the natural history of various malignancies. With our increased understanding of disease mechanisms and molecular techniques, the number of molecular signatures of cancer has increased exponentially with several signatures serving as both diagnostic and therapeutic targets.

In this chapter, we will explore molecular signatures of bladder cancer. During preparation of this section, we had several goals in mind. In addition to discussing molecular signatures of bladder cancer that are currently in use, it is important to be aware of those in development and foster critical thinking regarding signature investigation and bridging the gap between basic research and clinical application.

6.1 Molecular Signatures

Molecular signatures can be defined in many ways but are basically the characteristic features of the molecular composition of a cell or its surroundings. Crucial differences exist between normal and cancer cells that stem from discreet changes in specific genes that control tissue proliferation, apoptosis, invasion, as well as homeostasis (Harris 1996). There are a multitude of cancer related genes that have been discovered and implicated in the natural history of various malignancies. With our increased understanding of disease mechanisms and molecular techniques, the number of molecular signatures of cancer has increased exponentially with several signatures serving as both diagnostic and therapeutic targets.

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In this chapter, we will explore molecular signatures of bladder cancer. During preparation of this section, we had several goals in mind. In addition to discussing molecular signatures of bladder cancer that are currently in use, it is important to be aware of those in development and foster critical thinking regarding signature investigation and bridging the gap between basic research and clinical application (Table 6.1).

Molecular changes that lead to the development of bladder cancer can be broadly classified into three interrelated categories (Vrooman and Witjes 2009). The first are chromosomal alterations, which trigger the initial carcinogenic event, followed by tumor proliferation caused by loss of cell-cycle regulation, and derangements in apoptosis. This can result in metastases in which there is angiogenesis and loss of cell adhesion (Quek et al. 2003). The investigation of molecular signatures involved in cancer has employed molecular biology techniques evaluating the three basic pathways of gene expression: the gene, mRNA produced by the gene, and the protein coded by the mRNA.

Several genomic advances over the last decade have led to the identification of molecular signatures of bladder cancer. One of the more promising developments has been the advancement of gene expression microarray technology. Gene microarray chips have made it possible to measure mRNA expression levels for thousands of genes from a tissue sample by hybridizing fluorescently labeled complementary DNA from tissue to the chip (van de Rijn and Gilks 2004).

Proteomics is the characterization of molecular and cellular dynamics in protein expression and function on a global scale. Proteomic techniques have identified the functioning units of expressed genes providing a protein fingerprint (Pandey and Mann 2000). This fingerprint reflects the intrinsic genetic program of the cell and the impact of its immediate environment, making it a valuable resource for biomarker discovery. Proteomic technologies have been used to study cancers of various organs and advances in proteomic techniques have led to the discovery of several molecular signatures of bladder cancer (Wu et al. 2008; Shen et al. 2006; Lee et al. 2005; Ornstein and Tyson 2006; Li et al. 2005; Vlahou et al. 2001; Wang et al. 2005; Zhou et al. 2005).

6.2 Bladder Cancer

The 2010 estimated incidence of bladder cancer in the United States is 70,530 with approximately 14,100 deaths resulting from the disease (American cancer society: cancer facts and figures 2010). Despite what appears to be a relatively low incidence rate, the worldwide prevalence of bladder cancer is believed to be over one million (Lerner 2005). A majority of bladder cancers are superficial and respond well to local resection and adjuvant intravesical therapy, if required. Unfortunately, recurrence rates for superficial bladder cancer are high (50%–70%), with 10%–15% of these tumors progressing to muscle-invasive disease (Prout et al. 1992).

Cystoscopy, aided by cytology, is the mainstay for diagnosis of bladder cancer. Voided urine cytology has been utilized as a screening test for bladder cancer, since 1945, yet many studies have demonstrated its low sensitivity in screening populations (Fradet 1996; Rife et al. 1979). Cystoscopy is invasive, relatively expensive, and oftentimes inconclusive, especially in individuals with indwelling catheters or active inflammation because of the abnormal appearance of the bladder urothelium. High-grade carcinoma in situ can be missed by cystoscopy alone in some instances, delaying diagnosis and the initiation of treatment. These factors have led scientists to evaluate other methods to detect bladder cancer and predict its behavior.

While the death rate for bladder cancer has been relatively stable, or even increased slightly over the last decade, the mortality rate from prostate cancer has decreased, largely a result of screening measures, public awareness, and the ability to identify patients with aggressive disease in need of curative therapy. A dramatic stage migration has occurred with prostate cancer with most patients now presenting with low stage disease (Hankey et al. 1999; Han et al. 2003). In addition to urinary cytology, a number of markers have been available for the diagnosis of bladder cancer with a positive predictive value as good as, if not better than PSA, leading one to question why the mortality rate from bladder cancer has not fallen, mirroring that of prostate cancer (Araki et al. 2007). Investigators at the University of Miami reviewed radical cystectomy and radical retropubic prostatectomy databases during two consecutive 7-year periods to analyze the changes in pathologic stage (Araki et al. 2007). Patients were divided into two consecutive groups: group 1 (1992–1998) and group 2 (1999–2000). No differences were found in the clinical or pathologic staging between the two groups of patients undergoing radical cystectomy, with similar 5-year overall and disease-specific survival rates. Molecular signatures could aid in identifying patients with aggressive tumors with a higher likelihood of progression and cancer-specific mortality. Identification at earlier points in the natural history of the disease could impact observed mortality rates.

Bladder cancer treatment accounts for the greatest lifetime cost for cancer care among Medicare recipients, ranging from US$96,000 to US$187,000 per patient in 2001 (Botteman et al. 2003). With life expectancies increasing along with inflation, the cost of treating bladder cancer will continue to rise, presenting yet another incentive for the discovery and clinical implementation of reliable molecular signatures, which could assist caregivers in tailoring treatment regimens to individuals based on their risk profile. This could decrease healthcare costs associated with the treatment of bladder cancer, which is necessary, considering that increase in annual costs observed for treating cancer of the bladder and upper urinary tract between 1994 and 2000 outpaced inflation during the same period (Konety et al. 2007).

Radical cystectomy remains the most common treatment for patients with muscle-invasive bladder cancer. Despite advances in surgical technique, 5-year disease-specific survival remains 50%–60% (Shariat et al. 2006; Stein et al. 2001). There is significant morbidity associated with the treatment of muscle-invasive bladder cancer with complication rates of 17%–32% reported in contemporary series and a perioperative mortality rate of 1%–2% (Yafi et al. 2008; Rosario et al. 2000).

Table 6.1 Table caption

Test/marker	Marker detected	Specimen	Assay type	Manufacturer	Sensitivity (%)	Specificity(%)	Comments
Cytology (Harris 1996)	Tumor cells	Voided urine, Barbotage	Microscopy	Diagnostic reference laboratories	11–76	90–100	FDA approved
NMP-22	Nuclear mitotic apparatus protein	Voided urine	Sandwich ELISA Qualitative immuno-chromography	MATRITECH, Inc., Biosite/ Inverness	44–100	55–98	FDA approved Specificity and sensitivity depends on cutoff values
BTA-TRAK	Complement factor H/H-related protein	Voided urine	Sandwich ELISA	POLYMEDCO, Inc.	17–78	51–95	FDA approved Not useful with Hematuria/ proteinuria
Bta-stat	Complement factor H/H-related protein	Voided urine	Point-of-care /colorimetric	Bion Diagnostic Science, Inc.	57–83	68–72	FDA approved Not useful with Hematuria/ proteinuria
ImmunoCyt	Two-Mucin like proteins, and HMW CEA	Exfoliated cells, voided urine	Fluorescence	DiagnoCure, Inc	50–100	56–75	FDA approved Interobserver variability high Failure of test due inadequate cellularity
UroVysion	Aneupoidy in chromosome 3, 7, and 17. And loss of locus 9p21.	Exfoliated cells	FISH	Vysis, Inc	69–75	82–85 (Vrooman and Witjes 2009)	FDA approved Expensive

BCLA-4	Nuclear matrix protein-transcription factor	Voided urine	ELISA	Investigational	89–96	95100	
Telomerase	Human telomerase reverse transcriptase mRNA	Exfoliated urine	PCR, TRAP assay	Investigational Intergen, Oncor	46–92	69–99	Variability of telomerase among individuals. False positives with UTIs, and Urolithiasis Not FDA approved Independent of grade/stage
Bladder tumor fibronectin (BTF)	Fibronectin, degradation product of extracellular matrix	Voided urine	Solid-phase chemiluminescent immune-metric test	Investigational	78–85	80–91	Not FDA approved
Fibroblast growth factor 3	Mutation of fibroblast growth factor receptor 3	Urine	Immunoassay	Investigational			Detects low-grade and stage Potential prognostic factor
Survivin	Detect survivin mRNA	Urine	RT-PCR	Investigational	64–94	93–100	Not FDA approved
Reg1	Reg-1 mRNA	Urine	Immunoassay	Investigational	81	81	Not FDA approved

(continued)

Table 6.1 (continued)

Test/marker	Marker detected	Specimen	Assay type	Manufacturer	Sensitivity (%)	Specificity(%)	Comments
Urinary bladder test (UBC) Cytokeratins 8, 18, 20	Detection of cytokeratins in bladder cancer.	Exfoliated cells in urine	UBC-Rapid and UBC-ELISA UBC-Rapid is a point-of-care assay	IDL Biotech, Sollentuna	21–87	72–97	Poor detection of low-grade and low-stage disease
			UBCELISA is a 2-h sand wich ELISA	Sweden			Low specificity after intravesical therapy
Prothymosin alpha soluble Fas	Nuclear protein	Urine	Immunoassay	Investigational	Unknown	91	Not FDA approved. Elevation with inflammation
Matrix metallo-proteinases	Ratio MMP/ TIMP-2	Urine sediment	ELISA	Investigational	99–100	81–88	Not FDA approved

Molecular signatures that could predict disease progression and response to therapy could result in more patients being treated with bladder sparing modalities, reducing the morbidity associated with radical cystectomy.

6.3 Molecular Signatures of Bladder Cancer: A Historical Perspective

The search for molecular signatures of bladder cancer began over a decade ago to improve upon pathologic staging of bladder tumors. An appropriate staging is critical to a sound decision making regarding bladder cancer management because of the involved morbidity and cost of treatment.

Traditional pathologic staging of bladder cancer relies on pattern recognition and nomenclature, which can be subjective and lead to inter- and intraobserver variability (Mitra et al. 2005). The absence of muscle in some biopsy specimens and inappropriate sampling of the tumor base in large, exophytic tumors can also affect pathologic staging. This has led to the widespread acceptance of restaging TURBT prior to making decisions regarding radical surgery. There is also no uniformly accepted definition for micro-invasion, which could alter prognosis. These factors, among others, have stimulated interest in molecular signatures that could predict tumor behavior.

Knudson hypothesized that two independent mutations in the same gene could result in childhood retinoblastomas (RB) (Knudson 1971). This gene was later called the retinoblastoma (RB) gene. It is located at chromosome 13q14 and its protein product is a nuclear phosphoprotein, which has a critical role in senescence, cell-cycle regulation, and apoptosis (Knudson 1978; Mitra et al. 2007). Several reports have suggested RB's role in bladder cancer recurrence, progression, and survival, yet it alone cannot predict the behavior of bladder cancer.

The p53 protein is widely accepted as the most crucial molecule involved in cell-cycle regulation in bladder cancer. p53 is a tumor suppressor protein encoded by the TP53 gene on chromosome 17p13.1 (Mitra et al. 2006). It inhibits phase-specific cell-cycle progression (G1-S) through the transcriptional activation of p21 (Livingstone et al. 1992; el-Deiry et al. 1993). p53's prognostic potential lies in its association with recurrence, progression, survival, and possible resistance to chemotherapy. There have been several reports detailing p53's role in bladder cancer, yet, like RB, it alone is not sufficient as a predictor of tumor behavior.

Some investigators have utilized a polymerase chain reaction based assay for the detection of microsatellite DNA, which are 2–6 base pair short tandem repeats identified throughout the human genome (Shirodkar and Lokeshwar 2008). Loss of heterozygosity (LOH) and microsatellite instability form the basis of detection in microsatellite analysis. Several locations of microsatellite alterations, thought to play a role in bladder cancer, have been identified involving chromosomes 4p, 8p, 9p and q, and 11p (Karran 1996; von Knobloch et al. 2001). While microsatellite analysis has been shown to have a high sensitivity throughout the various grades and stages of bladder cancers, it is technically

difficult, expensive, and a consensus panel of patterns have not been agreed upon for detection. Further studies are in process to identify ideal panels for diagnosis and prognosis.

Innovative high-throughput microarray analysis has provided a new tool for the identification of genomic and proteomic signatures of bladder cancer. Thus far, microarray analysis has shown the potential to differentiate molecular subgroups with different clinical outcomes but most studies have been limited by less than ideal numbers of archived samples and their retrospective nature (Modlich et al. 2004; Sanchez-Carbayo et al. 2006; Blaveri et al. 2005). Investigators are also using proteomic techniques to identify novel signatures of bladder cancer (Munro et al. 2006). The discovery of more sensitive and specific markers of bladder cancer will expand with gene expression analysis and proteomic technologies.

6.3.1 Proteomics

Proteomics is among the technologies of the future in early diagnosis, surveillance, and prognosis of cancer (Jain 2008) referred by some authors as "oncoproteomics." The techniques involve extraction of proteins from blood or tissue, and analysis with high-resolution 2D electrophoresis (2-DE), or surface-enhanced laser desorption ionization (SELDI), or electrospray ionization (ESI), or mass spectrometry (MS).

Celis and Gromov et al. have published extensively using proteomic technology in urothelial (UC) and squamous cell carcinoma (SCC) of the bladder (Gromov et al. 2002; Celis and Gromov 2003; Celis et al. 2002). In a recent article, Gromov et al. showed using 2-DE that FG/FDPs as a biomarker has an accuracy of 99%, 97%, 96% for patients withsuperficial, early invasive, and highly invasive bladder cancer respectively. The results are particularly encouraging in the detection of low-grade, noninvasive tumors. Other proteomic markers such as, TIF (Tissue interstitial fluid), S100 (SCC), and A-FABP (adipocyte-type fatty acid binding protein) are also under investigation as molecular signatures in bladder cancer (Wu et al. 2007a).

Monro et al. and Wittke et al. have demonstrated using SELDI and antibody arrays, respectively, to detect with high accuracy differences in patients with urothelial carcinoma compared to healthy individuals (Munro et al. 2006; Wittke et al. 2007; Wittke et al. 2005). Proteomic technology has a great potential to decipher the heterogeneity of bladder cancer, and will help with development of novel noninvasive methods for diagnosis and monitoring of both primary and recurrent bladder tumor.

These techniques remain expensive, with variable sensitivity, specificity, and reliability in detecting urothelial carcinoma.

While several signatures have been identified, prospective validation studies are necessary to determine the true clinical utility of genomic and proteomic signatures of bladder cancer.

6.4 Epigenetic Signatures of Bladder Cancer

6.4.1 Introduction to Epigenetics

The concept of epigenetics was first introduced by C.H. Waddington in 1939 when he investigated the development of wings in normal and mutant strains of drosophila. He described it as "the casual interactions between genes and their products, which bring the phenotype into being" (Esteller 2008; Waddington 1939). Epigenetics was later defined as heritable changes in gene expression that are not due to any alterations in the DNA sequence (Holliday 1987).

There are several proposed epigenetic mechanisms, the best known of which is DNA methylation (Esteller 2008). The revelation that substantial DNA hypomethylation was found in genes of cancer cells compared with their normal counterparts led to other studies that identified hypermethylated tumor suppressor genes (Feinberg and Vogelstein 1983; Greger et al. 1989; Sakai et al. 1991; Herman et al. 1994; Merlo et al. 1995; Herman et al. 1995). It is now evident that DNA methylation occurs in a complex chromatin network and is influenced by modifications in histone structure (Bernstein et al. 2007; Kouzarides 2007; Fraga et al. 2005; Seligson et al. 2005). Other proposed epigenetic mechanisms include ATP dependent remodeling, RNA interference, and changes in local and higher order conformation of DNA (Atkinson and Armstrong 2008).

DNA methylation serves a critical role in the control of gene activity and nuclear architecture (Esteller 2008). DNA methylation occurs in humans in cytosines that precede guanines, otherwise referred to as dinucleotide CpGs (Herman and Baylin 2003; Weber et al. 2007). Dinucleotide CpGs exist in CpG-rich regions known as CpG islands, which span the 5′ end of the regulatory region of many genes. DNA methylation has a variable role in tumorigenesis. The degree of hypomethylation of genomic DNA increases as some tumors progress from benign proliferations of cells to invasive cancers. Three mechanisms proposed to describe the contribution of hypomethylation to tumor development are the generation of chromosomal instability, reactivation of transposable elements, and the loss of imprinting (Feinberg and Vogelstein 1983). DNA hypermethylation can also alter the expression of tumor suppressor genes resulting in tumorigenesis.

Histones are the main protein component of chromatin. There are four core histones (H2A, H2B, H3, and H4), which assemble to form the nucleosome that winds around 146 base pairs of DNA. Modification of histones is another, less well-characterized, mechanism of epigenetic modification. Acetylation and methylation of histones can have a direct effect on gene transcription, DNA repair, DNA replication and chromosomal organization.

Micro RNAs are short, 22 nucleotide, noncoding RNAs that regulate gene expression by sequence specific base pairing in the 3′ untranslated regions of target mRNA. They exhibit their influence through RNA interference, resulting in mRNA degradation or inhibition of translation (He and Hannon 2004). This is yet another epigenetic mechanism by which cell proliferation, apoptosis, and differentiation are regulated.

6.4.2 Epigenetic Mechanisms of Bladder Cancer

As a result of the high rates of bladder cancer recurrence and multifocality, some have suggested that there is a clonal nature for urothelial cell carcinoma, with tumors originating from a primary transformed progenitor cell (Denzinger et al. 2006; Sidransky et al. 1992; Junker et al. 2005). While bladder cancer stem cells have been hypothesized, the idea in bladder cancer tumorigenesis remains controversial.

The POU homeodomain transcription factor OCT-4 is a key regulator of self-renewal and differentiation in embryonic stem cells (Rosner et al. 1990; Scholer et al. 1990). OCT-4 levels decrease with differentiation and loss of pluripotency (Niwa et al. 2000; Matin et al. 2004). Atlasi et al. investigated the expression of OCT-4 in bladder cancer. Using RT-PCR, western blotting, and immunohistochemical analysis, they evaluated OCT-4 expression in 32 tumors, 13 nontumor tissues from the margins of urothelial tumors, and 9 normal urothelial samples (Atlasi et al. 2007). The sensitivity and specificity of OCT-4 expression in their study was 96% and 66%, respectively, with no significant correlation between the expression level, grade, and stage of cancer. Immunohistochemical analysis revealed OCT-4's localization within the nuclei of tumor cells with low immunoreactivity in normal urothelial cells. This suggests the existence of cancer stem cells in urothelial carcinoma, yet further studies are necessary to prove their existence.

There is a pronounced difference in the biologic potential of superficial and muscle invasive bladder cancer, which is indicative of the genetic alterations in each subtype. Two molecular pathways to the development of transitional cell carcinoma of the bladder were described by Spruck et al. (Spruck et al. 1994). They examined 216 bladder tumors for chromosome 9, loss of heterozygosity, and mutations of the p53 tumor suppressor gene. Changes in Ta tumors included loss of heterozygosity of chromosome 9 that was observed in 34% with a p53 mutation frequency of 3%. CIS in dysplastic urothelium had a loss of heterozygosity frequency of 12% with a pronounced p53 mutation rate of 65%, which is comparable to the observed p53 mutation rate of 51% in muscle-invasive tumors. These results led the authors to conclude that p53 mutations in CIS and dysplasia may explain their risk of progression and that bladder cancer tumorigenesis may, therefore, proceed through two distinct genetic alteration pathways responsible for generating superficial tumors with differing morphologies and pathologies. Mutational inactivation of p53 is thought to play a critical role in bladder cancer. It has been demonstrated that mutational spots within the p53 gene occur at CpG dinucleotides that are methylated, implicating 5-methylcytosine, and therefore, DNA methylation, as an endogenous mutagen (Rideout et al. 1990; Tornaletti and Pfeifer 1995). Several other genes have been found to be frequently hypermethylated in bladder cancer including p16, E-cadherin, RASSF1, SOX9, LOX1, and LOX4.

SOX9 is a transcription factor that is expressed in chondrocytes, central nervous system, and the urogenital system (Huang et al. 2000; Soderstrom et al. 2002). Using specimens from 10 patients with bladder tumors, 11 bladder cancer cell lines, and 101 primary bladder tumor specimens, they identified promoter DNA hypermethylation of SOX9. Hypermethylation was associated with tumor grade

and survival. The authors concluded that SOX9 hypermethylation could be used to stratify patients diagnosed with bladder cancer.

Lysyl oxidase is an amine oxidase that oxidizes primary amine substrates to reactive aldehydes (Wu et al. 2007b). It is well known for its role in the extracellular catalysis of lysine derived cross-links in fibrillar collagen and elastin (Kagan and Li 2003). Wu et al. identified 59 candidate hypermethylated genes, including two novel members of the LOX amine oxidase family (LOXL1 and LOXL4) that were hypermethylated in bladder cancer. LOXL1 and LOXL4 were frequently hypermethylated and lost expression in primary bladder tumors. LOXL1 and LOXL4 antagonize Ras activation of the ERK signaling pathway in bladder cancer cells suggesting that they can serve as tumor suppressor genes in bladder cancer.

Thus far, several genes have been detected in bodily fluids in patients with bladder cancer. DAPK, E-cadherin/CDH1, RARβ, RASSF1A, and p16/CDKN2A have been detected within urine, while p14ARF and p16/CDKN2A have been detected in plasma (Chan et al. 2002; Dominguez et al. 2003; Thievessen et al. 2003).

Epigenetics therapy of bladder cancer may one day be feasible. Scientists have begun to evaluate the role of DNA methylation inhibitors, such as 5-azacytidine, in reactivating silenced genes in human cancers (Liang et al. 2002). Investigators from the University of Southern California compared changes in gene expression using microarrays in fibroblasts and a human bladder cancer cell line after treatment with 5-aza-2′-deoxycytidine, one of the most potent inhibitors of DNA methylation (Jones and Taylor 1980). More genes were induced in tumorigenic cells compared to fibroblasts. This suggests that methylation inhibitors can reverse epigenetic changes in bladder cancer cells.

6.5 Nuclear Matrix Protein 22 (NMP22)

The nuclear matrix acts as an internal scaffold for the nucleus, providing structural integrity and playing a role in DNA replication, gene expression, and mitosis (Pardoll et al. 1980). Nuclear structural alterations are so prevalent in cancer cells that they are commonly used as markers of transformation for many types of cancer (Konety et al. 1998; Khanuja et al. 1993; Keesee et al. 1994; Donat et al. 1996). NMP22 is a component of the nuclear mitotic apparatus that is not cancer-specific and is found in all human cells. It is associated with the mitotic spindle and is thought to facilitate proper segregation and distribution of chromosomes during cell division (Yang et al. 1992). NMP22 was found to be present in greater concentrations in bladder cancer cell lines compared to normal urothelial cells and some have suggested that it correlates with the degree of differentiation (Keesee et al. 1996; Di Carlo et al. 2003).

The first commercially available test for NMP22 was developed by Matritech Inc. as a quantitative immunoassay, designed to detect complexed or fragmented forms of the nuclear mitotic apparatus in urine, using monoclonal antibodies. The newest generation (BladderChek) is a point-of-care assay that relies on qualitative immunochromographic detection of the protein

NMP22's reported sensitivity and specificity in the detection of all bladder cancers (primary and recurrent) ranges from 44% to 100% and 55% to 98%, respectively. The mean reported sensitivity and specificity was estimated to be approximately 67% and 74% by Konety, compared to 48% and 96% for cytology (Konety 2006). Investigators from the Cleveland Clinic recently addressed the question of whether or not NMP22 could enhance the positive predictive value of atypical cytology in groups of patients being screened or monitored for recurrence (Raina et al. 2008). Using NMP22 values with cutoffs of >10 U/mL for screened high-risk patients and >6 U/mL cutoff for those being monitored for recurrence, indexing atypical cytology results with NMP-22 increased positive predictive values. Considering all atypical cytology as positive, and indexing the result with NMP22, the specificity and PPV could be increased to 93% and 85%, respectively. Mansoor's report examining the role of NMP-22 combined with cytology in follow-up surveillance of recurrent superficial TCCa supports the Cleveland Clinic group's findings (Mansoor et al. 2008). NMP22 complemented cytology by its higher sensitivity for low-grade lesions.

The initial NMP22 assay was approved in 1996 for the diagnosis of bladder cancer recurrence in 1996 and for the diagnosis of suspected bladder cancer in 2000 (Konety 2006). The newer generation BladderChek point-of-care assay was approved for diagnosis in 2002 and for screening in 2003.

NMP22's strengths as a molecular signature of bladder cancer include its practicality, ease of use, cost effectiveness, and lack of expert/subjective interpretation (Nguyen and Jones 2008; Zippe et al. 1999). Its disadvantages include a lack of a universally accepted cutoff value, poor sensitivity for smaller tumors that typically recur, and suboptimal sensitivity when used alone for monitoring for disease relapse. One of the greatest features of both NMP22 assays is that values are not affected by bacillus Calmette–Guérin (BCG) therapy changes, which are limiting in cytology. While one would expect the clinical use of NMP-22 to be widespread, its varying sensitivity and false-positive results in the presence of benign urologic conditions has limited its utility (Shirodkar and Lokeshwar 2008).

6.6 Bladder Tumor Antigen

The most recent versions of the bladder tumor antigen (BTA) assays measure complement factor H-related protein in urine. Complement factor H-related protein is produced by human bladder cancer cells and is thought to enhance degradation of other complement factors (Malkowicz 2000). There are two commercially available versions of the BTA assay, BTA TRAK (Polymedco Inc, Cortlandt Manor, NY) and BTA stat (Bion Diagnostic Sciences Inc, Redmond, WA). BTA TRAK is an ELISA, whereas BTA stat test can be performed at the point-of-care in 5 min, without pretreatment of the voided urine sample (Black et al. 2006; Sarosdy et al. 1997).

Varying sensitivities and specificities have been reported in the literature. BTA stat sensitivities have ranged from 53% to 89% compared to 17% to 78% for the

BTA TRAK assay (Konety 2006). Specificity has ranged from 54% to 93% for BTA stat compared to 51% to 95% for BTA TRAK.

The FinnBladder study began in 1997 with the goal of accruing patients with a history of bladder cancer under surveillance. A recent update of the multi-institutional FinnBladder study was reported (Raitanen 2008). Voided urine samples of 501 patients were obtained prior to cystoscopy and split for culture, cytology, and BTA stat testing. The overall sensitivities and specificities for the BTA stat Test and cytology were 56.0% and 19.2% and 85.7% and 98.3%, respectively.

Jovanovic et al. examined the use of the BTA-Stat test in patients with suspected upper tract TCC (Jovanovic et al. 2007). Thirty-five patients with upper tract tumors (22 renal pelvic, 13 ureteral) were compared to 35 controls without evidence of malignancy. Selective upper tract and voided urine were examined. Ureteral sensitivity of BTA-stat was 62.9% compared to 57.1% for cytology. Voided urine BTA sensitivity was 51.4% compared to 48.6% for cytology. In their study, sensitivity depended upon histology of tumor with improved detection in patients with T2–T4 disease.

Babjuk concluded that cytology was better than BTA and UBC in a prospective study comparing the results of cytology, BTA trak, and UBC in individuals with pTapT1 bladder cancer (Babjuk et al. 2008). Urinary cytology fulfilled requirements for an adjunctive method to cystoscopy but BTA and UBC tests have a low sensitivity in the detection of bladder cancer recurrence and cannot be used routinely to reduce the number of cystoscopies during follow-up.

BTA TRAK was approved by the FDA for diagnosis in 1997 followed by BTA stat in 1998 (Konety 2006). While both have been demonstrated to have improved sensitivity over cytology, their specificity can be decreased by a history of a urinary tract foreign body, bowel interposition segment, other genitourinary cancer, and benign genitourinary conditions. False-positives have occurred in individuals with hematuria, proteinuria, infection, stones, and inflammation. Like NMP-22, its clinical utility has been limited by these factors.

6.7 Soluble Fas

Cytotoxic T-cell mediated and natural killer cell mediated apoptosis against tumor cells are aided by the interaction of Fas and Fas ligand. Tumor cells evade apoptosis by expressing sFas (circulating soluble Fas [sFas]). Mizutani et al. (Mizutani et al. 2001) examined the level of sFas in 169 patients with UCC using ELISA and concluded that it correlated with both disease progression and increase in tumor grade, and the elevated serum sFasL is associated with a greater risk of disease progression and recurrence . The overall sensitivity of sFas is reported to be greater than 75%. Svatek et al. demonstrated that sFas is an independent predictor of bladder cancer recurrence and invasiveness in patients with history of Ta UCC. When sFas was compared to NMP-22, sFas was found to be more specific (Svatek et al. 2006).

sFas and sFasL are novel molecular signatures that hold great potential in prognosis and possible surveillance of bladder cancer.

6.8 Fluorescence In situ Hybridization

Fluorescence in situ hybridization (FISH) is a technique that uses fluorescently labeled DNA probes to assess cells for genetic alterations (Halling and Kipp 2008). There are two general types of FISH probes: chromosome enumeration probes, which hybridize pericentromeric regions of chromosomes, enumerate the number of chromosomes in a cell, and locus-specific indicator probes, which hybridize to genes of interest. The number of copies of a given probe target are present in the nucleus of a cell are then estimated under microscopic examination.

The UroVysion (Abbott Laboratories) FISH assay is performed on exfoliated cells in urine. It detects aneuploidy for chromosomes 3, 7, and 17, and loss of locus 9p21. Its reported sensitivity ranges from 60% to 82% while its specificity has been reported to be between 79% and 96% (Sarosdy et al. 2002; Halling et al. 2002; Friedrich et al. 2003; Halling et al. 2000; Junker et al. 2006). Junker et al. reported their results with the UroVysion FISH assay regarding tumor stage and grade. The sensitivity of FISH and cytology were 36.1% and 15% in pTa, 65.2% and 25.7% in pT1, 100% and 66.7% in pT2-3 tumors, respectively. Concerning tumor grade, sensitivity was 37% and 14% in G1, 65.4% and 40% in G2, 91.7% and 50% in G3 tumors, for FISH and cytology, respectively.

The UroVysion FISH assay received FDA approval for the detection of recurrent bladder cancer in 2002. Approval was granted for the use of FISH in the detection of bladder cancer in individuals with hematuria, without a history of bladder cancer in 2005 (Halling and Kipp 2008).

FISH has been demonstrated to predict future recurrences in the presence of a negative cystoscopy (Skacel et al. 2003; Yoder et al. 2007). Investigators at the Cleveland Clinic followed 250 patients with urine cytology, concurrent multitarget FISH, and cystoscopic examination for recurrence. Of 81 cases (32.4%) with FISH-positive results, tumor recurrence developed in 60 (74.0%). Of 169 (67.6%) FISH-negative cases, recurrent urothelial carcinoma developed in 22 (13.0%). Mian et al. used FISH to stratify individuals into low- and high-risk groups of recurrence and progression based on their chromosomal pattern (Mian et al. 2006). 33.3% of patients with a low-risk pattern recurred (mean of 30.8 months) compared with 66.7% of those with a high-risk pattern (mean of 17.6 months). They concluded that patients with high-risk patterns had a shorter disease free survival and higher rate of progression.

More recently, Gudjonsson et al. (Gudjonsson et al. 2008) using UroVysion assay in 159 cases had an overall 30% sensitivity for biopsy-proven recurrence and 70% sensitivity for high-risk tumors (pT1 and CIS) and specificity was 95%. In metanalysis by Hajdinjak et al. (Hajdinjak 2008), comparing UV to cytology, the pooled sensitivity and specificity of all 14 studies involving 2477 tests in 35% UCC was 72% (69%–75%) and 83% (82%–85%), respectively. Cytology data had an overall sensitivity and specificity of 42% (38%–45%) and 96% (95%–97%), respectively. For *only* high-grade disease, UV and cytology had a sensitivity of 86% and 61%, respectively. Although results are promising, UroVysion cannot replace cystoscopy at this time as a sole surveillance tool for nonmuscle-invasive, but has some utility for detecting CIS (Gudjonsson et al. 2008).

FISH's disadvantages include its expense, the need for trained personnel, and need for intact cells. The clinical use of FISH remains lower than expected, most likely due to its cost and limited sensitivity in detecting low-grade tumors which can be missed by cytology (Vrooman and Witjes 2009). However, it appears to be a useful adjunct to cytology and cystoscopy in the follow-up of bladder cancer.

6.9 ImmunoCyt

Utilizing three fluorescently labeled monoclonal antibodies, ImmunoCyt detects two mucin-like proteins and a high molecular weight form of carcinoembryonic antigen reported to be specific cellular markers of bladder cancer in exfoliated urothelial cells in voided urine (Fradet and Lockhard 1997). Cells are then scored for fluorescence under microscopic examination.

Reported sensitivities have ranged from 39% to 86%, while the specificity has ranged from 73% to 84%. ImmunoCyt was approved by the FDA as an adjunct test for bladder cancer in 2000.

The efficacy of ImmunoCyt for monitoring superficial bladder cancer in a multicenter trial was reported by Messing et al. (Messing et al. 2005). The sensitivity and specificity were 81% and 75% for ImmunoCyt, respectively, compared with 23% and 93% for cytology. The improved sensitivity of ImmunoCyt was seen particularly in lower stage and lower-grade tumors.

The value of the ImmunoCyt/uCyt+ test compared to cytology in the detection and follow-up of carcinoma in situ was reported in 2005 (Mian et al. 2005). In a cohort of 35 patients receiving BCG therapy, both ImmunoCyt and cytology were positive at the time of diagnosis. Both tests detected recurrences, with sensitivities varying at different time points between 50% and 100%. Combining ImmunoCyt and cytology resulted in a sensitivity of 100%. ImmunoCyt's specificity decreased to 56% at the second surveillance cystoscopy, performed at 6 months. It improved to 89% in those receiving maintenance BCG therapies. The specificity of cytology was 88%–100%.

ImmunoCyt has several disadvantages including a high level of interobserver variability and an up to 17% rate of test failure due to inadequate cellularity of the specimens (Tetu et al. 2005; Vriesema et al. 2001). ImmunoCyt limitations have limited its use as a stand alone clinical marker.

6.10 BLCA-4

Understanding that nuclear structural alterations were present in cancer cells, investigators from the University of Pittsburgh examined normal and tumor bladder tissue samples from 24 patients undergoing surgery for bladder cancer (Getzenberg et al. 1996). Their nuclear matrix protein composition was analyzed

utilizing a computer-based gel analysis system. There were differences in nuclear matrix composition between areas of cancer and benign urothelium from the same bladder. Six nuclear matrix proteins were identified that were present in all of the tumors, yet absent in the adjacent normal tissue. Five of these nuclear matrix proteins were identified in three human bladder cancer cell lines. The most abundant bladder cancer–specific protein, BLCA-4, was sequenced and utilized to generate antipeptide antibodies. The gene that encodes BLCA-4 has been identified and sequenced, having homology with the ELK-3 gene, a member of the ETS transcription factor family.

BLCA-4 levels in urine were measured in urine obtained from patients with bladder cancer, benign urologic conditions, and prostate cancer using a second generation sandwich immunoassay (Van Le et al. 2005). A prospectively determined cutoff value of 0.04 absorbance units (OD) resulted in an assay sensitivity of 89% and specificity of 95%, which corroborated previous findings that cystitis does not correlate with elevated BLCA-4 levels in patients with spinal cord injury (Konety et al. 2000).

BLCA-4 may also prove one day to be a novel target for the treatment of bladder cancer. Myers-Irvin et al. examined the functional aspects of BLCA-4 and its potential role in bladder cancer pathobiology (Myers-Irvin et al. 2005). The authors stably transfected human urothelial cells with the gene encoding BLCA-4. After confirming expression, they found that overexpressing clones exhibited a 4.3-fold greater proliferation rate, and microarray analysis revealed interleukin(IL)-1α, IL-8, and thrombomodulin upregulation. Through the action of these substances, BLCA-4 may enhance malignant urothelial cells proliferation and invasion, in addition to playing a role in angiogenesis.

BLCA-4 is currently in development. Investigators are in the process of optimizing assay conditions for possible widespread clinical use. Additionally, studies are being performed evaluating BLCA-4 levels in individuals at higher risk for bladder cancer.

6.11 Telomerase

Telomeres comprises noncoding, repetitive DNA at the ends of chromosomes, featuring the nucleotide repeat TTAGGG in humans. The telomerase holoenzyme helps mediate chromosomal integrity by enzymatically maintaining telomeric DNA that would otherwise be progressively lost or degraded during repeated rounds of cell division, particularly in cancer cells (Cong et al. 2002). Most human cells do not express telomerase activity and lose telomeric DNA with each cell division (Kim et al. 1994).Telomerase, an enzyme present in greater than 80% of all cancer cells, has the potential to be a successful bladder tumor marker (Bennett 2008).

Telomerase is measured in exfoliated cells in urine by telomerase repeat amplification protocol or human telomerase reverse transcriptase assays (Alvarez and Lokeshwar 2007). The reported sensitivity of telomerase has ranged from 46% to

92% with a specificity ranging from 69% to 99% (Konety 2006). Variation is most likely due to the low stability of telomerase and human telomerase reverse transcriptase mRNA in urine.

Zachos et al. recently reported their findings from a prospective study to determine the correlation between expression of telomerase reverse transcriptase before and after BCG therapy with the clinical outcome of high-risk SBC patients treated with TURBT and adjuvant intravesical BCG (Zachos et al. 2009). They wanted to determine whether expression of telomerase reverse transcriptase, measured with immunohistochemistry, is associated with recurrence free survival (RFS) or development of invasive disease. Post-BCG telomerase reverse transcriptase expression was statistically significantly lower than pre-BCG expression. Pre-BCG nucleolar staining in more than 75% of cells was associated with decreased RFS, while post-BCG staining in more than 50% of the cells was associated with worse RFS and development of invasive disease. In multivariate analysis, post-BCG expression was independently associated with RFS and development of invasive disease. Immunohistochemical evaluation of telomerase reverse transcriptase may help define patients that will fail intravesical therapy and benefit from early radical cystectomy.

Telomerase's weaknesses as a molecular marker of bladder cancer stem from its low sensitivity and specificity for surveillance, poor reproducibility, variable stability, and potential for false-positives in the presence of urinary tract infections or urolithiasis. Telomerase as a marker of bladder cancer is not FDA approved and is currently in development.

6.12 Fibronectin

Fibronectin is a structural glycoprotein widely distributed in cells, plasma, and the extracellular matrix (Li et al. 2008). Proteases released by invasive and/or metastatic tumors degrade components of the extracellular matrix (Redwood et al. 1992; Katayama et al. 1989). Numerous reports have indicated fibronectin as a marker of bladder cancer. Bladder tumor fibronectin (BTF), an automatic assay for the detection of urinary fibronectin, has a reported sensitivity of 78%–85% and a specificity of 80%–91.3% in detecting bladder cancer (Mutlu et al. 2003; Menendez et al. 2005).

Liao et al. sought to evaluate whether the presence of urinary fibronectin predicts residual tumor load after TURBT (Li et al. 2008). Urine samples were collected from 167 consecutive patients with suspected bladder cancer admitted for TURBT. Specimens were obtained before and after surgery. BTF was analyzed with a solid-phase chemiluminescent immunometric test. Creatinine in urine was also determined and the BTF/creatinine ratio was calculated. The BTF sensitivity and specificity in determining the presence of residual tumor was 91.4% and 87.8% compared to 89% and 85.6%, using the BTF/creatinine ratio.

Bladder tumor fibronectin is an investigational assay and has not yet been approved by the FDA.

6.13 Survivin

Survivin is a member of the inhibitor of apoptosis proteins family of molecules. It has been reported to control mitotic progression and induce gene expression, altering tumor cell invasiveness (Li and Ling 2006; Altieri 2004). Survivin's expression in malignant epithelium along with mRNA and protein detection in urine underlies its potential as a molecular marker of bladder cancer.

Smith was the first to report survivin's utility as a bladder cancer marker (Smith et al. 2001). Survivin protein and mRNA were detected in the urine of all 46 patients with bladder cancer involved in the study. Survivin was not detected in the urine samples of 32 of 35 patients treated for bladder cancer, and had negative cystoscopy results. None of the healthy volunteers or patients with prostate, kidney, vaginal, or cervical cancer had detectable survivin in urine samples. Survivin was detected in 3/30 with benign genitourinary conditions.

Shariat et al. analyzed voided urine from 117 patients with bladder cancer undergoing cystoscopy and 92 controls for levels of Survivin and compared these results to cytology and NMP22 in the detection of bladder cancer (Shariat et al. 2004). The overall sensitivity, specificity, and positive and negative predictive values of Survivin for the diagnosis of bladder cancer (64%, 93%, 92% and 67%, respectively) were superior to those of NMP22 and cytology. Survivin had the highest specificity and positive predictive value for the detection of bladder cancer across each tumor stage and grade.

A follow-up study involving patients who underwent radical cystectomy correlated survivin overexpression with advanced pathologic stage, metastases to lymph nodes, lymphovascular invasion, number positive lymph nodes, percent-positive lymph nodes, disease recurrence, disease progression, and bladder cancer–specific mortality (Shariat et al. 2007). They found a high concordance rate of Survivin expression status between matched radical cystectomy and metastatic lymph node specimens (84%).

Survivin is currently an investigational marker and has not received FDA approval. While preliminary studies have proposed an improved sensitivity and specificity of Survivin compared to NMP22 and cytology, a significant amount of urinary survivin is contributed by the normal prostate (Davies et al. 2005). This may limit the application of this marker for bladder cancer, as there may not be a way to accurately estimate the prostatic contribution of survivin, especially among men with prostates of varying size.

6.14 Fibroblast Growth Factor Receptor 3 (FGFR3)

The fibroblast growth factor receptors (FGFRs) make up a family of four high affinity cell surface associated receptors that have been highly conserved throughout the evolution (Johnson and Williams 1993). They have a common structure, consisting of an extracellular domain that includes an amino terminal hydrophobic signal peptide followed by three immunoglobulin-like domains, a hydrophobic transmembrane

domain, and an intracellular tyrosine kinase domain. Mutations of FGFR3 in bladder cancer have been shown to be strongly associated with tumor of low-grade and stage (Knowles 2007; van Rhijn et al. 2002). Others have reported FGFR3's detection in urine, making it a potential marker of disease (van Rhijn et al. 2003; Rieger-Christ et al. 2003).

FGFR3 as a marker of bladder cancer is currently in development. It strength lies in its ability to detect low-grade tumors and potential as a prognostic factor.

6.15 Reg 1

Significant overexpression of Reg-1 mRNA and protein was previously reported to be associated with tumor progression, and poor prognosis in patients with colon, liver, and gastric cancer (Macadam et al. 2000; Yonemura et al. 2003). It has been suggested that the regenerative response resulting from Reg-1 expression may be responsible for inhibition of apoptosis.

Investigators from the Spanish National Cancer Research Center reported their experience with a 2D gel-based proteomic approach (2D-DIGE) coupled with mass spectrometry and database interrogation to explore the urine for markers of bladder cancer (Orenes-Pinero et al. 2007). They identified Reg-1 as a protein differentially expressed between bladder cancers and controls. They confirmed this association with immunoblotting of bladder cancer cell lines and immunohistochemistry of tissue samples. The association of Reg-1 with staging and clinical outcome was revealed by an independent series of bladder tumors in tissue microarrays. Furthermore, Reg-1 expression in urine was quantified with an ELISA and was able to discriminate patients with bladder cancer from controls with a sensitivity of 81.3% and specificity of 81.2%.

6.16 Cytokeratin/Urinary Bladder Cancer Test

Cytokeratins are intermediate filament proteins characteristic of epithelial cells. Twenty different cytokeratin isotypes have been described. The urothelium shows alterations in the expression and configuration of cytokeratin isotypes related to stratification and differentiation. The urinary bladder cancer (UBC) test detects fragments of cytokeratins 8 and 18 in increased concentrations in the urine of patients with bladder cancer (Mungan et al. 2000). There are currently two UBC tests, an ELISA and a rapid assay.

May et al. compared cytology, FISH, and the cytokeratin-detection test of UBC in routine clinical practice (May et al. 2007). The overall sensitivities of FISH, UBC-ELISA, and cytology were 53.2%, 40.3%, and 71.0%, respectively. In the 104 patients without TCC, the specificity of FISH, UBC-ELISA, and cytology was 74.0%, 75.0%, and 83.7%, respectively. Receiver operating characteristic analysis

showed an area under the curve for FISH, UBC, and cytology of 0.636, 0.577, and 0.773, respectively. Only cytology and FISH were significantly predictive of a TCC finding on histologic examination ($p < 0.001$ and $p = 0.003$, respectively).

Other cytokeratins have been suggested for use in bladder cancer screening, particularly cytokeratins 20. Bhatia et al. reported their experience with cytokeratin 20 as a marker of bladder cancer (Bhatia et al. 2007). Urine cytology smears were retrieved for 14 cases positive for bladder cancer, 14 negative, and five atypical cytology cases. They were stained with a monoclonal antibody for CK20. All benign cases were negative except for a few cases in which the umbrella cells were weakly to moderately positive. In all five cases of atypical, urine cytology the atypical cells stained positive with the antibody. These cases were later confirmed as TCC on histopathology of bladder wall biopsy. These results suggest a role for cytokeratin 20 as a marker of bladder cancer.

The UBC is currently an investigational assay and is not FDA approved. It has several disadvantages including the occurrence of false-positives in the presence of infection or urolithiasis.

6.17 Prothymosin Alpha

Prothymosin alpha is a nuclear protein found in essentially all mammalian tissue (Manrow et al. 1991). While its exact function is unknown, investigators have suggested a role in cell proliferation because of its presence in rapidly dividing cells and decrease in cells forced to subsist in a stationary phase (Fraga et al. 1993; Sburlati et al. 1993).

Tzai et al. performed prothymosin alpha immunohistochemical staining on 16 transitional cell carcinoma bladder specimens and three normal tissue specimens from adjacent areas free of tumor (Tzai et al. 2006). Reactivity was higher in cancer specimens, suggesting a role in bladder tumorigenesis, leading to the evaluation of urinary prothymosin alpha levels. Urine was obtained from 238 individuals with a history of urothelial cell carcinoma, 22 with nonurothelial tumors, and 211 individuals without evidence of cancer. Urine prothymosin alpha levels were persistently elevated when residual tumor was present after treatment. Inflammation as a result of infection caused a temporary elevation of urine prothymosin alpha levels that decreased with antibiotic therapy. The authors also found that prothymosin alpha was overexpressed in patients with urothelial cell carcinoma but not in patients with other tumors of the genitourinary tract.

6.18 Matrix Metalloproteinases

Degradation of the basement membrane and extracellular matrix is a well-known prerequisite for tumor invasion. Matrix metalloproteinases (MMPs) belong to a group of extracellular matrix degradation enzymes whose balance with tissue

inhibitor of metalloproteinases (TIMPs) maintains connective tissue homeostasis in normal tissue (Nelson et al. 2000). In neoplastic conditions, there is a perceived imbalance between MMPs and TIMPs, resulting in an excess of degradation leading to tumor invasion (Ray and Stetler-Stevenson 1994).

Eissa et al. assessed the usefulness of MMP-2 and MMP-9 levels in relation to TIMP-2 urine levels as a means of diagnosing bilharzial bladder cancer (Eissa et al. 2007). Voided urine samples were collected from individuals with bladder cancer, benign urologic conditions, and normal controls. Urine sediment was used for cytology while the supernatant was used for estimation of MMPs and TIMP-2 by ELISA and gelatin zymography. The sensitivity and specificity of cytology for identifying patient with bladder cancer in their study was 75% and 91.66%, respectively, but fell to 60% and 91.66% when looking at individuals with low-grade disease. The MMP-2/TIMP-2 ratio yielded a sensitivity and specificity of 99% and 81.7%, respectively, in all individuals with bladder cancer and improved the detection rate in low-grade disease with a sensitivity and specificity of 100% and 81.7%, respectively. The MMP-9/TIMP-2 ratio performed even better in low-grade tumors with a sensitivity of 100% and specificity of 88%. The MMP-9/TIMP-2 ratio was found to be 1.72-fold higher in bilharzial bladder cancer compared to nonbilharzial cancer ($p<0.05$). In contrast, urinary MMP-9 median level and MMP-2/TIMP-2 median ratio were higher in SCC (1.31-fold and 1.27-fold, respectively) than in TCC ($p<0.05$).

6.19 Hyaluronic Acid and HYAL-1 Hyaluronidase

Hyaluronic acid (HA), also referred to as hyaluronan, is a nonsulfated glycosaminoglycan that has been shown to be elevated in various tumors and promote tumor metastasis (Tammi et al. 2008). HA levels are elevated in a variety of tumors including those involving the breast, ovary, colon, and prostate. HA promotes the aggressive spread of cancer using several mechanisms including the following:

1. Upregulation when the epithelial cells undergo malignant transformation. HA supports cell proliferation, inhibits apoptosis, maintains intercellular space to facilitate nutrient diffusion, and enhances cell locomotion to stimulate invasion.
2. Enhancing epithelial/mesenchymal transition, releasing cells from their epithelial compartment for invasion.
3. Coating cancer cells and shielding them from the effect of cytoxic T-lymphocytes.
4. Stimulation of endothelial cell proliferation.

Lokeshwar and colleagues at the University of Miami were the first to critically evaluate HA's role in bladder cancer and reported its 83.1% sensitivity and 90.1% specificity in detecting bladder cancer, using a urine ELISA for HA and hyaluronidase (Lokeshwar et al. 2000). Investigators from this group also reported that the inhibition of HA synthesis in bladder cancer cells can inhibit tumor growth, invasion, and angiogenesis (Golshani et al. 2008).

Kramer et al. reported their findings after examining whether HA and HYAL-1—type hyaluronidase could predict progression to muscle-invasion and recurrence among patients with nonmuscle-invasive bladder cancer (Kramer et al. 2010). Using tissue microarrays prepared from archival bladder cancer specimens from 178 patients from collaborating institutions, HA and HYAL-1 expression were evaluated by immunohistochemistry. Follow-up information (mean 69.5 months) was available for 111 patients with nonmuscle-invasive bladder cancer. Utilizing univariate and multivariate analysis, the association of HA and HYAL-1 expression with recurrence and progression was examined. The authors reached several conclusions. HA and HYAL-1 expression increased with tumor grade, stage, and multifocality, yet only HYAL-1 staining was significantly associated with muscle-invasion and recurrence in multivariate analysis. This study had several limitations including its retrospective nature, limitation to two institutions, and the fact that some of the patients involved were treated with intravesical therapy after undergoing TURBT, which could have impacted their propensity to recur or progress.

The previously mentioned findings regarding HA and HYAL-1 are significant for a number of reasons and could have clinical utility. Assays for HAL and HYAL-1 could aid in the identification and stratification of individuals with bladder cancer, especially those with high grade T1 disease. Some have advocated early cystectomy for this group prior to pathologic evidence of muscle-invasion. HYAL-1, along with other molecular markers could aid in clinical decision making. It also could have a role in targeted therapy for BCG resistant nonmuscle-invasive disease.

6.20 Urothelial Cell Carcinoma Versus Squamous Cell Carcinoma

Urothelial cell carcinoma represents the predominant subtype of bladder cancer, accounting for greater than 90% of cases diagnosed in the United States. Squamous cell carcinoma is the second most common subtype of bladder cancer diagnosed in the United States but accounts for more cases in regions of the world where schistosomiasis is endemic. Urothelial cell carcinoma and squamous cell carcinoma behave differently, leading some to postulate different natural histories of UCC versus SCC.

Abdulamir et al. recently reported their findings comparing the molecular profiles of UCC and SCC (Abdulamir et al. 2009). They explored the role of p53, p16, bcl-2, ki-67, c-myc, Rb, and EGFR in schistosomal bladder tumors versus non-schistosomal bladder tumors and found distinct molecular profile of tumor development and progression, which could prove useful in tailoring therapy.

6.21 Conclusion

The number of patients presenting for with hematuria continues to comprise a significant portion of patients who seek urologic evaluation (Sangar et al. 2008). While there are a myriad of causes of both gross and microhematuria, bladder

cancer remains the most feared etiology of hematuria. Cystoscopy and cytology have persisted as the main tools in the armamentarium of urologists to workup patients with hematuria and diagnose bladder cancer. These examinations have accounted for significant healthcare costs with limited success in determining which individuals have cancer. Screening high-risk patients for the presence of molecular signatures could potentially identify those likely to have cancer and avoid the cost and morbidity of cystoscopy in those unlikely to have disease.

The discovery of molecular signatures of bladder cancer has grown exponentially with genomic and proteomic advancements. While some have proven clinical utility, a number are in need of multi-institutional trials to validate initial reports. There are point-of-care methods, useful in the follow-up of patients with a history of bladder cancer, and other assays requiring dedicated laboratory expertise. Widespread clinical use has been limited by varying cutoff values and altered sensitivity and specificity in the presence of benign urologic conditions. There are several exciting potential markers in the development including BLCA-4, Survivin, and FGFR3 among others.

While no marker to date has replaced cystoscopy with biopsy as the gold standard for the diagnosis of bladder cancer, improvements in microarray technology and DNA microsatellite analysis may lead to the discovery of better molecular signatures and potential targets of therapy, ultimately mitigating the cost and morbidity associated with contemporary bladder cancer management.

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Chapter 7
Economics of Bladder Cancer Diagnosis and Surveillance

Joshua Sleeper and Yair Lotan

Abstract Bladder cancer is the fourth most common cancer in men and the tenth most common in women in the United States with over 500,000 people currently living with a diagnosis of the disease. Recurrence rates range from 30% to 70% with progression of disease in 10%–30% even with successful resection. Low-grade disease tends to be a relapsing condition requiring lifelong monitoring. The relatively high prevalence of bladder cancer, high percentage of recurrence, and risk of progression leads to rigorous surveillance protocols and the intense utilization of resources resulting in bladder cancer being among the top five costliest cancers to treat.

With national healthcare expenditures growing at unsustainable levels, medical decision-making is increasingly being affected by economic concerns. Healthcare costs and availability are a major issue in the current political environment. The procurement of new technologies, pharmaceutical development, formulary choices by hospitals, and patients' ability or willingness to take medications are all being driven by financial issues. Institutions and healthcare plans face limited budgets, which require efficient utilization of resources. Thus, choices must be made between various diagnostic and treatment options available while considering both the efficacy and the cost. Inevitably cost-effectiveness analyses will have a greater role in the options available to clinicians and patients as the cost of new treatments and drugs rise faster than their marginal benefit.

Diagnosing and treating bladder cancer exacts a high cost on society that is likely to continue to grow along with the aging population. There is potential to reduce cost of care for bladder cancer by reducing costs associated with surveillance and earlier detection of the disease. Introduction of modifications to current care requires careful evaluation of both costs and benefits.

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7.1 Introduction

Bladder cancer is the fourth most common cancer in men and the tenth most common in women in the United States with over 500,000 people currently living with a diagnosis of the disease (Jemal et al. 2008). An estimated 68,810 new diagnoses in the United States were projected for 2008 (Jemal et al. 2008). Of these new diagnoses, approximately 70% will be noninvasive at diagnosis. Recurrence rates range from 30% to 70% with progression of disease in 10%–30%, even with successful resection (Messing 2007). Low-grade disease tends to be a relapsing condition requiring lifelong monitoring. The relatively high prevalence of bladder cancer, high percentage of recurrence, and risk of progression leads to rigorous surveillance protocols and the intense utilization of resources resulting in bladder cancer being among the top five costliest cancers to treat (Botteman et al. 2003).

With national healthcare expenditures growing at unsustainable levels, medical decision-making is increasingly being affected by economic concerns. Healthcare costs and availability are a major issue in the current political environment. The procurement of new technologies, pharmaceutical development, formulary choices by hospitals, and patients' ability or willingness to take medications are all being driven by financial issues. Institutions and healthcare plans face limited budgets, which require efficient utilization of resources. Thus, choices must be made between various diagnostic and treatment options available while considering both the efficacy and the cost. Inevitably cost-effectiveness analyses will have a greater role in the options available to clinicians and patients as the cost of new treatments and drugs rise faster than their marginal benefit (Meropol and Schulman 2007; Bach 2009). A major concern with new treatments is that they become less cost-effective than the older treatments when the cost rises faster than improvement in efficacy (Bach 2009). One consequence of reduced cost-effectiveness includes efforts by healthcare plans to make policy changes that limit access to treatments or reduce payments. This can lead to clinicians altering their practice patterns by reducing utilization of the affected treatment and increasing the use of another (Weight et al. 2008).

This chapter will review the economics of the diagnosis and surveillance of bladder cancer including the potential impact of screening high-risk populations and the emerging use of biomarkers. A brief discussion on cost analysis in health care is included.

7.1.1 Cost Analysis in Health Care

Economic analysis in health care attempts to address specific issues. Questions of cost versus benefit or effectiveness of specific treatments lend themselves these analyses. The fact that there is a fixed pool of resources coupled with the potential trade-off of cost and benefit is currently an important issue. There are several issues that apply to all economic analyses. These issues include the perspective of the analysis, outcomes using cost versus charge, and discounting.

7.1.1.1 The Importance of Perspective

Cost analyses can be constructed in different ways based on the factors that are included in the analysis. In order to determine which costs to include in an analysis, the perspective of the payer needs to be determined. Typically, there are three "payer" perspectives: that of society, the hospital, and the patient.

The perspective of the patient is the most difficult and subjective to evaluate as it depends on factors related to the patient's finances and insurance. Individuals who have insurance or the ability to pay may be more likely to seek medical care before a condition is urgent. Insurance coverage and benefits may impact a patient's willingness to initiate and continue treatment of a condition. Conversely, individuals lacking insurance or the ability to pay may delay seeking treatment or be unable to tolerate the cost of treatment.

The hospital's perspective is usually the easiest to measure based on the ability to itemize and account for resource use. Costs can be individualized to the patient based on the resources, personnel, medications, supplies, and equipment used during a treatment. There are also general and fixed costs such as hospital administration, facility maintenance, and amortization of capital equipment. In evaluating the cost-effectiveness of surgical approaches, for example, the cost of equipment can play a significant role. A hospital usually does not get additional payment for a robotic prostatectomy but has to purchase the robot and pay for maintenance (Lotan et al. 2004). Additionally, the different cost centers within the hospital need to be evaluated individually as new treatments may be more expensive for some areas in the hospital, such as the operating room, but result in savings overall (Lotan et al. 2002). A broader examination of the financial implications of new technologies and treatments is necessary as different cost centers are affected in different ways.

The perspective of society involves both direct and indirect costs. The nationalization of health care results in a larger portion of direct costs being shared by society. Private insurance companies pass increasing costs on to employers and participants through higher premiums. Indirect costs result from loss in productivity of patients and care givers during an illness and the recovery period. These indirect costs can be difficult to measure but can be substantial. Additionally, the morbidities and effects on quality of life associated with a treatment all have indirect costs that may not be accounted for immediately.

7.1.1.2 Cost Versus Charge

Articles dealing with economic evaluation of healthcare services often use either *cost* or *charge* data. This can be very confusing because cost and charge represent significantly different measures. The charge for a service, procedure, or medication incorporates the cost of an item, indirect costs, and profit margins. The cost of an item represents that actual resources used to acquire an object. Often, there is a significant difference between the charge and cost of an item and conclusions drawn from analyzing charge may not be applicable when evaluating the cost.

There are several problems with using charge data. Charge data include profit margins, which are arbitrarily determined. Different departments in a hospital use different cost-to-charge ratios such that the radiology department may charge twice as much as the cost for an imaging study but the pharmacy may charge five times as much for a medication. Another consideration is the fact that most hospitals do not get paid the actual amount that they charge due to Medicare set rates and insurance contracts. The reimbursement varies as per hospital and geographic location such that comparing costs is a more uniform means of evaluating differences between treatment or management options.

Unfortunately, even the use of costs can vary based on utilization rates of capital equipment and reduced rates for bulk purchases. For example, if one pays more than US$1 million for a robot and uses the procedure ten times per year then that cost is distributed over those ten patients. However, if one uses the robot 100 times per year then the cost is tenfold lower per patient (Lotan et al. 2004). This applies to capital equipment as well as other resources such as room and board. Since hospitals have fixed costs for nursing, building maintenance, security, janitorial services, etc., an increase in bed utilization will reduce the cost of inpatient care on a per-patient basis. Furthermore, when comparing cost within an institution or country, there is need to understand that the conclusions may not be accurate in other economic settings or with other cost assumptions (Lotan et al. 2005).

7.1.1.3 Discounting

In many analyses, there is a time component so that outcomes occur in the future. This applies to many cancer-related analyses in which the main outcome such as survival occurs at a different time point from the initial treatment. Discounting is a method of comparing future costs with current costs (Cairns 2001). Discounting is necessary because there is greater value to a benefit that one receives immediately as opposed to one that will be received in 1 year's time (Fleming et al. 1993; Gold et al. 1996). Most cost analyses apply a yearly discounting rate around 3% to future costs and future years of life (Gold et al. 1996; Siegel et al. 1997). This is based on typical annual inflation rates between 0% and 5% in the United States.

Most cost analyses in urology involve comparison of costs associated with procedures or techniques but discounting is very important for cost-effectiveness analyses evaluating screening or chemoprevention where the initial costs are high but benefits may take a long time to materialize (Lotan et al. 2006; Svatek et al. 2008).

7.1.2 Overall Bladder Cancer Costs

Bladder cancer is still mainly a surgical disease as the majority of new diagnoses are localized to the bladder. Almost all patients with cystoscopic or radiologic findings consistent with localized bladder cancer are initially treated with a transurethral resection. Further treatment decisions are guided by the pathological findings.

Some patients require subsequent staging procedures, chemotherapy, or more radical surgery. At the very least, the diagnosis of bladder cancer commits a patient to a rigorous and frequent surveillance regimen. The costs associated with the diagnosis, treatment, complications of treatment, and surveillance make bladder cancer one of the most expensive cancers to treat (Botteman et al. 2003; Avritscher et al. 2006).

In 2003, Botteman et al. conducted a review of the literature on the costs associated with the diagnosis and treatment of bladder cancer (Botteman et al. 2003). Bladder cancer was the fifth most expensive cancer to treat in the United States accounting for 7% of all cancer expenditures in 1989. Direct costs for all cancers including inpatient and outpatient care, medications, and devices were estimated at US$74 billion in 2005 (Meropol and Schulman 2007). Assuming constant proportional spending on bladder cancer, this would amount to direct costs of more than US$5 billion.

On a per-patient basis, bladder cancer is the most expensive cancer based on Medicare expenditures from diagnosis until death costing between US$82,640 and US$194,286 per patient (in 2001 US dollars) (Botteman et al. 2003). More recently, Avritscher and colleagues evaluated the cost of care for bladder cancer in 208 patients and found the mean cost per patient was US$65,158 over a 3.7-year period from the arrival at MD Anderson Cancer Center (Avritscher et al. 2006). They then modeled the lifetime direct medical costs of a cohort of patients with bladder cancer with both superficial and muscle-invasive disease, including patients younger than 65, starting with diagnosis. Two scenarios were modeled, the "best case" where patients with superficial disease had progression at the expected rate, and the "worst case" where all patients progressed and eventually died of their disease. Because of longer survival, the patients that progressed at the expected rate had the highest cost of US$120,684 versus a cost of US$99,270, if all patients had disease progression. In this analysis, hospital admissions and surgical procedures accounted for 50% of the total costs. Surveillance and treatment of recurrences, when combined, accounted for 60% of total expenditures and 30% of the total cost was associated with complications.

Total costs vary with stage at diagnosis (Han and Schoenberg 2000; Avritscher et al. 2006; Cooksley et al. 2008). Patients presenting with muscle-invasive disease incur diagnostic and treatment costs that are between two and three times higher than patients with noninvasive disease. The initial evaluation and treatment, surveillance, and treatment of recurrences are all significantly higher in patients with muscle-invasive disease. These patients undergo more invasive procedures, have higher complication rates, and have longer and more costly inpatient stays. Thus, diagnosing patients at an earlier stage and identifying recurrences before progression could reduce treatment and surveillance costs. Furthermore, reducing the number of recurrences could decrease the cost of management.

Resource utilization of patients with bladder cancer has also changed over time. The Urologic Diseases in America study examined various aspects and trends in health care resource use of patients with bladder cancer between 1992 and 2001 (Konety et al. 2007). A decrease in inpatient care with an increase in the use of ambulatory surgery and outpatient visits was observed. Expenditures for physician

office visits increased 239% between 1994 and 2000. The use of cytology almost tripled and cystoscopy increased every year, likely due to the increased availability of flexible cystoscopes. Staging by computed tomography (CT) almost tripled, as that imaging modality replaced retrograde and intravenous urography, which both decreased. Inpatient services still accounted for more than 60% of all expenditures.

7.1.3 The Economics of Bladder Cancer Diagnosis and Screening

In appropriate populations, the presence of hematuria or other clinical findings in the absence of other causative factors initiates the investigation for bladder cancer. The typical evaluation includes cystoscopy, with or without cytology, and upper tract imaging (Gorssfeld et al. 2001a, b). Cystoscopy is still the gold standard for the diagnosis and surveillance of bladder cancer; however, it is costly and invasive. On the other hand, up to 18% of apparently normal individuals have some degree of hematuria (Mohr et al. 1986; Mariani et al. 1989; Messing et al. 1995a, b; Grossfeld et al. 2001a, b). The prevalence of bladder cancer in patients with microscopic hematuria is low and ranges between 2% and 5% (Grossfeld et al. 2001a, b). Thus, routine evaluation in all patients with microhematuria results in an invasive evaluation with cystoscopy and imaging for 95% of patients with microscopic hematuria without any malignancy identified. Within the last decade, multiple bladder tumor markers have been developed to improve detection of bladder cancer (Lokeshwar et al. 2005). At this time, these markers have demonstrated improved sensitivity over cytology but suffer from reduced specificity. Their role in management of bladder cancer has not been encouraged by guideline panels and requires further evaluation (Lokeshwar et al. 2005).

Analyzing the costs associated with the evaluation of patients suspected of having bladder cancer should take into consideration the expense of not only the diagnostic tests but the various costs associated with office visits, lab work, radiology, etc. How to account for these direct costs in cost analysis can be difficult to answer. For example, performing a cystoscopy results in a number of areas where expenses can occur. The cost of the equipment and the expense of maintenance and sterilization could be examined. Disposable supplies, assistants, and the use of preprocedure antibiotics all have their own costs. Radiology exams also vary in true direct costs. One only has to consider the difference in the expense of the state-of-the-art magnetic resonance imaging technology versus the equipment required for intravenous urography. Thus, the majority of studies that examine and model costs use Medicare or private insurance reimbursement rates.

7.1.3.1 The Costs Associated with Diagnosing Bladder Cancer

The American Urological Association best practice policy recommends that in the absence of other inciting factors, patients 40 years of age or older and younger

patients at risk for developing urothelial carcinoma presenting with microscopic hematuria or irritative voiding symptoms should undergo evaluation (Grossfeld et al. 2001a, b). Gross hematuria should prompt a full evaluation, regardless of age or risk factors due to the high risk of malignancy (around 10%) (Mohr et al. 1986; Mariani et al. 1989; Messing et al. 1995; Grossfeld et al. 2001a, b; Bruyninckx et al. 2003). Cystoscopy and some form of upper tract imaging is accepted as the standard of care. Cytology is often used as an adjunct to cystoscopic evaluating. The development of urine-based tumor markers has provided an alternative to cytology; however, their use in asymptomatic patients for screening purposes is not recommended. At this time, there are limited data on the most cost-effective strategy to evaluate patients suspected of having bladder cancer.

Imaging of the upper tracts can be performed by intravenous or retrograde urography, CT or magnetic resonance imaging, and ultrasound. However, studies on the most cost-effective imaging modality are lacking. Before the proliferation of cross-sectional imaging technology, intravenous or retrograde urography and ultrasound were the primary means of upper tract evaluation. Ultrasound was found to be more cost-effective than intravenous urography when both were used with cystoscopy in diagnosing bladder cancer as the initial imaging modality in the evaluation of asymptomatic, microscopic hematuria (Corwin and Silverstein 1988). Currently, the majority of patients undergo CT urography as the initial imaging study (Silverman et al. 2009). Compared to intravenous urography, CT has been shown to be more sensitive and specific in diagnosing both urologic and nonurologic causes of microscopic hematuria (Gray Sears et al. 2002; Silverman et al. 2009). CT urography has also been shown to be nearly as sensitive and specific as cystoscopy in detecting bladder tumors in certain groups of patients with gross hematuria (Sadow et al. 2008). An obvious deficiency of imaging is the ability to detect flat lesions such as carcinoma in situ. However, the performance of CT urography raises the question of whether cystoscopy can be avoided in specific groups of patients with negative imaging. On the other hand, since kidney cancer is rare in patients with microhematuria, one might question the cost-effectiveness of upper tract imaging in evaluation of these patients (Mariani et al. 1989).

The routine use of cytology has been questioned due to the relatively low sensitivity for low-grade tumors, problems with reliability, interobserver variability, and inconclusive findings (Paez et al. 1999; Raitanen et al. 2002; Nabi et al. 2003). Atypical findings are problematic because they raise concerns with both the patient and the physician about the possible presence of cancer (Raitanen et al. 2001; Nabi et al. 2004). Furthermore, cytology is not inexpensive (cost US$50–US$80) due to the cost of processing the cells, collection, and the need for a skilled pathologist (Lotan et al. 2006).

The availability of urine-based tumor markers with improved sensitivity over cytology has raised the question of whether replacing cytology may result in improved cost-effectiveness of evaluating patients at risk for bladder cancer. When one considers use of any additional test such as urine-based tumor markers, one needs to evaluate not only cost but also performance characteristics. Even if a test is

inexpensive, a significant number of false-positive results will necessitate additional and potentially more expensive tests that raise the overall cost of evaluation.

There are only a few studies that have evaluated the cost-effectiveness of using urine-based markers in the diagnosis of bladder cancer. Zippe et al. evaluated the performance of NMP22 in detecting bladder cancer in patients who presented with hematuria or other symptoms with comparison to cystoscopy and cytology (Zippe et al. 1999). They found that if the indication for cystoscopy was a positive NMP22, then a cost-savings of US$28,302–111,072 (in 1998 US Dollar) (depending on the type of insurance carrier) would have been achieved. Of note, in this study, the sensitivity of NMP22 was 100% with a specificity of 85%. The performance characteristics of NMP22 in this study were superior to those reported in the literature since the overall sensitivity in a large multicenter trial for the point-of-care NMP22 (BladderChek test) was 55% and the specificity of 85% was at the high end of most studies (Grossman et al. 2005; Lotan and Roehrborn 2003; Hong and Loughlin 2008). The use of markers to detect cancer in lieu of cystoscopy would be ideal if the markers had a high sensitivity and specificity. The cost of missing a cancer diagnosis is harder to estimate and must be factored into any cost-effectiveness analysis.

One other consideration is the incorporation of markers into predictive models such as nomograms, which take into consideration both the marker status as well as clinical factors. There is growing evidence that patients with microscopic hematuria are not referred to urologists for evaluation on a routine basis and this could potentially lead to delays in diagnosis (Nieder et al. 2008; Johnson et al. 2008). A nomogram for bladder cancer detection incorporating the NMP22 BladderChek test has been developed but awaits validation prior to recommending routine clinical use (Lotan et al. 2009). The cost-effectiveness of such models will require evaluation since the added cost of markers needs to be balanced against any potential benefits.

7.1.3.2 Screening for Bladder Cancer

Even though bladder cancer is the fourth most common cancer in men, wide spread screening is currently not accepted as the standard of care. However, given that the risk factors for bladder cancer are well known, screening populations at the highest risk may be appropriate. Cancer screening should result in the identification of malignancies before they have progressed beyond the condition of being curable. For bladder cancer screening to be considered effective, the disease must be detected at an earlier stage and the earlier diagnoses should lead to treatments that improve survival. Even though screening for prostate, colon, and breast cancer is prevalent, controversy still remains regarding the benefit on overall mortality (Kaplan 2005). There is good evidence that diagnosing bladder cancer before muscle invasion improves survival (Stein et al. 2001; Messing et al. 2006; Gupta et al. 2008). Early detection of bladder cancer would reduce cancer-related mortality based on the 5-year survival rates of superficial versus muscle-invasive disease (American

College of Surgeons. Commission on Cancer National Cancer Database. Available at http://www.facs.org/cancer/ncdb/index.html. Accessed September 7, 2005).

The costs associated with screening are also critical in examining the feasibility of cancer screening programs. The widespread screening for prostate, lung, colon, and cervical cancer can result in costs approaching US$50,000 per life-year saved (Lotan et al. 2006; Svatek et al. 2006). Screening costs are affected by several factors including disease prevalence, benefit of screening, and both the cost and performance of the test. The prevalence of a disease in a population directly affects the performance of the screening evaluation. Screening for diseases that are rare in a given population results in a lower positive predictive value and a larger number of unnecessary tests per disease found. The benefit of screening for a specific cancer should result in a significant gain in life-years for a screening program to be cost-effective. For example, finding breast cancer in a 40-year-old woman results in a larger gain in life-years compared to finding prostate cancer in a 75-year-old man. The cost of the screening test is especially relevant given advances in cross-sectional imaging and molecular diagnostics. The more costly a screening test is and the more frequent the test is performed on a patient, per-patient expenditures and cost per life-year gained can rise dramatically. For example, the use of whole body CT screening results in only a 6-day gain in life expectancy yielding a cost-effectiveness ratio of US$151,000 per life-year gained (Beinfeld et al. 2005). Additionally, the screening test should have an appropriate sensitivity and specificity to identify life threatening cancers early on while avoiding unnecessary investigations of false-positives.

7.1.3.2.1 Screening Based on the Presence of Hematuria

Assuming that identifying factors can be found in urine, screening for bladder cancer should be relatively easy. Examining the urine for blood or atypical cells would be an obvious starting point. Early attempts at bladder cancer screening involved evaluating patients for hematuria by urine dipstick. Prospective studies by Messing and colleagues evaluated the use of a chemical reagent strip that identified hemoglobin in the urine in 1575 men older than 50 years of age in 1987 and from 1989 to 1992 (Messing et al. 1995a, b). Identification of hematuria elicited a standard evaluation of cystoscopy as well as imaging of the kidneys and ureters. A total of 258 (16.4%) of the screened patients underwent a hematuria evaluation and 21 (8.1%) were found to have bladder cancer. Similar proportions of superficial low-grade (stage Ta and T1) and invasive high-grade (stage≥T2) were found in the screened cohort and the tumor registry. However, the men who underwent screening had a significantly lower proportion of invasive cancers (10%) than those found in unscreened men (60%; $p=0.002$). A 2006-update of the study found that no bladder cancer specific deaths had occurred in the screened cohort versus 20.4% of the men with bladder cancer in the unscreened group (Messing et al. 2006).

The use of urine dipstick testing for hematuria represents an inexpensive and readily available test that is relatively examiner independent. The limiting factor in

the widespread utility of this test is the low-positive predictive value. In the study, more than 90% of the patients found to have hematuria had no evidence of cancer. This yields a positive predictive value for urine dipstick screening for hematuria of only 8.3% resulting in a large number of costly and unnecessary evaluations. However, an analysis of the cost per cancer found was similar to other current screening programs (Messing et al. 1995a, b).

7.1.3.2.2 Screening by Using Bladder Tumor Markers in High-Risk Populations

The inadequacy of screening by using cytology or testing the urine for hemoglobin has led to the development of several molecular urine-based markers for detecting bladder cancer. Some have received FDA approval for the use in detecting bladder cancer in patients with high risk. These markers have been found to be more sensitive for low-grade tumors and equivalent to cytology for high-grade tumors and carcinoma in situ (Lotan and Roehrborn 2003). The positive predictive value is higher compared to the detection of hematuria but still only approaches 20% (Svatek and Lotan 2008). The positive predictive value increases in populations at increased risk for bladder cancer, thus limiting the screening to high-risk populations can improve detection rates and reduce the number of false-positive results.

The cost-effectiveness of using NMP22 to screen populations with different incidence rates of bladder cancer has been investigated through economic modeling (Lotan et al. 2006; Svatek et al. 2006). Svatek et al. examined what the cost per cancer detected would be in populations and stratified by age, sex, and incidence rates (Svatek et al. 2006). Costs were calculated based on Medicare reimbursement rates in 2005. They found that widespread screening in all patients 50 years of age and older, regardless of risk, would result in costs per cancer detected of more than US$100,000. Applying the same model to a high-risk population with a 6% annual incidence lowered the cost per detection dramatically resulting in an average cost of only US$3934. These results are comparable to other widespread screening programs. In examining the effect of incidence rates on cost, it was observed that costs increase dramatically as the incidence falls below 4% in a given population. Additionally, the performance of the marker played a role in cost per detection. A lower sensitivity would have resulted in fewer cancers being detected resulting in a higher cost per detection. Conversely, decreased specificity leads to unnecessary evaluations and increased cost. In another analysis, NMP22 screening in a population with a 4% incidence of bladder cancer resulted in greater than US$100,000 in cost-savings for the population and a gain of 3 life-years per 1000 patients (Lotan et al. 2006). Modeling showed that screening is a cost-effective strategy when cancer incidence is greater than 1.6%. Similar to the cost per cancer detection, the cost of the marker and performance are important. Finally, the screening strategy has to result in tumor downstaging with improved survival. Based on these analyses, screening high-risk populations with appropriate bladder tumor markers would result in costs per cancer detected similar to programs currently in place for colon, breast, and prostate. Ongoing studies are necessary to evaluate the

incidence of bladder cancer in high-risk population and the performance of urine-markers in these patients.

7.1.3.3 The Economics of Bladder Cancer Surveillance

Bladder cancer is a disease with a high recurrence and progression rates (Heney et al. 1983; Millan-Rodriguez et al. 2000). As a consequence, surveillance of bladder cancer is frequent and due to the use of cystoscopy and cytology, it can incur significant costs. As noted above, an analysis of lifetime cost of treating bladder cancer found that surveillance alone accounted for 21% of all costs (Avritscher et al. 2006). Furthermore, the cost of treating recurrences accounted for another 39% of total costs. There have been efforts to reduce surveillance costs by reducing the number of recurrences, reducing risk of progression, and reducing number of cystoscopic procedures.

Strategies to reduce the number of recurrences can actually start at the time of initial transurethral resection (TUR). The primary mode of cystoscopy at this time is white light (WL) but there is concern that visualization is not adequate resulting in incomplete resection of tumor (Brausi et al. 2002; Grimm et al. 2003). Fluorescent cystoscopy (FC), which is based on topic application of porphyrines acting as fluorescing substances, has been used to improve visualization of tumors. Two randomized trials of FC versus WL have found better recurrence-free survival rates in patients with FC (Daniltchenko et al. 2005; Denzinger et al. 2007). A study by Burger et al. prospectively randomized 301 patients with noninvasive bladder cancer to standard WL or FC transurethral resections of the bladder (Burger et al. 2007). Over a median follow-up of 7.1 years, disease recurrence was found in 42% and 18% of WL and FC patients, respectively ($p=0.0003$). The number of TUR procedures per patient in the WL and FC groups was 2.0 and 0.8, respectively. Because cost of FC is €135, overall costs were significantly lower (by €1195) in FC patients based on fewer TURs. Further studies are needed to determine if the use of FC would prove to be cost-effective. At this time, the use of FC is not approved for use in the United States.

Once initial TUR is completed, several randomized clinical trials found a decrease in recurrence after a single instillation of a chemotherapeutic agent (mitomycin C or epirubicin) immediately or within 24 h after TUR (Solsona et al. 1999; Sylvester et al. 2004; Gudjonsson et al. 2009). A meta-analysis of the literature found that a single instillation of chemotherapy can reduce risk of recurrence by 39% (Sylvester et al. 2004). Considering the high cost of TURBT and relatively low cost and toxicity of a single dose of mitomycin C, this approach is very cost-effective. Several studies suggest that approximately 8.5 patients need to be treated to prevent one recurrence (Sylvester et al. 2004; Oosterlinck and Sylvester 2008).

Most urologists perform cystoscopy on 3 months intervals after initial diagnosis for a period of 2 years regardless of initial stage and grade. There have been several studies that have evaluated the potential for urine-based tumor markers to reduce the number of cystoscopic procedures (Lachaine et al. 2000; Lotan and

Roehrborn 2002; Lodde et al. 2006). Since low-grade noninvasive tumors progress very infrequently (<5%), the use of markers alternating with cystoscopy can save money with low risk that more aggressive tumors will be missed (Lotan and Roehrborn 2002). Even a marker with a sensitivity of 50% and specificity of 70%, which are well within range of current markers, can result in significant cost savings as long as a marker cost is less than $264 (Lotan and Roehrborn 2002, 2003). This strategy was cost advantageous over a wide range of marker sensitivity and specificity, recurrence, and progression rates. Lachaine et al. evaluated the use of NMP22 in a modified surveillance strategy and found that 64% of patients avoided one cystoscopy (Lachaine et al. 2000). While the strategy saved money, 8.9% of patients experienced a 3-month delay before diagnosis of a recurrence. A study by Lodde et al. evaluated the utility of ImmunoCyt/uCyt+ in combination with cytology to reduce the number and cost of cystoscopies in the follow-up of patients with urothelial cancer of the bladder (Lodde et al. 2006). Cytology and ImmunoCyt/uCyt+ together had a sensitivity of 86.6% and a negative predictive value of 95.2%. Of the cystoscopies performed during the 26 months of follow-up, 69.7% were negative. While this study supported the cost-effectiveness of ImmunoCyt/uCyt+ and cytology every 6 months combined with annual cystoscopy, it was based on costs in Italy. The cost of the test in the United States is greater than the cost of cystoscopy in the United States, so such an approach will not be cost-effective in every setting. The use of urine-based markers alternating with cystoscopy has not been tested prospectively, so the potential cost-effectiveness has yet to be proven. One further consideration is that the 2008 European Association of Urology (EAU) guidelines on nonmuscle-invasive bladder cancer suggests that for low-grade tumors with a negative cystoscopy at 3 months, there is no need for another cystoscopy until 9 months and yearly afterward (Babjuk et al. 2008). As such, use of markers alternating with cystoscopy may just add more cost without additional benefit.

The use of adjuvant therapies in patients with high-grade noninvasive tumors has been shown to reduce recurrences and progression (Herr et al. 1995; Bohle et al. 2003; Witjes and Hendricksen 2008). While the use of BCG has been shown to be effective in managing patients with noninvasive tumors, there are no studies evaluating the cost-effectiveness of this approach. There is also an absence of studies evaluating the use of chemotherapy in patients with muscle-invasive disease. The use of multimodal therapy with chemotherapy has not been fully embraced by the urologic community. A study by David et al. evaluated the use of perioperative chemotherapy (within 4 months before or 4 months after surgery) in 7161 stage III patients with bladder cancer (David et al. 2007). Only 11.6% of patients received any chemotherapy include 10.4% receiving adjuvant chemotherapy and 1.2% receiving neoadjuvant chemotherapy. Several studies have evaluated the use of neoadjuvant chemotherapy and there are multiple reviews on the topic (Calabro and Sternberg 2008; Bellmunt et al. 2008). A meta-analysis of 11 randomized controlled trials that compared neoadjuvant chemotherapy plus local treatment with the same local treatment alone evaluated 3005 eligible patients and found a 5% absolute improvement in survival at 5 years (Neoadjuvant chemotherapy in

invasive bladder cancer—update of a systematic review and meta-analysis of individual patient data. Advanced Bladder Cancer (ABC) Meta-analysis Collaboration (2005)). The data on adjuvant chemotherapy are more limited as there are fewer studies and these are limited by small sample size, trials that stopped early, patients that did not receive initial treatment, or salvage chemotherapy. A meta-analysis based on 491 patients from six trials found an overall hazard ratio for survival of 0.75 (95%CI 0.60–0.96, $p=0.019$) suggesting a 25% relative reduction in the risk of death for chemotherapy compared to controls (Adjuvant chemotherapy for invasive bladder cancer (individual patient data). Cochrane Database Syst Rev (2006)). Since the cost of chemotherapy exceeds US$50,000 per patient (Lotan et al. 2006), one can surmise that the small survival benefit will result in a very high cost per life-year saved. The cost-effectiveness of using chemotherapy in patients with metastatic disease is even lower due to the rare cure rates in this patient group (von der Maase et al. 2000). It is important to note that the willingness to pay for a treatment and cost-effectiveness are viewed differently based on the disease state. For example, the cost-effectiveness of screening approaches is viewed with great scrutiny while the cost-effectiveness of treating known disease is not evaluated as carefully. Future attempts to reduce costs in medical care may target therapies with low cost-effectiveness. It is possible that very expensive therapies with small benefits may be the first to be eliminated including expensive chemotherapeutic regimens.

7.1.3.4 Conclusions

Diagnosing and treating bladder cancer exacts a high cost on society that is likely to continue to grow along with the aging population. There is potential to reduce cost of care for bladder cancer by reducing costs associated with surveillance and earlier detection of disease. Introduction of modifications to current care requires careful evaluation of both costs and benefits.

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Chapter 8
Prognostic Markers for Bladder Cancer

Tomonori Habuchi

Abstract At present, there is a need for good prognostic or predictive markers for bladder cancer. These markers are required for monitoring treatment response, recurrence, and survival after transurethral resection of bladder tumor (TUR-BT), intravesical therapy, radical cystectomy, radiation therapy, and systemic chemotherapy. Although significant correlations between various laboratory molecular markers and treatment outcome or survival have been demonstrated, these tests have not yet been adopted in standard practice. Usefulness of candidate molecular markers as independent prognostic markers still has to be determined in large prospective comparative studies. Definition of universal and robust criteria for test positivity, a clearly defined patient population, standardization of laboratory techniques to evaluate markers, establishment of definitive criteria for evaluating test positivity, clearly specified endpoints, and suitable statistical methods will help to apply accurate independent prognostic indicators in clinical management of patients with bladder cancer.

8.1 Overview

Traditional clinical and pathological parameters, such as tumor grade, stage as well as vascular and lymphatic extension, provide important prognostic information but have limited ability to predict tumor recurrence and progression, development of metastases, response to therapy, or survival. Although a substantial number of potential molecular markers for prediction of clinical course and outcome have been identified in recent molecular biological and genetic studies, no single molecular marker is reliable enough to predict clinical course and outcome of bladder cancers and replace standard clinical and pathological parameters. The independent

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prognostic value of a new marker needs to be demonstrated using a sufficient number of subjects in relevant staging and grading categories to determine each marker's prognostic independence. Furthermore, the marker must add some predictive capacity beyond the standard offered by clinical and pathological parameters.

Good molecular prognostic markers are needed for monitoring treatment response, recurrence and survival after transurethral endoscopic treatment (TUR-BT), TUR-BT + intravesical therapy, radical cystectomy, radiation therapy, and systemic chemotherapy for advanced disease. Furthermore, prognostic or predictive markers for response to bacille Calmette-Guérin (BCG) instillation therapy and progression from nonmuscle-invasive disease to muscle-invasive disease or from muscle-invasive bladder cancer to metastasis also need to be established.

In the first part of this section, representative bladder cancer prognostic markers are presented according to the disease situation, i.e., (1) intravesical recurrence after TUR-BT, (2) progression to muscle-invasive disease after endoscopic management for nonmuscle-invasive bladder cancer, (3) response to intravesical BCG instillation therapy, (4) outcome after radical cystectomy for muscle-invasive or locally advanced disease, and (5) response to systemic chemotherapy for advanced disease.

Recently identified candidate molecular prognostic or predictive markers, including those that will not be described here, are listed in Table 8.1.

In the second part, major representative candidate molecular markers classified by function and methodology are summarized; however, an extensive description of the biological role of each marker is not provided.

8.2 Prognostic Markers According to Clinical Situation

8.2.1 Intravesical Recurrence After TUR-BT

Low-grade, nonmuscle-invasive transitional cell carcinomas are mostly treated with TUR, with or without adjuvant intravesical chemotherapy or BCG instillation. Since most of these patients (60%) have intravesical recurrences and may have a risk of progressive muscle-invasive disease, molecular markers to predict recurrence have long been awaited by both patients and physicians.

Many potential markers have been evaluated by immunohistochemistry (IHC). However, there is no definitive marker that will consistently predict recurrence. Although most markers, as evaluated by IHC, may provide useful information, the criteria in such evaluation (strongly positive, weakly positive, or negative; ratio of positive cells) may not be clearly objective. Despite these defects, there is a relatively large literature supporting the fact that the Ki-67 labeling index is a good prognostic marker for prediction of recurrence in nonmuscle-invasive bladder cancer patients (Liukkonen et al. 1999; Gontero et al. 2000; Wu et al. 2000; Stavropoulos et al. 2002; Santos et al. 2003). On the other hand, accumulating literatures indicate that the presence of a fibroblast growth factor receptor 3

Table 8.1 New candidate molecular markers classified by clinical situation in bladder cancer

Target molecule	Method	Associated outcome	Literature
Intravesical recurrence after TUR-BT			
FGFR3 mutation	DNA sequence	Intravesical recurrence	van Rhijn et al. (2003)
APAF-1, IGFBP-3	DNA methylation	Intravesical recurrence	Christoph et al. (2006)
DAP kinase	DNA methylation	Intravesical recurrence	Tada et al. (2002)
Various genes	DNA methylation	Intravesical recurrence	Friedrich et al. (2005)
mRNA expression profile	cDNA array	Early and late recurrence	Dyrskjot et al. (2003)
Interleukin-6/10 ratio	ELISA, urine	Intravesical recurrence	Cai et al. (2007)
Carbonic anhydrase IX	IHC	Intravesical recurrence	Klatte et al. (2009)
NF-kappaB polymorphism	PCR genotyping	Intravesical recurrence	Riemann et al. (2007)
E-cadherin polymorphism	PCR genotyping	Intravesical recurrence	Lin et al. (2006)
Glutathione peroxidase 1 polymorphism	PCR genotyping	Intravesical recurrence	Zhao et al. (2005a, b)
Progression to muscle invasive disease after TUR-BT and intravesical therapy			
IMP3	IHC	T1 or less to muscle invasion or metastasis	Sitnikova et al. (2008)
MRP-1/CD9	IHC	Ta-1 to muscle invasion	Mhawech et al. (2003)
Gamma-catenin	IHC	Progression in T1 tumors	Clairotte et al. (2006)
Thrombospondin-1	IHC	T1 or less to muscle invasion or metastasis	Goddard et al. (2002)
Promoter of 5 genes	DNA methylation	T1 or less to muscle invasion or metastasis	Yates et al. (2007)
mRNA expression profile	cDNA array	Ta-1 to muscle invasion	Dyrskjot et al. (2007)
Response to intravesical BCG instillation therapy			
HSP90	IHC	BCG treatment response	Lebret et al. (2007)
Outcome after radical cystectomy for muscle-invasive or locally advanced disease			
Carbonic anhydrase IX	IHC	Overall survival	Klatte et al. (2009)
DOC-2/DAB2 expression	IHC	Tumor recurrence, cancer-specific mortality	Karam et al. (2007)
HIF-1alpha polymorphisms	PCR genotyping	Cancer-specific survival	Nadaoka et al. (2008)
Endothelin (B) receptor	IHC	Disease-free survival	Wulfing et al. (2005)

(continued)

Table 8.1 (continued)

Osteoprotegerin	ELISA	Disease-specific survival	Mizutani et al. (2004)
Topoisomerase II alpha	FISH, IHC	Overall survival	Simon et al. (2003)
Urokinase-type plasminogen activator	ELISA	Progression, disease-specific survival	Shariat et al. (2003b)
RhoA, RhoB, ROCK-I, ROCK-II	Western Blot	Disease-free survival, overall survival	Kamai et al. (2003)
Response to systemic chemotherapy for advanced disease			
Emmprin, Survivin	cDNA array, IHC	Poor outcome after cisplatin-based chemotherapy	Als et al. (2007)

(*FGFR3*) gene mutation is linked to a favorable disease course and low-grade bladder cancer with low malignant clinical features, such as absence of concomitant carcinoma in situ (CIS). van Rhijn et al. (2003) reported that the combination of *FGFR3* mutation and MIB-1, an antibody against Ki-67, status in patients with nonmuscle-invasive disease is an independent prognostic variable for recurrence-free survival, in addition to progression-free and cancer-specific survival. Since the presence or absence of *FGFR3* mutation is more objective and may be an all-or-none marker, *FGFR3* mutation may be a good predictive marker from the clinical and practical standpoint.

Molecular genetic analyses revealed that hypermethylation of certain genes, such as apoptotic protease-activating factor-1, insulin-like growth factor-binding protein-3 (Christoph et al. 2006), death-associated protein kinase (DAPK) (Tada et al. 2002), suppressor of cytokine signaling (SOCS)-1, signal transducer and activator of transcription (STAT)-1, B-cell leukemia/lymphoma-2 (Bcl-2), tissue inhibitor of metalloproteinases (TIMP)-3, and E-cadherin (Friedrich et al. 2005), is significantly associated with intravesical recurrence after transurethral treatment and may provide independent prognostic markers. Because there are many tumor-suppressor genes whose hypermethylation may be associated with higher recurrence rate and evaluation of hypermethylation status is technically objective, combinations of these hypermethylation markers may be more effective in predicting prognosis of intravesical recurrence. Microsatellite alterations that suggested loss of tumor-suppressor genes, such as certain chromosomal loci or DNA mismatch repair defects, may also be candidate predictive markers. Migaldi et al. (2005) showed in univariate analysis that the presence of microsatellite alterations at the analyzed loci was associated with reduced risk of tumor recurrence and disease progression. However, the number of patients in the study was small (45), and the results were not confirmed by multivariate analysis. Furthermore, because of so many candidate loci, microsatellite analysis is still uncommon and is not yet established as predictive markers.

Quantitative evaluation of mRNA levels of various genes in cancer cells may provide significant objective predictive marker(s), and cDNA microarray analysis can provide a powerful tool for characterizing gene expression on a whole genome basis. In a recent study, a panel of 26 genes was identified from a large cDNA microarray analysis of bladder tumors that discriminated between early and late recurrences in patients with superficial Ta tumors (Dyrskjot et al. 2003). However, a validation study using real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) showed that data on these 26 genes were not reproducible in the cohort studied by Dyrskjot et al. (Schultz et al. 2006). As suggested in other types of cancers, the prognostic value of microarray results should be interpreted with caution and validated carefully in other sets of samples selected without bias (Michiels et al. 2005; Schultz et al. 2006). Another approach is measurement of target proteins in serum or urine by enzyme-linked immunosorbent assay (ELISA). Because ELISA is reproducible and objective, it is hoped that good markers for predicting bladder cancer, such as prostate-specific antigen in prostate cancer, are discovered. Cai et al. (2007) reported that the interleukin-6/10 ratio was an

independent prognostic factor of recurrence in multivariate analysis. Test sensitivity and specificity of the interleukin-6/10 ratio were 0.83% and 0.76%, respectively. Zhao et al. (2005a) reported that in patients with superficial bladder carcinoma, high angiogenin levels measured by ELISA were associated with shorter recurrence-free survival ($P<0.01$) and increased recurrence risk (hazard ratio of 2.85) in multivariate Cox regression analysis. Mizutani et al. (2004) reported that serum osteoprotegerin (OPG) might be a good predictor of recurrence; however, multivariate analysis was not performed. Furthermore, none of these markers measured by ELISA has been established in multicenter studies.

8.2.2 Progression to Muscle-Invasive Disease After Endoscopic Management for Nonmuscle-Invasive Bladder Cancer

There are two major goals in treatment of nonmuscle-invasive tumors; one is prevention of intravesical recurrence and the other is prevention of progression to muscle-invasive or metastatic disease, which indicates a life-threatening condition. In patients with nonmuscle-invasive bladder cancer, the average risk of progression to muscle-invasive disease is 10–20%, and patients progressing to muscle-invasive disease eventually require radical cystectomy and urinary diversion. Therefore, there is a great need for biomarkers that can accurately distinguish superficial tumors with high probability of progression from those that will remain indolent. Using such biomarkers, it would be possible to decide a patient's prognosis and effectively target individuals most likely to benefit from the therapy.

Almost all candidate molecular markers for predicting progression from nonmuscle-invasive tumors are assessed by IHC. Recent IHC studies indicated that the expression level or score for motility-related protein-1 (MRP-1/CD9) (Mhawech et al. 2003), intermodulation product 3 (IMP3) (Sitnikova et al. 2008), gamma-catenin (Clairotte et al. 2006), Ki-67 (MIB-1) (Santos et al. 2003; Stavropoulos et al. 2002; Blanchet et al. 2001), p53 (Rodriguez-Alonso et al. 2002), and thrombospondin (TSP)-1 (Goddard et al. 2002) may be candidate predictive markers for progression from Ta-1 tumors to muscle-invasive or metastatic disease. However, because of the difficulty in defining positive staining, judgment, tissue sampling, selection bias, and lack of multi-institutional studies, no established predictive markers are in clinical use. Among these candidates, the Ki-67 (MIB-1) index or score is associated with the most consistent results, although there have been no multi-institutional studies.

The nucleotide-based approach may be more objective, but laboratory procedures may be more cumbersome. Yates et al. (2007) analyzed the methylation status of 17 gene promoters by a quantitative methylation-specific PCR method in 70 Ta-1 patients and reported that the methylation status of the *E-cadherin* gene (*CDH1*) promoter and the extent of methylation of the 5-locus panel were significantly associated with tumor progression. However, further multivariate analyses with a larger number of cases will be required to establish hypermethylation as a predictive

marker for progression in patients with nonmuscle-invasive disease. A study by van Rhijn et al. (2003) indicated that the combination of *FGFR3* mutation and Ki-67 provided an independent prognostic factor for disease progression (246 Ta-1 tumors, 40 T2-4 tumors) to muscle invasion or metastases. However, because no data were presented on focused analysis of Ta-1 disease, the role of *FGFR3* mutation and Ki-67 as a progressive marker in Ta-1 disease was not established.

In a multicenter study including 404 patients with nonmuscle-invasive disease, cDNA-microarray-based mRNA expression analyses showed that an 88-gene expression signature was highly significantly correlated with progression-free and cancer-specific survival ($P<0.001$ and $P=0.001$, respectively) (Dyrskjot et al. 2007). Furthermore, the expression signature was shown to be an independent factor for progression in multivariate Cox regression analysis.

8.2.3 Response to Intravesical BCG Instillation Therapy

Intravesical instillation of BCG has been shown to improve the short-term outcome of high-risk superficial bladder cancer. Furthermore, progression to muscle-invasive disease may be prolonged or prevented in certain patients with high-risk superficial bladder cancer. However, early BCG failure is associated with worse prognosis, and survival may be better in patients treated with first-line cystectomy compared to those with invasive disease after failed BCG therapy. Therefore, a good prognostic marker for predicting response to BCG therapy is urgently needed.

Literature on predictive markers for response to BCG instillation therapy is limited. This is partly because the definition of BCG response is rather ambiguous and many treatment biases might influence the results. In 33 patients with high-grade nonmuscle-invasive disease, Lebret et al. (2007) reported that low-heat shock protein 90 (HSP90) expression could be useful in predicting BCG failure. Expression of p53 protein has been the most frequent target of investigation, but results regarding its predictive usefulness are contradictory. In 102 high-risk patients with Tis, Ta, and T1 tumors treated with BCG, p53 overexpression was shown to be an independent predictor of recurrence (Saint et al. 2004). In another study including 198 patients with tumors at stages Tis to T1, p53 overexpression in residual tumors after BCG instillation therapy was the only independent marker for disease progression in BCG nonresponders in multivariate analysis, whereas p53 overexpression before BCG therapy did not predict response to BCG therapy (Lacombe et al. 1996). On the contrary, several studies indicate the absence of such predictive value. Overexpression of p53 had no predictive value for recurrence and progression in 29 T1G3 bladder cancer patients treated with BCG therapy (Peyromaure et al. 2002); it did not act as a predictor of recurrence or progression in 47 patients with superficial bladder tumors at high risk for recurrence or progression, treated with 6 weekly intravesical BCG instillations (Zlotta et al. 1999). Therefore, the role of p53 overexpression as a prognostic and predictive marker after intravesical BCG instillation remains unclear.

8.2.4 *Outcome After Radical Cystectomy for Muscle-Invasive or Locally Advanced Disease*

Although radical cystectomy is currently the standard treatment for localized, muscle-invasive bladder cancer in most countries, the oncologic outcome is not satisfactory, the 5-year recurrence-free and overall survival in male and female patients being reported as 66–68% and 58–66%, respectively (Stein et al. 2001). Because of these unsatisfactory results, neoadjuvant or adjuvant chemotherapy was tested. However, neoadjuvant chemotherapy provided an overall survival advantage of only 5–8% in recently published studies and meta-analyses, and there are no convincing data regarding the advantage of adjuvant chemotherapy after radical cystectomy. In this difficult situation, molecular markers for predicting the outcome of therapy will be of great value in future application of pre- or postoperative additional therapy.

As in other situations, most of the many predictive markers for muscle-invasive disease are based on IHC studies. Multivariate analyses indicate that promising candidate molecular targets of predictive markers may include Twist and E-cadherin (Fondrevelle et al. 2009), carbonic anhydrase IX (CAIX) (Klatte et al. 2009), p53 and/or p21 (Shariat et al. 2004), p53, p21, and pRb (Chatterjee et al. 2004), p53 (see Sect. 8.3.4, and Tsai et al. 2003), c-erbB-2 (Haitel et al. 2001; Kruger et al. 2002a, b) as well as tenascin-C (Brunner et al. 2004).

Molecular targets were recently identified by expression analysis on a whole genome basis using a cDNA microarray, and IHC was then performed for promising molecules. Sanchez-Carbayo et al. (2006) analyzed mRNA transcript profiles using oligonucleotide arrays in 105 bladder tumors, including 72 invasive lesions. They found that a genetic profile consisting of 174 probes was significantly associated with lymph node metastasis and overall survival, simultaneously. They further identified a new candidate marker, synuclein, and showed that it had a significant association with tumor staging and clinical outcome by IHC (Sanchez-Carbayo et al. 2006).

In the clinical setting, serum or plasma markers may be ideal because of their convenience and lack of intersample and interlaboratory bias. Shariat et al. (2003b) explored the predictive value of plasma urokinase-type plasminogen activator (uPA) and its receptor in 51 patients who underwent radical cystectomy for muscle-invasive cancer or Tis, Ta, or T1 cancer refractory to intravesical therapy. They found that preoperative uPA was independently associated with metastasis to regional lymph nodes ($P=0.017$), lymphovascular invasion ($P=0.019$), disease progression ($P=0.030$), and death from bladder cancer ($P=0.038$). Mizutani et al. (2004) reported that, among patients with muscle-invasive bladder carcinoma, the 5-year disease-specific survival rate was greater for those with low-serum OPG levels compared to those with high-serum OPG levels; however, no multivariate analysis was performed.

Another approach is genetic polymorphism. Although analysis of DNA polymorphisms is generally used for identification of genetic susceptibility factors or predictive markers for drug responses and adverse effects, polymorphisms may affect the prognosis and outcome of cancer treatment. Recent studies have shown that the tumor microenvironment plays a significant role in determining progression,

metastasis, treatment outcome, and prognosis in cancer. The tumor microenvironment is strongly affected by host genetic factors, i.e., host genetic polymorphisms; for example, after radical cystectomy, patients with the hypoxia-inducible factor-1 (*HIF-1*) alpha gene variant allele had significantly worse disease-free and disease-specific survival than those without the variant allele (Nadaoka et al. 2008). Furthermore, presence of the variant allele was an independent predictor of disease-free survival in multivariate analysis using a Cox proportional hazards model (Nadaoka et al. 2008). Since genotyping of single-nucleotide polymorphisms is relatively easy and robust and interlaboratory discrepancy is rare, establishment of single-nucleotide polymorphisms as predictive markers may be useful and practical from the clinical point of view.

8.2.5 Response to Systemic Chemotherapy for Advanced Disease

In general, patients with advanced bladder cancer with metastatic lesions or locally inoperable disease may be treated with cisplatin-based systemic chemotherapy. At present, there are two standard chemotherapeutic regimens involving cisplatin: methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) and (gemcitabine and cisplatin (GC). There are no established molecular markers for predicting response, outcome, prognosis, and adverse effects for systemic chemotherapy under these conditions. Until date, the association between p53 positivity and response to cisplatin-based chemotherapy remains unknown; this issue is discussed later (see Sect. 8.3.4).

Using a cDNA microarray technique, Als et al. (2007) evaluated gene expression signatures in original primary tumors from 30 patients, who later received systemic chemotherapy for metastatic or locally advanced disease, and identified a set of 55 genes with distinct expression highly correlated with survival time after chemotherapy. Among the 55 candidate genes, emmprin and survivin were selected for validation by IHC, and multivariate analysis revealed that emmprin and survivin expression were independent prognostic markers for poor outcome. Although the results and conclusions of the study should be validated in further multi-institutional studies, the strategies used in the study may provide a rational and standard approach to identify prognostic markers for systemic chemotherapy, and predictive markers identified in this way may also be applicable to predict response to neoadjuvant or adjuvant chemotherapy.

8.3 Candidate Molecular Predictive Markers Classified by Target Molecules and Methodology

According to the target molecules and methodology, prognostic markers are classified into the following groups: (1) chromosomal alterations and DNA/nucleotide-based markers, (2) cDNA microarray mRNA expression analysis and gene expression

signatures, (3) proto-oncogenes/oncogenes, (4) tumor-suppressor genes, (5) cell cycle regulators, (6) angiogenesis-related factors, and (7) extracellular matrix adhesion molecules.

8.3.1 Chromosomal Alterations and DNA/Nucleotide-Based Markers

Molecular genetic methods, such as loss of heterozygosity analysis, microsatellite alteration analysis, fluorescence in situ hybridization analysis (Pycha et al. 1999), and comparative genomic hybridization, have made it possible to identify loss of tumor-suppressor genes and gain of oncogenes in bladder cancer.

Microsatellite alteration: Migaldi et al. (2005) examined microsatellite alterations at 19 microsatellite loci that were previously reported to be associated with bladder tumorigenesis in 51 superficial papillary bladder cancers in young patients. They found that the presence of microsatellite alterations at the analyzed loci was associated with reduced risk of tumor recurrence ($P = 0.04$, log-rank test) and disease progression ($P = 0.02$) in univariate analysis but not in multivariate analysis. Vaish et al. (2005) indicated that the presence of microsatellite instability in primary tumors was significantly more frequent in patients who had one or more recurrences. However, no multivariate analysis was performed. Until date, no large prospective study has demonstrated that this type of genetic analysis has an independent prognostic value in patients with bladder cancer. The complicated laboratory process required in this type of analysis has hampered the widespread application of genetic analysis in clinical settings.

Abnormal DNA methylation: Abnormal methylation patterns may also provide candidate molecular markers, although laboratory procedures may be too cumbersome for routine clinical use. Several studies focusing on methylation status of well-known candidate genes have been published to predict intravesical recurrences (Tada et al. 2002; Friedrich et al. 2005; Christoph et al. 2006) and progression from nonmuscle-invasive disease to muscle-invasive disease (Yates et al. 2007) or overall survival in general (Kim et al. 2008). Among these, Friedrich et al. (2005) used an extensive approach evaluating abnormal methylation status in 20 cancer-associated genes. They identified six genes (*SOCS-1*, *STAT-1*, *BCL-2*, *DAPK*, *TIMP-3*, and *CDH1*) for which methylation was associated with intravesical recurrence.

DNA polymorphisms: Although DNA polymorphisms, including single-nucleotide polymorphisms, are generally used to predict disease susceptibility or response or adverse event in certain drugs, they may also serve as predictive markers for disease progression. Until date, polymorphisms of the nuclear factor-kappaB gene (*NFKB*) (Riemann et al. 2007), *CDH1* (Lin et al. 2006), and the glutathione peroxidase 1 gene (*GPX1*) (Zhao et al. 2005b) were reportedly associated with intravesical recurrence. *HIF-1*alpha polymorphisms were associated with cancer-specific and disease-free survival after radical cystectomy, as described above (Nadaoka 2008). The more extensive and whole genome approach may provide

clinically useful predictive information because laboratory techniques to evaluate polymorphisms are evolving and becoming rather robust and rapid.

8.3.2 cDNA Microarray mRNA Expression Analysis and Gene Expression Signature

As stated above, gene expression signatures identified by cDNA microarray mRNA expression analysis on a whole genome basis have been used to identify prognostic or predictive markers (signatures) for intravesical recurrence after treatment of nonmuscle-invasive disease (Dyrskjot et al. 2003), progression from nonmuscle-invasive disease to muscle-invasive or advanced disease (Dyrskjot et al. 2007), prognosis after radical treatment for locally advanced disease (Sanchez-Carbayo et al. 2006), or prognosis after systemic chemotherapy for advanced disease (Als et al. 2007). While some signatures were confirmed to be significant in a multicenter validation study, this study did not confirm previous significant results in some cases. Owing to the large number of genes analyzed by microarray analysis, there may be false-positive associations. To establish a particular candidate expression signature as a clinically applicable predictive marker, the signature should be validated in large independent cohorts of patients.

8.3.3 Proto-oncogenes/Oncogenes

Epidermal growth factor receptor (EGFR): Several investigators have shown a positive association between overexpression of EGFR and high-grade, high-stage bladder cancer, thus indicating that EGFR expression identified by IHC is an independent prognostic factor in patients with advanced bladder cancer (Neal et al. 1985; Messing 1990; Lipponen and Eskelinen 1994; Nguyen et al. 1994; Mellon et al. 1995) However, EGFR expression determined by IHC was not a significant predictor of progression in patients with Ta-1 bladder cancers in multivariate analysis (Liukkonen et al. 1999), and it had no prognostic significance in 43 muscle-invasive bladder cancer patients treated with cystectomy (Ravery et al. 1997).

HER-2 (*human EGFR-related 2*): The oncoprotein HER-2, which is encoded by *c-erb-B2*, was found to be frequently expressed in bladder cancer (Lipponen et al. 1991). Using the IHC method, Sato et al. (1992) found that HER-2 overexpression was an independent prognostic factor in 88 patients with muscle-invasive bladder cancer, and Kruger et al. (2002b) found that it was a significant and independent prognostic factor of tumor-specific survival in 145 patients with muscle-invasive bladder cancer in multivariate analysis. However, other investigators found that the HER-2 expression level provided no additional prognostic information regarding tumor stage and grade for bladder cancer in 95 (Mellon et al. 1996) and 89 patients

(Underwood et al. 1995), respectively. In 80 patients with muscle-invasive bladder cancer treated with radical cystectomy, no significant difference in median survival between patients with HER-2-negative and HER-2-positive results was found (Jimenez et al. 2001). In summary, the level of expression and prognostic significance of HER-2 protein in urothelial cancer varies among studies, some revealing no prognostic relevance and others suggesting a better or worse prognosis according to the chemotherapeutic regimen in patients with locally advanced bladder cancer (Gandour-Edwards et al. 2002).

H-Ras: Mutations in the H-*ras* gene at codons 12 and 61 have been implicated in up to 10% of bladder cancers (Knowles and Williamson 1993). However, no study has shown any prognostic value in the presence of mutation. While one study indicated that overexpression was correlated with early recurrence of superficial Ta or T1 bladder cancer (Fontana et al. 1996), this prognostic effect was not clearly shown in other studies; contradictory results have also been observed (Moriyama et al. 1989; Ye et al. 1993).

Bcl-2: In 62 low-grade pTa papillary bladder cancer patients, no significant relationship was discovered between bcl-2 expression level and intravesical recurrence (Pich et al. 2002). In 58 patients treated with TUR-BT, Bcl-2 expression did not offer any additional prognostic information in prediction of either recurrence or progression compared to that offered by conventional prognostic factors (Stavropoulos et al. 2002). In 77 patients who underwent cystectomy, no significant correlation was present between Bcl-2 expression and overall survival (Shiina et al. 1996). In a study of 109 patients with invasive bladder carcinoma treated with preoperative radiation therapy without concurrent chemotherapy, Bcl-2 overexpression was significantly associated with disease progression and upstaging of the tumor during radiation therapy (Pollack et al. 1997). However, Bcl-2 overexpression had no significant effect on response to a combination of platinum-based chemotherapy and radiation therapy (Pollack et al. 1997; Rodel et al. 2000). At present, the Bcl-2 expression level evaluated by IHC does not appear to provide any additional significant information in patients with nonmuscle- and muscle-invasive disease.

FGFR3: Molecular genetic analyses have shown frequent presence of *FGFR3* point mutation in bladder cancers, especially in those with a low-grade and low-stage phenotype (Cappellen et al. 1999; van Rhijn et al. 2001). Presence of *FGFR3* mutation when combined with the MIB-1 expression score might be a significant independent prognostic variable for recurrence- and progression-free survival as well as cancer-specific survival (van Rhijn et al. 2003). In 80 patients with papillary urothelial neoplasms of low malignant potential, strong expression of FGFR3 evaluated by IHC indicated low risk of intravesical recurrence (Barbisan et al. 2008). On the other hand, van Oers et al. (2007) found that the presence of *FGFR3* mutation was not an independent marker of recurrence in pTa G1 tumors, although the mutation was frequently found in low-grade noninvasive papillary tumors. Further multi-institutional large study is desired to determine whether *FGFR3* mutation has significant prognostic independence, because the mutation plays a significant role in genesis of low-grade noninvasive papillary bladder tumors.

8.3.4 Tumor Suppressor Genes

p53: Mutated p53 protein often results in a prolonged half-life compared to wild-type p53; it can be detected by IHC. Comparison of p53 detection by IHC and molecular genetic analysis of *p53* gene mutation at the nucleotide level has shown that p53 accumulation correlates with the mutated *p53* gene (Esrig et al. 1993). However, there may be a considerable number of discordant cases of *p53* gene mutation confirmed by DNA sequencing and "altered p53" evaluated by IHC (Watanabe et al. 2004). Owing to the complicated process of molecular analysis, many groups have investigated the prognostic value of p53 by IHC. A high level of variability between laboratories and observers may exist in the gray zone of low p53 positivity between 1and 20% (McShane et al. 2000). More recent meta-analysis showed that "p53 overexpression" by IHC was associated with risk of recurrence in nonmuscle-invasive disease [HR (hazard ratio) = 1.6, 95% confidence interval (CI) = 1.2–2.1], progression (HR = 3.1, 95% CI = 1.9–4.9), and mortality (HR = 1.4, 95% CI = 1.2–1.7) (Malats et al. 2005). However, the characteristic background of patients and disease condition were heterogeneous, and the definition of recurrence, progression, or mortality was not clear and uniform in the meta-analysis (i.e., intravesical and systemic recurrences as well as progression from nonmuscle- to muscle-invasive disease and from locally advanced disease to systemic disease were mixed) (Malats et al. 2005). Furthermore, publication bias should also be considered. Taken together, care should be taken when interpreting the large amount of research data on the association between p53 expression by IHC and clinical outcome.

p53 as a prognostic marker in patients with Nonmuscle-invasive bladder cancer: With regard to T1 disease, p53 positivity was associated with a significantly shorter progression-free interval in 43 patients (Llopis et al. 2000), and it was among the independent cancer-related survival variables in 175 patients (Rodriguez-Alonso et al. 2002). Absence of a role for p53 positivity as an independent prognostic variable for recurrence or progression in Ta or T1 bladder cancer has been reported in several studies (Vatne et al. 1995; Pfister et al. 1999; Wu et al. 2000; Gontero et al. 2000). In general, as described above, the role of p53 overexpression as a prognostic and predictive marker for intravesical BCG instillation in nonmuscle-invasive disease remains unclear and questionable (see Sect. 8.2.3). Therefore, the role of p53 positivity as an independent prognostic marker in patients with nonmuscle-invasive bladder cancer, with or without BCG treatment, has not been clearly demonstrated. Meta-analysis by Schmitz-Dräger et al. (2000) showed about only 50% positive multivariate analyses of p53 overexpression as a prognostic marker of progression in T1 bladder cancer.

p53 as a marker in patients with Muscle-invasive Bladder Cancer: Esrig et al. (1994) reported that nuclear p53 positivity by IHC was an independent predictor of recurrence and overall survival in multivariate analysis, stratified according to grade, pathological stage, and lymph node status, in 253 patients treated with radical cystectomy. However, p53 positivity was not predictive of disease-free survival in

node-positive disease in 59 patients with pathological lymph node-positive bladder cancer treated with cystectomy (Fleshner et al. 2000). Furthermore, p53 positivity had no independent prognostic value over clinical stage and mitotic index in multivariate survival analysis in 212 patients (Lipponen 1993). The Bladder Tumor Marker Network also evaluated a series of 109 patients with G2 or G3, T2 to T3 disease and found no prognostic value of p53 staining (Lianes et al. 1998). On the other hand, in 109 patients with pT2N0M0 bilharzial-related bladder cancer, p53, together with MIB-1, Bcl-x, and Bax, was an independent predictor of progression-free survival in the urothelial carcinoma group ($n=49$), and p53 positivity and proliferation cell nuclear antigen were independent predictors in the squamous cell carcinoma group ($n=60$) (Haitel et al. 2001). Among the contradictory results regarding the predictive significance of p53 expression in muscle-invasive bladder cancer, the extensive review by Schmitz-Dräger et al. (2000) of all published research on the association of p53 positivity and prognosis of bladder cancer patients demonstrated that only two of the seven studies in muscle-invasive bladder cancer considered p53 to be an independent prognostic marker for progression.

p53 as a response marker to chemotherapy or radiotherapy: Several studies exploring the possible association between p53 positivity and response to systemic chemotherapy or radiation therapy for advanced bladder cancer have been published. In 111 patients with muscle-invasive bladder cancer treated with neoadjuvant MVAC therapy, p53-positive tumors had lower response to chemotherapy (Sarkis et al. 1995). In support of this finding, Kakehi et al. (1998) reported that p53-negative tumors responded favorably to cisplatin-based combination chemotherapy in 60 patients with advanced bladder cancer. In another study of 60 patients receiving cisplatin-based systemic chemotherapy for advanced urothelial cancer, p53 negativity was correlated with complete or partial remission, thus indicating that tumors with intact p53 responded significantly better (Jankevicius et al. 2002). In 50 primary bladder cancers with metastatic lesions, p53 positivity was not correlated with response to cisplatin-based chemotherapy or survival (Sengelov et al. 1997). In contrast, a preliminary study showed that p53-positive tumors responded better to adjuvant chemotherapy in patients treated with radical cystectomy (Cote et al. 1997). Taken together, the relationship between p53 positivity and response to systemic chemotherapy remains unclear.

With regard to the predictive value after radiation therapy, Ogura et al. (1995) showed no significant correlation between p53 positivity and radiosensitivity in 60 patients with muscle-invasive bladder cancer. In a study of 131 patients with T2 to T4 bladder cancer treated with external full-dose radiotherapy, p53 positivity was not an independent prognostic factor for overall survival (Osen et al. 1998). Lack of association between p53 positivity and response to radiation therapy was also found in two other studies (Pollack et al. 1997; Rotterud et al. 2001). Therefore, it is unlikely that p53 expression has significant prognostic value with respect to radiation response in bladder cancer.

Combination of p53 with other potential markers: The significance of p53 expression combined with other potential markers was explored in several studies. Cote et al. (1998) examined p53 and retinoblastoma (Rb) protein expression by

IHC in 185 bladder cancer patients who underwent radical cystectomy. They found that patients having tumors with both p53 and Rb alterations had significantly increased rates of recurrence ($P<0.0001$) and survival ($P<0.0001$) compared to those with no alterations, and those with alterations in only one of these proteins had intermediate rates of recurrence and survival. In 59 Ta and T1 bladder cancer patients, there was significant increase in progression and decreased overall survival in patients having tumors with both p53 and Rb alterations (Cordon-Cardo et al. 1997). These studies suggest an independent yet synergistic role for p53 and Rb expression in bladder cancer progression.

In a study examining the combined value of p53, p21, and pRb expression in 154 bladder cancer patients, the number of altered markers (p53, p21, and pRB) was significantly associated with time for recurrence and overall survival in multivariate analysis (Chatterjee et al. 2004). In another study examining p53, p21, pRB, and p16 expression in 80 bladder cancer patients who underwent radical cystectomy, the incremental number of altered markers was independently associated with increased risk of bladder cancer progression and mortality (Shariat et al. 2004).

Although these studies were retrospective and included a relatively small number of subjects, analysis of the interaction of different cell cycle regulatory proteins in combination with p53 may increase the benefit of p53 determination as a prognostic marker.

Rb: In 38 patients with muscle-invasive tumors, overall survival was independently higher in patients with normal Rb protein tumors compared to those with altered Rb protein (negative or weak) tumors (Cordon-Cardo et al. 1992). In 43 patients with locally advanced bladder cancer treated with surgery and chemotherapy, a significantly poorer tumor-free survival rate was found in those with an altered Rb protein tumor (Logothetis et al. 1992). In 45 patients with T1 bladder tumors, loss of Rb protein expression combined with altered p53 expression was associated with significantly poorer progression-free survival (Grossman et al. 1998). However, all these studies were by IHC and included a relatively small number of patients; no extensive results have been published yet.

8.3.5 Cell Cycle Regulators

p21: In 96 patients with superficial bladder cancer, negative p21 expression was an independent predictor of reduced overall survival but not of disease-free survival in multivariate analysis (Migaldi et al. 2000). In 242 patients who underwent cystectomy, the p21 IHC labeling index was an independent predictor of tumor recurrence and survival in multivariate analysis (Stein et al. 1998). Furthermore, patients with p53-positive/p21-negative tumors demonstrated a higher rate of recurrence and worse survival compared to those with p53-positive/p21-positive tumors (Stein et al. 1998). In contrast, in 186 patients with Ta or T1 tumors, low p21 positivity was associated with a better prognosis, although this relation was not statistically significant. In a multicenter study including 207 patients with Ta or T1 bladder

cancer, p21 expression by IHC provided no additional prognostic information to that provided by established prognostic factors in prediction of tumor recurrence and progressive disease (Liukkonen et al. 2000). In 47 nonmuscle-invasive bladder cancer patients treated with 6 weekly intravesical BCG instillations, high p21 expression was associated with shorter recurrence-free intervals (Liukkonen et al. 2000). Therefore, the role of p21 expression as a prognostic and predictive variable remains controversial.

p27: p27 levels were significantly higher in low-grade, superficial, papillary, and slowly proliferating tumors (Korkolopoulou et al. 2000). In 120 patients with bladder cancer, decreased p27 expression was associated with poor overall survival, and p27 status combined with Ki-67 expression had the strongest relationship with overall survival in patients with muscle-invasive tumors in multivariate analysis (Korkolopoulou et al. 2000). In a series of 96 patients with Ta or T1 bladder cancer, low p27 expression was significantly correlated with decreased disease-free and overall survival and was an independent predictor of reduced disease-free survival (Sgambato et al. 1999). In 145 consecutive patients with bladder cancer, low IHC p27 expression levels were independent predictors of shortened disease-free and overall survival in multivariate analysis (Kamai et al. 2001). Furthermore, significant correlations were observed between low IHC p27 expression and early recurrence in 86 patients with Ta or T1 disease (Kamai et al. 2001). Taken together, decreased p27 expression in bladder cancer appears to have some prognostic value, although more studies are needed before drawing any conclusion on the predictive significance of p27 expression level.

Ki-67 (*MIB-1*): Using the monoclonal antibodies Ki-67 and MIB-1, the Ki-67 antigen detected by IHC accumulates in the nuclei of proliferating cells from the G1 phase to mitosis but not in the nuclei of quiescent or resting cells (Gerdes et al. 1984). A large number of studies have defined the Ki-67 labeling index as an independent prognostic marker of bladder cancer progression and recurrence in multivariate analysis. In 159 patients with Ta or T1 bladder cancer, a high Ki-67 index (≥18%) and multifocality were significantly related to recurrence- and progression-free survival and were independent prognostic factors in multivariate analysis (Santos et al. 2003). In 58 patients with Ta or T1 tumors, the Ki-67 index, and not p53 and Bcl-2, was an independent prognostic factor for recurrence in patients treated with TUR alone (Stavropoulos et al. 2002). In 207 patients with Ta or T1 disease, MIB-1 score and papillary status, and not p53 and EGFR expression, were independent predictors of progressive disease and cancer-specific survival in multivariate analysis (Liukkonen et al. 1999). In 192 patients with Ta or T1 disease, Ki-67 and multifocality, and not p53 positivity, were found to be independent prognostic factors of recurrence in multivariate analysis (Gontero et al. 2000). In a study examining Ki-67, Bcl-2, and p53 expression in 93 cases of low-grade nonmuscle-invasive bladder cancer, the Ki-67 labeling index was the only significant prognostic indicator of tumor recurrence in univariate and multivariate analyses (Wu et al. 2000). These documents consistently indicate that Ki-67 expression is a promising marker for recurrence and progression in nonmuscle-invasive bladder cancer. Therefore, larger prospective studies with a standardized method and positive

criteria, especially of the cutoff value of the labeling index, may be required. On the other hand, Ki-67 expression as a prognostic marker in patients with locally advanced or metastatic bladder cancer remains unconfirmed.

Cyclin D1: Among 96 patients with Ta or T1 tumors, those having tumors with low cyclin D1 expression together with low p27 and high Ki-67 expression had extremely high rates of recurrence (Sgambato et al. 2002). However, another study including 392 patients with pTa or pT1 tumors showed that cyclin D1 positivity by IHC was not linked to risk of recurrence or tumor progression (Wagner et al. 1999). In a multicenter IHC study of 207 patients with Ta or T1 bladder cancer, the cyclin D1 expression level was an independent predictor of tumor recurrence (Liukkonen et al. 2000). However, it did not provide additional prognostic information on disease progression (Liukkonen et al. 2000). More recently, in 74 patients with Ta, Tis, and/or T1 bladder cancer, cyclin D1 immunoreactivity was not associated with any pathological characteristics or clinical outcome and provided no independent prognostic value (Shariat et al. 2007).

8.3.6 Angiogenesis-Related Factors

Vascular endothelial growth factor (*VEGF*): Crew et al. (1999) showed that VEGF was present in higher concentrations in the urine of patients with bladder cancer compared to that of control subjects. They also found that urinary VEGF levels determined by ELISA were correlated with tumor recurrence rates in 61 patients with tumor stage T1 or less (Crew et al. 1999). On the other hand, in 185 patients with pTa or T1 tumors, VEGF expression by IHC was not correlated with the risk of tumor recurrence or patient survival (Chow et al. 1999). Inoue et al. (2000) reported that VEGF expression determined by IHC was an independent prognostic factor for disease recurrence in multivariate analysis of 55 patients with muscle-invasive bladder cancer treated with neoadjuvant MVAC chemotherapy and radical cystectomy.

TSP-1: TSP-1 is an extracellular matrix glycoprotein that is a potent inhibitor of angiogenesis. In 163 patients treated with radical cystectomy, decreased TSP-1 expression by IHC was an independent predictor of disease recurrence and overall survival, after stratification for tumor stage, lymph node status, and histological grade (Grossfeld et al. 1997). Furthermore, TSP-1 expression was significantly associated with p53 expression status and microvessel density counts (Grossfeld et al. 1997). In a study of 220 patients with nonmuscle-invasive bladder cancer, reduced perivascular TSP-1 staining by IHC at initial presentation was an independent predictive factor for subsequent development of muscle-invasive or metastatic disease (Goddard et al. 2002).

Cyclooxygenase (*COX*)*-2*: In 108 patients treated with radical cystectomy, COX-2 expression determined by IHC was found in 31% of cases and was correlated with local invasion, lymphovascular invasion, and recurrence (Shirahama et al. 2001). However, its expression was not an independent prognostic marker for

survival (Shirahama et al. 2001). An IHC study including 39 patients with CIS and 34 with stage T1 tumors showed that COX-2 expression was not associated with clinical outcome in the T1 patients (Shariat et al. 2003a). In patients with CIS, COX-2 expression was significantly associated with disease recurrence using cut-offs of 0% and >10% positive cells and with disease progression using a >20% cutoff (Shariat et al. 2003a). In 37 patients with initial T1G3 bladder cancer who underwent complete TUR, followed by intravesical BCG instillation therapy, COX-2 expression was a statistically significant variable in predicting both recurrence and disease progression (Kim et al. 2002).

CAIX: CAIX is a hypoxia-inducible member of the carbonic anhydrase family that regulates intracellular pH, cell proliferation, cell adhesion, and tumor progression. In 340 patients with tumors of various grades and stages, high CAIX expression was correlated with higher stage. For patients with nonmuscle-invasive cancer treated with TUR, higher CAIX expression was associated with poorer recurrence-free survival. In patients with T1 tumors treated with TUR, higher CAIX expression had a 6.5-fold higher risk of progression to muscle-invasive disease ($P = 0.006$) (Klatte et al. 2009). In patients who underwent cystectomy, higher CAIX expression was associated with worse overall survival ($P = 0.003$) and was the strongest independent prognostic factor of overall survival in multivariate Cox models (Klatte et al. 2009). The role of CAIX expression as a predictive marker should be further evaluated in a larger number of studies from other laboratories (Klatte et al. 2009).

8.3.7 *Extracellular Matrix, Adhesion Molecules, Cell Surface Markers, and Related Proteins*

Matrix metalloproteinase (*MMP*), *tissue inhibitors of MMP, and emmprin*: A preliminary study evaluating the expression of *MMP-2*, *TIMP-2*, and membrane-type *MMP-1* by RT-PCR analysis in 41 patients with bladder cancer indicated that high levels of *MMP-2*, *TIMP-2*, and membrane-type *MMP-1* expression were strongly associated with decreased survival (Kanayama et al. 1998). In 97 patients with urothelial cancer, the serum ratio of MMP-2 to TIMP-2 determined by ELISA was a significant and independent factor for recurrence in advanced urothelial cancer patients in univariate and multivariate analyses (Gohji et al. 1998).

Emmprin is a modulator of MMPs and is upregulated in bladder carcinoma compared to benign urothelium (Muraoka et al. 1993). Excess expression has also been correlated with tumor progression and development of metastases in several types of cancer. In 124 locally advanced or metastatic bladder cancer patients who received cisplatin-based systemic chemotherapy, multivariate analysis identified emmprin and survivin expression as independent prognostic markers for poor outcome, together with the presence of visceral metastases (Als et al. 2007).

Taken together, the balance between the activity of MMPs, that of their inhibitors (i.e., TIMP), and that of promoters (emprinn) in serum or tissues may provide distinct information on progression in patients with bladder cancer.

E-cadherin: Decreased E-cadherin expression is generally correlated with increased muscle invasion and distant metastasis as well as with higher tumor grade and stage (Bringuier et al. 1993; Otto et al. 1994). Decreased E-cadherin expression has been shown to be associated with overall and recurrence-free survival (Bringuier et al. 1993; Lipponen and Eskelinen 1995; Ross et al. 1995; Syrigos et al. 1998). In 77 patients who underwent radical cystectomy, E-cadherin expression determined by IHC was significantly associated with disease progression and cancer-specific survival, and E-cadherin and stage were independent predictors of disease progression (Byrne et al. 2001).

uPA: The urokinase plasminogen activation (uPA) system plays an important role in tumor invasion and metastasis by mediating proteolysis, adhesion, and migration of tumor cells. In 52 patients undergoing TUR-BT, multivariate analysis indicated that elevated uPA content in tumor tissue was the most important risk factor for invasion and metastasis compared to tumor stage, grade, multiplicity, and size (Hasui et al. 1996). In 51 patients who underwent radical cystectomy, preoperative plasma uPA level, but not urinary uPA level, was independently associated with metastasis to regional lymph nodes, lymphovascular invasion, disease progression, and disease-specific survival (Shariat et al. 2003b).

8.4 Conclusion

Although significant correlations between various laboratory markers and tumor progression have been demonstrated, these markers have not yet been adopted in standard practice and should not significantly influence treatment decisions for individual patients. This is probably a result of the lack of standardized technology to assess the accuracy of a particular prognostic marker, the lack of definitive criteria for evaluating test positivity, a clearly defined patient population, the requisite clinical and pathological standard tests for staging and grading of each tumor, and clearly defined endpoints and statistical methods. For example, technical aspects, such as when and how the specimens are obtained, reagents used to detect the marker, and interpretation of results, are critical when assessing the usefulness of various prognostic markers in predicting response to treatment.

Therefore, the definition of universal and robust criteria for test positivity, a clearly defined patient population, standardization of techniques used to evaluate markers, and clearly specified endpoints and statistical methods will help to provide accurate independent prognostic indicators in the clinical management of patients with bladder cancer. Furthermore, multivariate analysis using sufficient number of subjects in the relevant staging and grading categories to determine each marker's prognostic independence is required.

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Chapter 9
Molecular Nomograms for Predicting Prognosis and Treatment Response

Steven Christopher Smith and Dan Theodorescu

Abstract Human bladder cancer constitutes a heterogeneous disease characterized by, among its most common variant in Western societies, urothelial carcinoma (UC), two distinct nosologic entities: These are non muscle-invasive UC (NMIUC) and muscle-invasive UC (MIUC), which present specific management questions and are mediated by different molecular pathologic mechanisms. Logically, if differential molecular machinery can be demonstrated to underpin these pathologic states, theoretically, molecular biomarkers can be discovered to prognosticate disease course or predict susceptibility to therapeutic intervention. Thus, while NMIUC may be managed with some success through endoscopic resection, with or without adjuvant immuno- or chemotherapy (Dalbagni 2007), prediction of recurrence or evolution to MIUC is an essential prognostic challenge, as is prediction of metastatic recurrence post cystectomy in de novo MIUC cases. Finally, selection of chemotherapy among locally advanced inoperable or metastatic cases, as well as in the adjuvant or neoadjuvant settings, presents a compelling need for molecularly guided therapy. In this chapter, we present and discuss the opportunity for molecular biomarkers of prognosis or prediction of therapeutic success, focusing on novel, multiplexed molecular biomarkers rather than single targets, and spotlighting cases where such strategies are tailored to the key clinical climacterics outlined above. Molecular data, or a combination of molecular data and traditional clinicopathologic data (Shariat et al. 2008a), can be used to construct models, which upon validation in large patient cohorts, are capable of contributing important prognostic or predictive information for the management of patients. Loosely defined, such a strategy may be called a nomogram (Shariat et al. 2008b).

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9.1 Introduction

Bladder cancer, the fourth most common solid malignancy diagnosed in the USA (Jemal et al. 2008), is costly and morbid (Botteman et al. 2003). Bladder cancer includes several histologic variants, but the most commonly diagnosed in the Western World is transitional cell carcinoma, now known as urothelial carcinoma (UC) (Kumar et al. 2005). Interestingly this disease has a biphasic presentation, where approximately 70% present with non muscle-invasive tumors and the remainder present with muscle-invasive disease. Nonmuscle- invasive UC (NMIUC), i.e., stages Ta, Tis, and T1, is treated by endoscopic transurethral resection of bladder tumor, with or without the use of intravesical chemo- or immunotherapy, resulting in excellent outcomes (10-year disease-specific survivals of 85% and 70% for Ta and T1 stage disease, respectively) (Dalbagni 2007). In contrast, MIUC is treated aggressively by radical cystectomy and bilateral pelvic iliac lymphadenectomy with curative intent (Stein and Skinner 2006). This aggressive procedure may be preceded by neoadjuvant chemotherapy or succeeded by adjuvant chemotherapy, though due to the aggressive nature of invasive bladder cancer, these regimens result in treatment failure in approximately half of the patients within 2 years (Ghoneim and Abol-Enein 2008).

9.1.1 From Classical Prognostic Factors to Nomograms

Prognostic factors have been described for both NMIUC and MIUC based on clinicopathologic parameters, building on the overall notion that stage, or in a rudimentary sense, presence or absence of invasion of the lamina propria, is the most important prognostic factor for this disease (Fleming et al. 1997). For NMIUC tumors, several studies have evaluated factors associated with risk of tumor recurrence (~80% of patients without intravesical therapy) and progression (~30%) (Holmang et al. 1995; Lutzeyer et al. 1982; Millan-Rodriguez et al. 2000; Heney et al. 1983), as well as disease-specific mortality, reviewed in (Pasin et al. 2008). Regarding progression, these include grade, which has been associated more with progression or disease-specific mortality than recurrence (Millan-Rodriguez et al. 2000), as well as stage (T1 versus Ta), and presence or absence of concomitant *carcinoma in situ* (Kiemeney et al. 1994). Regarding recurrence, number of tumors has been found to be prognostic (Heney et al. 1983), even included in a prognostic nomogram (Ali-El-Dein Osahe-Hiianmag 2003). Additionally, lack of *early* recurrence (i.e., not present on first surveillance cystoscopy post-transurethral resection) has been associated with low rates of recurrence (Fitzpatrick et al. 1986).

In the case of muscle-invasive bladder cancer, multiple case series have examined prognostic factors for recurrence and disease-specific and overall survival *post-cystectomy*. A comprehensive recent series of >1,000 cases spanning three decades found key prognostic roles for stage, nodal status (including extent of

nodal involvement), and extravesical extension (Stein et al. 2001). Additional factors involved include tumor size (>3 cm), elevated creatinine, and lymphovascular invasion (Ennis et al. 2000; Slaton et al. 1999; Bassi et al. 1999; Lotan et al. 2005). Meanwhile, for locally advanced cases, the only prognostic factors for response to chemotherapy identified include Karnofsky performance status less than 80% and the presence of visceral metastasis (Bajorin et al. 1999) or multiple sites of visceral metastasis (Joaquim 2002).

An important trend in recent studies has been to employ large case series, providing substantial statistical power and good patient follow-up, to study the contributions of clincopathologic and demographic prognostic factors, like those above, to prognosis. Such data have then been used to construct prognostic models, where, for a given patient, individual clinicopathologic factors may be input into a formula that estimates prognosis based on the *a priori* studies. While such estimates cannot predict outcome perfectly, they do give physicians and patients a better and more accurate way to estimate prognosis for their risk-benefit decisions of whether to undergo treatment. This kind of tool is called a *nomogram*.

Examples of recent important work in this area include a nomogram developed by Sylvester et al., based on >2,500 patients in European trials, that used six clinicopathologic factors to estimate probability of recurrence and progression in NMIUC. Not surprisingly, these include some of the factors above: number of tumors, tumor size, prior recurrence rate, T stage, *carcinoma* in situ, and grade (Sylvester et al. 2006). For MIUC, a key recent report constructed a clinical nomogram for estimation of risk of recurrence postcystectomy including the factors of patient age, sex, time from diagnosis to surgery, pathologic tumor stage and grade, tumor histologic subtype, and regional lymph node status (Bochner et al. 2006). An intriguing artificial intelligence method using neural networks to predict recurrence-free probability in NMIUC has also been reported recently, which included substantial contributions of previous recurrence rate and number of tumors (Cai et al. 2007).

9.1.2 Molecular Nomograms?

Underlying heterogeneity in the biology of urothelial tumors, despite similar clinicopathologic characteristics, likely reduces the accuracy provided by traditional nomograms. Input from validated molecular components may improve this situation. Such a tool, providing *prognostic* estimation of a patient's disease course or *predictive* estimation of therapeutic effect based on analysis of molecular data on prior series may be called a *molecular nomogram*. As mentioned above, it must not perforce contain only molecular characteristics, but may combine both and optimize the prediction only with those with independent predictive power. Suggestive of the ability to include measurements of molecular characteristics to classical nomograms, Shariat et al. recently published a nomogram using measurement of nuclear matrix protein 22, NMP22, as well as cytology, age, and gender to predict

probability of disease recurrence in patients with NMIUC (Shariat et al. 2005). Such tools are likely to become ever more important as novel, targeted molecular therapies provide hope for patient subsets whose tumors show dysregulation of specific molecular pathways.

In the discussion below of studies making headway towards these goals, it is important to note that we will use several terms to describe the molecular component of a nomogram. For example, we will describe the discovery of *biomarkers,* which generally refers to a molecule whose status can be measured that reports or "marks" a clinical characteristic of interest, just as does NMP22, in the study above (Shariat et al. 2005). Often, gene expression studies define a *signature*, which simply refers to a number of biomarkers that can be measured for the same purpose. Theoretically, either a single biomarker or a whole signature could be built into a nomogram using traditional clinicopathologic data. Also, either could stand alone to provide independent predictive information. Either way, the possibility of using molecular data to provide personalization of patient care is compelling, and important progress along these lines is being made.

9.1.3 Molecular Pathogenesis of Bladder Cancer: Opportunities for Biomarker Discovery

The advent of molecular technologies, starting with the classic techniques of DNA sequencing, specific protein detection in the settings of immunoblotting and immunohistochemistry (IHC), and quantitation of specific mRNA species by northern blotting, have ushered in a radically different age in our understanding of cancer. Particularly, the past decade of evolution of technologies for high throughput molecular profiling, whether of DNA, mRNA, microRNA, or protein species, has seen an exponential growth in our knowledge of the molecular constellation that characterizes disease states. Bladder cancer, though relatively understudied compared to other common, costly malignancies (Sangar et al. 2005), has yet seen significant advances in our understanding of its molecular pathology, as reviewed recently (Sanchez-Carbayo and Cordon-Cardo 2003).

Given the biphasic clinical picture presented above, finding a molecular model where noninvasive and invasive diseases are mediated through different pathways (Wu 2005) is not surprising. Authors have observed substantial correlations between molecular profiles and pathologic classifications (Lopez-Beltran et al. 2008), and such defining molecular lesions have been shown individually to bear prognostic value in patients (Wu 2005). In the case of NMIUC, a body of evidence from both analysis of clinical specimens and in vitro and in vivo models suggests that activation of the canonical mitotic pathway downstream from receptor tyrosine kinases and the Ras oncogene is causally related to this disease (Wu 2005; Oxford and Theodorescu 2003). Early studies found this to be the case, from the first cloning of the mutant *HRAS* oncogene from the papillary carcinoma grade III-derived cancer cell line model, T24, in 1982 (Santos et al. 1982). In the intervening years,

a variety of studies have reported differing activating mutation rates of the *HRAS, NRAS,* and *KRAS* paralogs (Oxford and Theodorescu 2003), with recent studies finding rates varying between 15% and 30% (Czerniak et al. 1992; Jebar et al. 2005), depending on the case series and the relative composition of noninvasive versus invasive tumors. Consistent with its having a causal role in the pathogenesis of NMIUC, urothelial-specific expression of this oncogene in mice has been shown to trigger the formation of papillary urothelial hyperplasia that progresses low-grade, superficial papillary tumors (Mo 2007; Zhang et al. 2001).

Upstream of Ras are a variety of receptor tyrosine kinases, which when activated by ligand with associated phosphorylation changes, recruit *son of sevenless*-family proteins which catalyze the conversion of signaling inactive GDP-bound Ras to active GTP-bound Ras (Downward 2003). One of the most interesting findings of recent years has been that of mutation of fibroblast growth factor receptor 3 (*FGFR3*) in bladder tumors (Cappellen et al. 1999), especially NMIUC tumors (Billerey et al. 2001). Interestingly, it appears from a large series that mutation of FGFR3 versus mutation of one of the Ras genes is a mutually exclusive event (Jebar et al. 2005), suggesting that they mechanistically *phenocopy* or are redundant. Intriguingly, and suggestive of how differences in molecular circuitry may underlie differential clinical behavior, FGFR3 mutation has been associated strongly with lower grade and stage in UC (Billerey et al. 2001). While different studies have found different relationships between FGFR3 mutation and recurrence (Hernandez et al. 2006; Kompier et al. 2009; van Rhijn et al. 2001), a large recent series suggests that FGFR3 mutation is associated with a good prognosis regarding survival in UCs of the upper and lower genitourinary tract (van Oers et al. 2009). Another recent series suggests that while recurrence rates may not differ substantially between mutant and wild type FGFR3 tumors, mutations are retained in >80% of recurrences of mutant tumors, and recurrent mutant tumors are of a lower stage and grade than wild type recurrences (Kompier et al. 2009).

In contrast, muscle invasive bladder cancer has been associated with loss of tumor suppressor function, perhaps most strikingly, p53 (Wu 2005; Dinney et al. 2004), a central regulator of cell cycle, apoptosis, DNA repair, and programmed cell death (Mitra et al. 2007). For example, one early report that largely began the molecular definition of this "dual track" pathway of urothelial carcinogenesis observed high rates of p53 mutation in dysplasia, *carcinoma* in situ, and invasive lesions, while other lesions, including LOH (loss of heterozygosity, see below Section 9.3.2) of chromosome 9 were observed in papillary Ta lesions (Spruck et al. 1994). Supportive data for this notion include a key report that found that detection of p53 nuclear accumulation (a hallmark of p53 mutations, which often result in buildup of a more stable mutant protein) was found to be a prognostic factor for recurrence of T1–T4 stage cancer, independent of traditional clinicopathologic factors (Esrig et al. 1994), and also the finding that p53 staining may identify a population of NMIUC tumors with high risk for progression to invasive disease (Masters et al. 2003). Loss of the RB tumor suppressor has also been strongly implicated in invasive lesions (Cairns et al. 1991). Consistent with both of these key regulators having an important role in invasive disease, pathologic studies have suggested that

they may have cooperative effects in progression (Cote et al. 1998), while two transgenic mouse model studies have shown that urothelial expression of the SV40 large T antigen, a viral oncoprotein, which inhibits these key pathways, causes development of *carcinoma* in situ and invasive lesions (Grippo and Sandgren 2000; Zhang et al. 1999).

Finally, given the richness of complexity in cancer biology, it bears mentioning that there are myriad exceptions to the theme of noninvasive disease being mediated by receptor tyrosine kinase-Ras pathways while invasive disease is mediated through loss of tumor suppressors. Two examples that come to mind include loss of expression at the *CDKN2A* locus (the p16/INK4a tumor suppressor) often occurring early in noninvasive disease (Orlow et al. 1995, 1999) and the findings of overexpression of epidermal growth factor receptor and other EGFR-family members (tyrosine kinase receptors like FGFR3, that activate the Ras pathway) in muscle-invasive tumors (Kiyoshima et al. 2005; Neal et al. 1990) and those with poor prognosis (Chow et al. 2001).

Despite compelling findings that different molecular pathways are associated with or can be demonstrated in model systems to even cause NMIUC as compared to MIUC, it bears mentioning that substantial heterogeneity in natural history exists even with these groups. Within NMIUC, approximately two-thirds of treated patients suffer from recurrence, while one- fourth to one-third suffer either the secondary development of, or progression of their primary tumor to, invasive disease (Dalbagni 2007). In a large case series of patients with invasive disease treated by radical cystectomy (Stein et al. 2001), substantially different outcomes are observed comparing patients by tumor stage, where the probability of 5-year recurrence-free survival ranges ~80% in T2 to ~45% in T4a. Involvement of regional lymph nodes, which includes >20% of such patients, is a strong, poor prognostic factor, imparting a 35% probability of 5-year recurrence-free survival as compared to ~80% probability in nodal negative patients (Stein et al. 2001). In short, there is a greater heterogeneity of tumor behavior than clinicopathologic characteristics or single molecular targets (as described above) can alone predict.

Thus, if different molecular pathways can define the two clinical patterns of bladder cancer and direct their clinical behavior, then there must exist subcircuits in these prevailing pathways that may modify their clinical behavior within matched disease states. Molecular profiling of bladder cancers from the same clinical stage, coupled with analysis based on follow-up information, should, in principle, be able to discover biomarkers that are associated with such disease course and be independent of classical clinicopathologic variables. After independent and prospective validation, prognostic strategies based on these biomarkers might be of use to plan clinical management, develop new interventions, or rationally design clinical trials – in short, to personalize cancer management. Addition of these to nomograms that include the traditional factors mentioned above (Shariat et al. 2008a) would appear to be the ideal way to provide enhanced prediction.

Discovery and validation of biomarkers of prognosis or treatment response prediction has used novel technologies (see Sect. 9.2). For management of noninvasive disease, authors have explored the essential prognostic questions of whether a NMIUC

will (1) recur or (2) progress to muscle-invasive disease (see Sect. 9.3, Molecular Nomograms for NMIUC). In contrast, for muscle-invasive disease, investigation has focused on (3) prediction of response to therapies, especially cisplatin-containing chemotherapeutic regimens (see Sect. 9.4 Molecular Nomograms for MIUC). Novel, predictive approaches may allow systematic identification and prediction of efficacy of chemotherapeutic drugs for personalized therapies (see Sect. 9.5).

Such personalized strategies for prognostic outcome prediction have already been applied in other tumor types. For example, in breast cancer, several molecular assays are now commercially available that risk-stratify patients based on a molecular nomograms derived from gene expression studies (Dowsett and Dunbier 2008). One of these assays, Oncotype DX, not only stratifies estrogen-receptor-positive, lymph node-negative patients based on risk of disease recurrence (Paik et al. 2004), but also, likely due to its inclusion of many genes related to hormone response (Dowsett and Dunbier 2008), predicts magnitude of benefit of addition of chemotherapy to tamoxifen (Paik et al. 2006). While in bladder cancer there are not yet similar molecular nomograms for prognosis and prediction, they are on the horizon (Zieger 2008). The sections below provide some perspective into how such assays are being developed and how they might be implemented in the clinic.

9.2 Key Technologies in Biomarker Development

If any predictive or prognostic tool based on or containing molecular measurements, i.e., a molecular nomogram, is to be developed and implemented for UC, it would logically require the ability to profile clinical material of a cancer patient (tissue, serum, urine, etc.) for biochemical species (DNA, RNA, protein, etc.) in a multiplexed fashion. Such a technology would then provide a constellation of datapoints that together, based on a validated algorithm, may be interpreted to provide useful information regarding a disease's prognosis or therapeutic susceptibility. Though the technologies and analysis employed for development of such assays are complex and comprise their own area of science, *Bioinformatics*, a very basic understanding of the assays in question is necessary to interpret the results. For a more complete understanding of this topic, we refer the reader to several excellent texts (Pevsner 2003; Xia 2007; Sensen 2005). What follows is a brief primer, designed for the clinician, on a few key technologies in the area of genomics and proteomics, including only those that have been applied to the studies of prognosis and therapeutic prediction discussed herein.

Few technologies have revolutionized molecular profiling to the extent that microarray gene expression profiling has, by allowing high throughput determination of a substantial subset of the mRNA complement of a cell or tissue type in question, which can be compared across conditions or among individuals. Not surprisingly, there now exists an entire suite of microarray studies of bladder cancer, many of which were designed to discover biomarkers of prognosis or predict therapy. A DNA microarray is a highly miniaturized grid of hundreds to tens of thousands

of nucleic acid probes, representative of many known biologic sequences, affixed to a solid framework. Such probes sample the mixture of RNA species present in the analyte based on the principle that the base pairs of DNA have the property of hybridizing to their complementary sequences, allowing gene-specific hybridization and quantification thereof. Hence, RNAs are isolated and stabilized from a biological sample, and through one of several strategies, "labeled," usually fluorescent, RNAs reflecting the same abundance of the initial species are generated from them. Finally, this mixture is hybridized to the entire array, specific mRNAs hybridize to specific probes, and the abundance of each of hundreds to tens of thousands, depending on the platform, may be calculated based on the intensity of fluorescence at the site of the probes complementary to them (Fig. 9.1a).

Two popular platforms of DNA microarrays are *oligonucleotide microarrays*, where short DNA probes of approximately 20 base pairs are synthesized directly on a glass chip (Lockhart et al. 1996) and *cDNA microarrays*, where cDNA probes complementary to known genes are spotted on specially prepared microscope slides (Schena et al. 1995). Both types have been adapted to study bladder cancer, and both types include custom and commercially available applications. Several groups have employed commercial oligonucleotide DNA microarrays (Dyrskjot 2003; Dyrskjot et al. 2003; Sanchez-Carbayo et al. 2006a), which represent a popular approach with standardized data analysis, allowing convenient interinstitutional data sharing (Barrett et al. 2005). In contrast, cDNA microarrays employ simultaneous co-hybridization of different wavelengths (color) fluorescently labeled nucleic acids isolated from individual "test" samples and the same "reference" sample (Schena et al. 1995; Duggan et al. 1999). The data output of this sort of assay is generally in the form of a ratio between the gene expression from the test sample to the reference sample. While cDNA arrays are commercially available, many also have been custom-designed and constructed to assay expression of specific genes of interest (Cheung et al. 1999), as one might envision being the case for development of a molecular nomogram.

Development of the software and hardware necessary to perform data analysis for such a massive number of parallel measurements has become really a science of its own, reviewed recently (Butte 2002; Roberts 2008; Wang 2008). Myriad programs have been written that organize, categorize, and infer higher level relationships between microarray data to the end of finding meaningful biology inside such a complex cloud of data (Quackenbush 2002; Wu 2001). Two general types of approaches necessary for understanding biomarker discovery projects in bladder cancer include analyses that are *unsupervised* or *supervised*. Unsupervised approaches are designed to identify similarity between samples based on expression data, with no a priori grouping of the samples, and allowing subsequent examination of whether samples of similar interesting characteristics are grouped together based on gene expression alone. One key type of algorithm for visualization of such relationships includes hierarchical clustering that uses programs, which create nested groups of samples based on their similarity of their gene expression (Eisen et al. 1998). Often, clustering is performed in two dimensions, where genes are also sorted based on their similarity of gene expression across the samples (Fig. 9.1b).

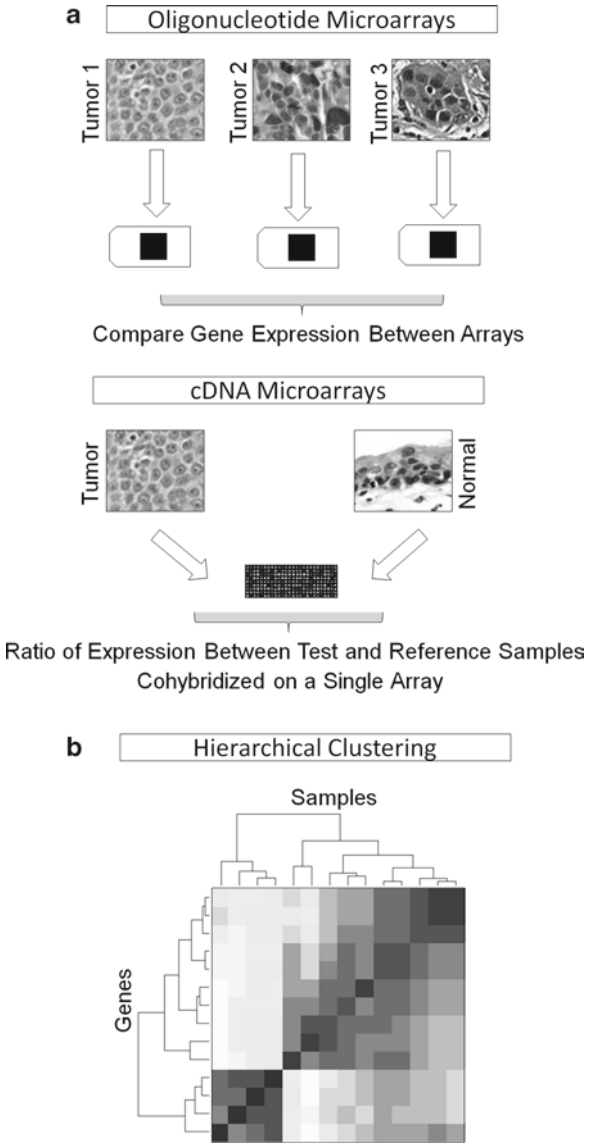

Fig. 9.1 Different microarray technologies, similar high-throughput data. (**a**) Oligonucleotide microarrays employ short ~20 base pair probesets complementary to thousands of genes to interrogate fluorescently labeled RNA species isolated from biological samples, here represented by three muscle invasive urothelial carcinoma (*MIUCs*). Each array is scanned by a high resolution scanner, and after normalization and adjustment by computer software, quantitative data for expression of each probeset's cognate transcript may be compared between samples. In contrast, cDNA microarrays function through co-hybridization of differentially fluorescently labeled test and reference samples, here illustrated by comparison of a MIUC to normal urothelium, though cell line RNA or any other abundant reference may be used. These arrays output data in the form of ratios of transcript expression between test sample to reference, and test samples may be compared between arrays because of the use of the same reference. (**b**) Hierarchical clustering represents a popular means of visualization of the relationships between samples across gene expression and gene expression across samples. Algorithms group samples and genes based on a similarity metric (e.g., correlation), and distance between samples on the cluster tree is proportional to their degree of difference

In contrast, and perhaps most adaptable to targeted discovery of gene expression patterns associated with clinicopathologic characters of interest (i.e., recurrence of a NMIUC) is supervised data analysis. Such a strategy often tries to develop a defining pattern or "signature" of gene expression that classifies tumors based on a known property. One important distinction in the case of such a discovery strategy is between signatures whose performance is only evaluated through cross-validation, roughly various strategies splitting a dataset into "training" (for supervised development of the signature) and test sets (for evaluation of such a signature), and signatures developed then validated on truly independent samples.

The question of validation and generalization of these kind of signatures deserves some attention and speaks to the core of the issue of translating them from the arena of investigation into validated, interinstitutionally evaluated signatures that could be appropriately integrated with traditional clinicopathologic factors into something that might be called a "molecular nomogram." The great advantage of microarray-based biomarker discovery is the ability to screen thousands of targets for association with characteristics of interest. However, this property also provides some of its greatest challenges, related to developing nosologic, prognostic, or predictive nomograms based on such a high number of potential variables. Because of problems with false discovery rate (FDR), where, given so many variables, many significant differences may be discovered by chance alone (Gusnanto et al. 2007), and "overfitting," where classification algorithms perform better when they include more variables because of the fit a dataset's "noise" rather than true biologic signal (Soukup et al. 2005; Wiemer and Prokudin 2004). In particular, correction for multiple hypothesis testing becomes indispensable, as traditional p-values become meaningless when thousands of hypotheses are tested (Pawitan et al. 2005; Shaffer 1995). In the end, the wide variety of algorithms, microarray platforms, differences in institutional protocols for analyte processing, not even to mention the importance of differences in genetic background between patient populations (Hunter 2006), calls for rigorous, independent, and interinstitutional validation of molecular nomograms for cancer patients. Additionally, the robustness of gene-expression-based signatures is commonly validated and extended through use of different technologies, including quantitative real time RT-PCR and IHC.

Such validation has greatly benefited from the development of tissue microarrays (TMAs) for expression of proteins of interest (Kononen et al. 1998; Shergill et al. 2004). In TMAs, hundreds to even thousands of tissue cores from representative areas of traditional paraffin tissue blocks are carefully inserted in a grid into a new paraffin block. These TMA blocks can then be sectioned, treated, and stained for IHC with specific antibodies in the same manner as any tissue section. The advantage of this technique is in both throughput – hundreds of sections may be sampled in the same assay at a fraction of the cost of staining individual sections – and standardization – all cores on a slide are stained with the same antibody solution, concentration, and treated identically. Then, as has been done in prior biomarker studies of bladder cancer (Shariat et al. 2004), serial sections may be stained for other molecules of interest providing multiplexed data that can, in theory, be used to build predictive nomograms.

9.3 Towards Molecular Nomograms for Nonmuscle-Invasive Urothelial Carcinoma

9.3.1 Microarray Studies

Gene expression studies targeted to discover biomarkers for diagnosis, classification, and prognosis in bladder cancer began with a microarray study of cell lines by Sanchez-Carbayo et al., in 2002. This study employed cDNA microarrays including ~9,000 genes run for nine bladder cancer cell lines derived from various stages and grades of tumors, including one cell line, SCABER, derived from a squamous cell carcinoma of the bladder. They found differential expression between the cell lines based on stage, grade, and histology. In particular, expression of keratin 10 was high in invasive tumor-derived cell lines compared to a noninvasive tumor-derived cell line, while the bladder squamous cell carcinoma-derived cell line, SCABER, evinced increased expression of caveolin 1. The group next employed TMAs of bladder tumors to validate the clinical significance of these genes, finding that they were associated with the presence of squamous differentiation, stage, and tumor grade. They also used a hierarchical clustering strategy to examine the relationship of these cell lines to each other, finding that invasive tumor-derived cell lines clustered differentially in concordance with their known lesions of the p53 and RB pathways, hallmarks of invasive tumor status (Wu 2005; Spruck et al. 1994). Consistent with the notion of molecular subcircuits within prevailing noninvasive or invasive molecular pathways modifying clinical behavior (see Sect. 9.2), they identified expression of three genes *zyxin*, *E-cadherin*, and *moesin*, which were different from these p53/RB-related groups. Expression of these genes, again assayed on a TMA platform, was significantly associated with bladder cancer stage and grade. Expression of *moesin* was found to be significantly associated with overall survival in a subset of 69 patients where clinical follow-up was available (Sanchez-Carbayo et al. 2002).

In a landmark study, Dyrskjøt et al. used oligonucleotide microarray analysis of 40 bladder tumors of varying stage and grade and normal urothelium (Dyrskjot et al. 2003). Using ~1,700 genes which were differentially expressed between tumors and normal urothelium, the group performed an unsupervised hierarchical cluster analysis. This finding separated the tumors into clusters representing three major stages, Ta, T1, and T2-4, as did the same algorithm when applied to a subset of 88 differentially expressed genes selected for being annotated at the time by the Cancer Genome Anatomy Project as "cancer-related." Based on the robustness of this finding to different subsets, the group went on to use a cross-validation strategy (see Sect. 9.2) to discover a stage classifier model using 32 genes to obtain correlation to pathologic staging. Intriguingly, they found that when they applied this classifier to the data from which it was generated, it classified several cases of Ta tumors that were noted on pathologic evaluation to exhibit concurrent *carcinoma* in situ as T2+.

When this classifier was applied to an independent test set of 68 samples profiled with a different microarray platform, they observed accurate classification of 84% of stage Ta, 50% of stage T1, and 74% of stage T2+ tumors, while

the Ta tumors misclassified as stage T1 or T2 showed a statistically significantly increased rate of progression. Compellingly, given the prognostic importance of progression of Ta tumors, they derived a 26-gene progression classifier, which correctly classified 75% of NMIUC tumors based on future recurrence in cross-validation studies.

The Cordon-Cardo group also studied the same diagnostic problem, classification of NMIUC versus MIUC, using a larger cDNA microarray platform (Sanchez-Carbayo et al. 2003). Again, employing hierarchical clustering in an unsupervised manner, early-stage tumors clustered together and separately from invasive UC, while gene expression data also segregated noninvasive lesions into papillary lesions versus *carcinoma* in situ, consistent with the Ørntoft group's findings (Dyrskjot et al. 2003) and with prior studies suggesting differential molecular etiologies for Ta and Tis lesions (Spruck et al. 1994). Additionally, similar to before, they were able to use gene expression data to find a subgroup of early-stage tumors that evinced expression profiles similar to organ-confined invasive lesions. The Cordon-Cardo group, focused additionally on validating, on the level of IHC with TMAs, some of the key targets differentially expressed between noninvasive- and muscle-invasive cancer. The genes, *cytokeratin-20*, *neuropilin-2*, *p21,*and *p33ING1* were analyzed usingTMAs. While expression of *cytokeratin-20*, *neuropilin-2*, *p21*, and *p33ING1* were all significantly correlated with tumor stage and grade, only *p33ING1* was associated with overall survival (Sanchez-Carbayo et al. 2003).

In summary, these groundbreaking studies proved several principles relevant to the development of molecular nomograms for NMIUC. First, they discovered that there exist genes whose expression pattern is dramatically different between noninvasive and invasive bladder cancer (Dyrskjot et al. 2003; Sanchez-Carbayo et al. 2002; Sanchez-Carbayo 2003). Secondly, they found that in some cases, genes expression in NMIUC tumors that appears similar to invasive tumors might be able to predict recurrence or progression (Dyrskjot et al. 2003). Finally, they proved that such targets might be able to be detected using IHC or other technologies (Sanchez-Carbayo and Cordon-Cardo 2003; Sanchez-Carbayo et al. 2003; Sanchez-Carbayo 2003). These findings would lay the foundation for approaches to target even more closely the key clinical questions for management of superficial bladder cancer: prognostication of recurrence and progression.

9.3.2 Recent Reports: Towards Molecular Signatures of Recurrence and Progression

Since the time of the above reports, several additional studies have approached the discovery of prognostic signatures for superficial cancer in a more targeted manner. To the issue of key molecular lesions characterizing superficial cancer (Wu 2005), Lindgren et al. employed parallel gene expression profiling, mutational analysis of *p53*, and *FGFR3*, and loss of heterozygosity (LOH) analysis of chromosome 9

(Lindgren et al. 2006). Chromosome 9 LOH, a common lesion in bladder cancer, often reflects loss of the *CDKN2A* locus, which expresses the tumor suppressor/cell cycle regulator p16/INK4a (Hoglund et al. 2001). (LOH, which means the loss of one of the two copies of a chromosome that is normally present in cells, generally reflects loss of a normal functional allele within the region, while the remaining allele(s) may be mutated or otherwise nonfunctional. This is common in the case of tumor suppressors, where loss of a remaining functional allele in the context of genomic instability would then confer a selective advantage to cells. This may occur over an extended region of a chromosome, just as entire arms of chromosomes may also be lost). Their careful analysis allowed them to subcategorize even NMIUCs, with three clusters enriched for grade 1, grade 2, and grade 3 tumors. Interestingly, correlating their mutation data (46% of tumors were FGFR3 mutation positive) to microarray data, they found that mutation of FGFR3 was associated with increased expression of this key oncogene. Interestingly, they identified clusters of genes which were expressed differentially based on mutations of FGFR3 and p53, where a cluster of cell cycle genes and a cluster of protein synthesis/ribosomal-related genes were increased in activity in tumors bearing mutant FGFR3 but wild type p53. Regarding their LOH analysis, they identified a set of >100 genes with differential expression to find whether the tumor harbored on LOH of chromosome 9; as one would expect, analyses showed that they were enriched for transcripts from that chromosome. Finally, and more specifically to the issue of molecular prediction, they identified 49 genes with differential expression between tumors with and without recurrence (Lindgren et al. 2006). In summary, this report provided key connections between prevailing molecular lesions in superficial cancer and how they interface with differences in gene expression that may provide prognostic data for tumors.

Another report targeted even more closely a key clinical phenotype in NMIUC: progression. Wild et al. reported microarray profiling studies of 67 bladder cancers, finding 225 genes with a high level of correlation to distinct classes of tumors, including a unique pattern of expression in Ta and a higher degree of gene expression similarity between stage T1 and pT2 tumors than between T1s and Tas (Wild et al. 2005). Interestingly, a pattern of gene expression could be identified characterizing invasive solid growth pattern, distinguishing it from invasive papillary growth, including the genes *FGFR3* and *CTSE*. Most importantly, however, Wild et al. developed a predictor for progression using 31 genes. In cross-validation studies, this algorithm exhibited accurate classification of 33/42 tumors, sensitivity 85.7%, specificity 71.4%. Refining this signature down to 10 genes and employing a threshold for strength of prediction, they observed correct classification of 31/33 cases with prediction calls above threshold. In terms of the key issue of validation of their microarray results, they validated the role of *CTSE* as a papillary growth biomarker ($P < 0.0001$) on the level of IHC on a large tissue microarray set of bladder cancers (>700).

The most mature discovery project of molecular nomograms for NMIUC consists of two recent reports by the Dyrskjøt et al. group. The first report profiled,

using a massive >60,000 probe oligonucleotide microarray platform, 29 bladder tumors, including 16 patients with later disease progression and 13 without later progression, and used a cross-validation scheme based on the 100 best classifying genes to develop a 45-gene signature of progression (Dyrskjot et al. 2005). Most importantly, they evaluated this signature in an independent set of 74 tumors, including 60 tumors from patients with no progression of the disease (median follow-up time, 56 months) and 14 tumors from patients with later disease stage progression (median time to progression, 13 months). Mimicking how such technology might be implemented in an actual clinical setting, this group developed a 60 base pair "60-mer" oligonucleotide microarray including both the 45-gene progression signature and their previously reported 32-gene stage classifier, which had provided prognostic information (Dyrskjot et al. 2003). Applying this classifier to the tumor set profiled on this platform and using as a criterion for classification that a tumor's distance between classes be greater than 30%, they classified 51 of 74 test samples, finding a significant concordance between actual and predicted progression ($P<0.03$). This difference was also apparent by Kaplan-Maier curves estimating progression risk over time ($P=0.03$). Using their prior 32-gene cancer stage classifier, they found again a significant correlation between disease outcome and stage classifications made ($P<0.004$).

This same group reported recently a multinational and multi-institutional validation study of the signatures developed above (Dyrskjot et al. 2007). First, because they meant to employ a similar 60-mer platform for validation, they constructed a 60-mer microarray platform using all genes from every signature they had reported to that point (Dyrskjot et al. 2003; Dyrskjot et al. 2005; Dyrskjot et al. 2004). They then reassessed from among these genes as based on their performance on the new platform by reprofiling all of the prior cases and regenerating expression signatures. This resulted in a 52-gene stage classifier, a 20-gene recurrence classifier, and an 88-gene progression classifier. Importantly, maintaining strict independence of training and test sets, they evaluated tumors of 404 patients from Denmark, Sweden, Spain, France, and the United Kingdom, comparing predictions with diagnoses and outcomes. For the 52-gene stage classifier, a highly significant concordance was noted between their predictions and actual pathologic diagnoses, $P<0.0001$, and the aforementioned phenomenon, where noninvasive tumors misclassified as invasive tumors exhibited substantially reduced disease-specific survival. For the 88-gene progression signature, they observed a cumulative probability of disease progression of 40% in progression- predicted cases, whereas in cases without the prediction the probability was <15%, $P<0.001$. Interestingly, and speaking of the utility of molecular nomograms providing added prognostic information as compared to traditional clinicopathologic risk factors, the authors found that the predictions were independent of age, sex, stage, grade, and intravesical treatment in multivariate analysis. This resulted in correct classification of 37 of 56 progressing cases and 158 of 238 nonprogressing cases, or sensitivities and specificities both 66%, with positive and negative predictive values of 32% and 89%,respectively (Dyrskjot et al. 2007).

One recent report used a meta-analysis of a whole suite of bladder cancer microarray studies to develop a signature of progression among NMIUCs (Wang et al. 2009). This undertaking, by Wang et al., compiled most of all the aforementioned studies into a master dataset with gene expression profiling representing 631 samples and 241,298 probe sets, lending substantial statistical power to detect genes related to a variety of disease states, including tumor grade, muscle invasion, recurrence, progression to higher stage, positive lymph node status, death from disease as well as overall aggressiveness. From these meta-profiling data, they developed a 96 gene quantitative RT-PCR based assay and applied it to an independent set of 107 bladder cancer patients, including 96 cancers (42 superficial nonprogressing tumors, 54 invasive or progressing to invasion), and 11 benign bladder samples. Of the 96 targets assayed, 57 were significantly associated with muscle invasion, and this subset was used as a predictive signature for progression.

This group employed this 57-gene signature to classify Ta and T1 tumors as high- or low- progression risk, and examined whether these groups exhibited different progression outcomes. They found a higher rate of progression at 2 years in the high progression risk-classified tumors as opposed to the low-progression risk group (45% versus 12% $P=0.003$). This effect also remained significant when applied to a T1 stage subset of tumors (61% versus 22% progression for low-risk patients; $P=0.02$), suggesting that the predictive information garnered from such a signature may add key information beyond stage status alone as a risk factor for progression. The authors further noted that among the T1 stage patients at a 1-year time point, the risk of progression in high-progression-risk-classified patients was 61% compared to 7% in low-progression-risk-classified patients. In multivariate analyses, they found that though stage and gender proved to predict progression to muscle invasion most significantly, the 57 gene signature continued to provide significant independent predictive information ($P=0.002$). Another group recently published a urine-based quantitative RT-PCR-based assay for the detection and classification of bladder cancer, suggesting that such a methodology might be adaptable to a noninvasive testing system (Holyoake et al. 2008).

In summary, these studies, focusing on classification and prediction of recurrence or progression in superficial bladder cancer prove the principle that molecular profiling can discover molecular biomarkers that characterize disease stages with high fidelity. A few of these studies employed advanced statistical and informatic methodologies to validate signatures in independent test sets (Dyrskjot et al. 2003; Dyrskjot et al. 2005), or most compellingly, in the setting of multi-institutional prospective validation (Dyrskjot et al. 2007; Wang et al. 2009). Last, a couple of studies have shown that signatures derived from these studies can actually be implemented through quantitative RT-PCR or other assays into what may actually be characterized as a "molecular nomogram" for detection (Holyoake et al. 2008) or prognostication of progression from superficial disease (Dyrskjot et al. 2007; Wang et al. 2009). Below, we examine what similar studies have shown in the case of invasive bladder cancer, focusing on prediction of therapy or survival outcomes postcystectomy.

9.4 Towards Molecular Nomograms for Muscle-Invasive Urothelial Carcinoma (MIUC)

Muscle-invasive, stage T2+ bladder cancer is commonly treated with radical cystectomy and pelvic lymphadenectomy with curative intent, with locally advanced, particularly nodal-positive cases treated with multiagent chemotherapy (Ghoneim and Abol-Enein 2008). However, this results in evolution of metastatic recurrence, most commonly at an extrapelvic site, in approximately half of the patients within 2 years (Stein and Skinner 2006). Recurrence happens at a higher frequency in nodal-positive patients (a key prognostic factor for survival), but also occurs in nodal-negative patients(Stein et al. 2001). Thus, key questions for the management of these patients include, generally, prognostication of metastatic recurrence risk, as well as, more specifically, the related decision of whether to treat (i.e., prognostication for nodal-negative or other low-risk patients) and how to treat (i.e., therapeutic prediction in inoperable, nodal-positive, and other high-risk patients). While this area perhaps has not matured to the same extent as has development of molecular nomograms for NMIUC, key studies suggest that these applications are both possible and promising.

9.4.1 Immunohistochemical Technologies Applied to MIUC

In a key report proving the principle for the use of TMAs and IHC for multiple targets to discover signatures of prognosis, Shariat et al. performed IHC for p53, p21, pRB, and p16 on serial sections of paraffin embedded archival tissue from 80 patients resected at cystectomy (Shariat et al. 2004). They then performed multivariate analyses, incorporating both immunohistochemical staining data and patient clinicopathologic factors into models and examining their independent contribution to prognosis. They generated a model that included these markers as combined variables, finding that both p53/p21 status and lymph node status were independently associated with cancer progression ($P \leq 0.047$) and death ($P \leq 0.036$). However, suggesting again that multiplexing biomarkers for the development of prognostic nomograms will prove fruitful, they found that an increased number of dysregulated immunohistochemical biomarkers was also independently associated with higher risk of cancer progression and mortality (Shariat et al. 2004). Similarly, more recently, the same group has found that p53, Bcl-2, caspase-3, and survivin provide distinct correlations with tumor stage, grade, and lymphovascular invasion. Again, the total number of altered markers was an important prognostic factor in disease recurrence and disease-specific survival (Karam et al. 2007), and as was the case in their most recent report, suggesting that an increased number of dysregulated cell cycle markers increased the prognostic accuracy of TNM staging-based prognostic nomograms for disease recurrence and cancer-specific mortality by 10.9% and 8.6%, respectively (Shariat et al. 2008a).

9.4.2 Microarray Studies of Survival Outcomes in MIUC

As we saw for superficial bladder cancer, the use of high throughput expression profiling can enable the detection (Holyoake et al. 2008), classification (Dyrskjot et al. 2003; Holyoake et al. 2008), and prognostic risk-stratification for human bladder cancer patients (Dyrskjot et al. 2003; Dyrskjot et al. 2005; Dyrskjot et al. 2007; Wang et al. 2009). Consistent with this phenomenon being general to MIUC as well, one early study began to show that gene expression differences might exist with high- and low-risk MIUCs. Modlich et al. profiled 20 invasive and 22 superficial bladder tumors with a cDNA-based microarray platform (Modlich et al. 2004). They found, as had been shown in other studies, that gene expression differences characterizing invasion versus superficial disease may be discovered in such studies. However, they characterized a new class of genes, which were differentially expressed *among invasive tumors* based on their status as high risk. Not only did they find that these poor prognosis cases clustered together on a distinct branch of dendrogram, but they also found that two clusters of genes determined this difference. One set of genes coded for proteins related to cell cycle regulation, transcription, cell-matrix, and cell-cell adhesion. Another key cluster of genes coded for targets controlling the cell cycle, cell proliferation, and growth (Modlich et al. 2004).

Importantly, a recent report, again by Sanchez-Carbayo et al., defined gene expression changes associated with poor prognosis and outcome within invasive bladder cancers (Sanchez-Carbayo et al. 2006a). In this study, they employed oligonucleotide microarrays to profile 33 superficial, 72 invasive lesions, and 52 normal bladder mucosae. Not surprisingly, given the aforementioned results, they were able to develop a stage classification scheme that yielded an accuracy of 82.3% in cross-validation studies, using the top 25 genes most differentially expressed between superficial and invasive disease. However, consistent with the concept of subprograms of gene expression underlying survival differences within invasive stage disease, they found that they could, with 90% overall accuracy in cross-validation studies, predict disease outcome, using 100 genes most significantly differentially expressed between survival groups. Finally, using a supervised approach to find the "overlap" of genes related to both positive nodal status (a very significant negative prognostic factor (18)) and poor overall survival, they uncovered a poor outcome signature of 174 microarray probes. Evaluating the contribution of these probes individually and as a group in terms of their ability to predict lymph node status and disease-specific survival, they found that these 174 probes were significantly associated with lymph node status and with disease-specific clinical outcome (both $P < 0.0001$).

This group has also used transcriptional profiling to provide targets for detecting bladder cancer from patient serum proteins using antibody arrays, a platform where commercial antibodies, specific for the target of interest, were printed onto nitrocellulose covered slides, to which labeled serum proteins were hybridized and fluorescently imaged for quantification (Sanchez-Carbayo et al. 2006b). The first antibody array, containing 254 antibodies against 183 microarray targets was used to evaluate

differences between serum samples from 37 bladder cancer patients and 58 controls, finding that serum protein profiles, analyzed by hierarchical clustering, could distinguish patients harboring a bladder tumor from normal controls with a sensitivity of 89.2% and a specificity of 96.5%. They then selected a subset of these antibodies that had proven useful in the first analysis to examine whether they provided prognostic information, this time among only the 37 cancer patients. They identified, using an unsupervised hierarchical clustering strategy applied to data from this second smaller array, two distinct clusters, patients that were high versus low risk, and found that this strategy could distinguish patients based on overall survival ($P=0.05$) (Sanchez-Carbayo et al. 2006b).

9.4.3 Recent Reports: Molecular Signatures Predicting Response to Therapy

Combination chemotherapy may be used for muscle-invasive bladder cancer in the neoadjuvant or adjuvant settings with cystectomy, as well as primary therapy in the setting of advanced, inoperable, and metastatic disease (Milowsky et al. 2008). However, combination chemotherapy has been associated with substantial toxicity; for example, the classic four-drug regimen methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) has been associated with mortality in the range of 3–4% (Sternberg et al. 1985; von der Maase et al. 2000). Because of this toxicity, the limited responses observed, and the fact that the intersection of these parameters means operationally that a substantial fraction of treated high-risk patients will suffer from substantial side effects without benefit, a molecular nomogram predictive of therapeutic response would be of great utility. Several studies have made great progress towards this end.

One early study from our group proved the principle that gene expression-based predictive models could not only be predictive of single-drug therapeutic response, but could also be combined to successfully predict combination responses (Havaleshko et al. 2007). This study derived dose-response data in vitro for the drugs cisplatin, gemcitabine, and paclitaxel, across a panel of 40 bladder cancer cell lines and used oligonucleotide microarrays to profile their baseline gene expression to develop prediction models. We then validated single-drug response predictions for the cell lines in the setting of cross-validation and tested doublet responses in vitro, comparing empirically determined responses to those predicted *in silico*. We observed a highly significant concordance between prediction and behavior, proving that, in principle, this kind of strategy might work for bladder cancer (Havaleshko et al. 2007).

Closer to the bedside, combination neoadjuvant chemotherapy has been shown significantly in trials (Grossman et al. 2003) and trended in a meta-analysis (Neoadjuvant chemotherapy in invasive bladder cancer 2003) to have survival value for patients with invasive disease. Notably, survival advantage was associated with lack of detectable residual tumor in the cystectomy resection specimen

(Grossman et al. 2003). To the end of prediction of response in this setting, Takata et al. recently employed a cDNA microarray platform to profile gene expression from biopsies of 27 invasive bladder cancer patients who were subsequently treated with MVAC neoadjuvant chemotherapy (Takata et al. 2005). Based on supervised analyses designed to discover genes differentially expressed between 14 MVAC responders (defined as downstaging ≤ T1) and 13 nonresponders (defined as ≥ T2), they identified a set of 50 genes, 25 overexpressed, 25 underexpressed that differed significantly between these groups. Splitting their data into an 18-sample learning and 9-sample test sets, they developed a 14-gene MVAC response classifier based on the learning samples that correctly classified 8 of 9 test set samples. They then went on to validate so that they could also use quantitative RT-PCR to assess expression of these genes for classification purposes (Takata et al. 2005).

However, such signatures are of much greater potential value if they are independently and prospectively validated on novel test sets. To that end, recently, the same group performed a validation study of their predictive platform on 22 additional cases, profiled as before (Takata et al. 2007). They found that their signature correctly predicted response in 19 of 22 additional cases, providing a sensitivity of ~100% with a specificity of ~73%. Most importantly, in terms of potential application of this classification scheme for invasive bladder cancer patients, in this independent test set, the positive predictive value for the assay was approximated at ~79% (11 of 14 cases), while the negative predictive value was approximated at ~100% (8 of 8 nonresponders identified).

A second key study also examined cisplatin-based combination chemotherapy (both MVAC and gemcitabine cisplatin (GC) doublet therapy, which has been shown to have similar efficacy with reduced side effects (von der Maase et al. 2005). Als et al., affiliated with the aforementioned Dyrskjøt/Ørntoft group, used an oligonucleotide microarray platform to profile the gene expression of 30 patients with locally advanced or metastatic invasive bladder cancer (T4b, N2-3 or metastatic M1), and compared gene expression in a supervised manner amongst cases based on survival (Als et al. 2007). A set of 55 genes were discovered to be differentially expressed between these groups; interestingly, all 55 were underexpressed in patients with long-term survival. Also, they did not note any difference in the genes correlated with survival between patients treated with MVAC versus GC, suggesting that gene expression differences likely underlie the similar efficacy of these regimens. To answer the question whether any of these genes might be adaptable to traditional immunohistochemical validation, they selected two, emmprin and survivin, for IHC, and found that they were highly significantly associated with overall survival singly or in combination (all cases $P \leq 0.001$). Lastly, addressing the issue of specificity of these genes in response to these drugs as opposed to some other survival parameter, they found that emmprin positive and negative tumors exhibited differential response rates of 39% versus 74%, while survivin positive and negative tumors exhibited response rates of 47 and 70%, respectively. Double-negative tumors exhibited a high response rate of 82%, whereas double-positive tumors exhibited only a response rate of 27%. This worked out to an odds ratio for response in double-negative tumors of 11.9 (95% CI, 3.2–42.3).

Based on these findings, the authors proposed that if emmprin and survivin immunohistochemical detection were independently validated in large patient cohorts and staining were interinstitutionally standardized, these markers might be used to stratify patients into high and low likelihood of response groups, with likely responders treated by traditional cisplatin-based methods and likely nonresponders assigned to investigational therapies (Als et al. 2007).

In summary, while molecular nomograms for prognosis and prediction of therapeutic response in invasive bladder cancer have perhaps not reached the level of maturity of certain recent reports for the prognostication of, for example, progression of superficial tumors, the above studies prove key principles. Given the efficacy of cisplatin-based combination chemotherapy for this disease (Neoadjuvant chemotherapy in invasive bladder cancer 2003) and value of neoadjuvant chemotherapy in this disease (Grossman et al. 2003), which must be weighed against risk in delaying definitive surgical management (Weight et al. 2009) and toxicity (von der Maase et al. 2000), the key reports from the Takata group suggest that with independent and interinstitutional validation and standardization, molecular nomograms like their gene expression signature could truly be adapted to enable rational patient selection (Takata et al. 2005; 2007). In the setting of advanced and inoperable disease, the gene expression signature and validated immunohistochemical markers developed by Als et al. might prove equally useful in stratifying patients for standard of care versus investigational therapy. Below, we will conclude this chapter by entertaining several very recent innovations that hold great promise regarding the development of systematic ways to predict therapeutic responses for approved and investigational agents and for discovery of bladder cancer as a potential new therapeutic indication for agents under investigation in other disease types.

9.5 New Strategies

In the 40 years since former US President Richard Nixon declared war on cancer in 1971, a tremendous amount of data has been collected and analyzed regarding cancer cellular biology. One key initiative during the 1980s, predicated by the development of immortal cell lines derived from a variety of cancers, was the creation of the US National Institutes of Health's National Cancer Institute's in vitro screening of a panel of 60 cell lines (the NCI-60) for tens of thousands of candidate therapeutic compounds (Shoemaker 2006; Paull et al. 1989). While this screen has proven fruitful in many ways, including its contributions to the development of novel, approved compounds, such as the proteasome inhibitor, bortezomib (Rajkumar et al. 2005), application of genomics technology has led to several recent, promising strategies for drug development (Weinstein et al. 1997), including in the case of bladder cancer (Crunkhorn 2007). These strategies have employed various advancements in bioinformatics to usurp a vexing program that had hindered the field, i.e., the inability to predict patient trial outcomes a priori based

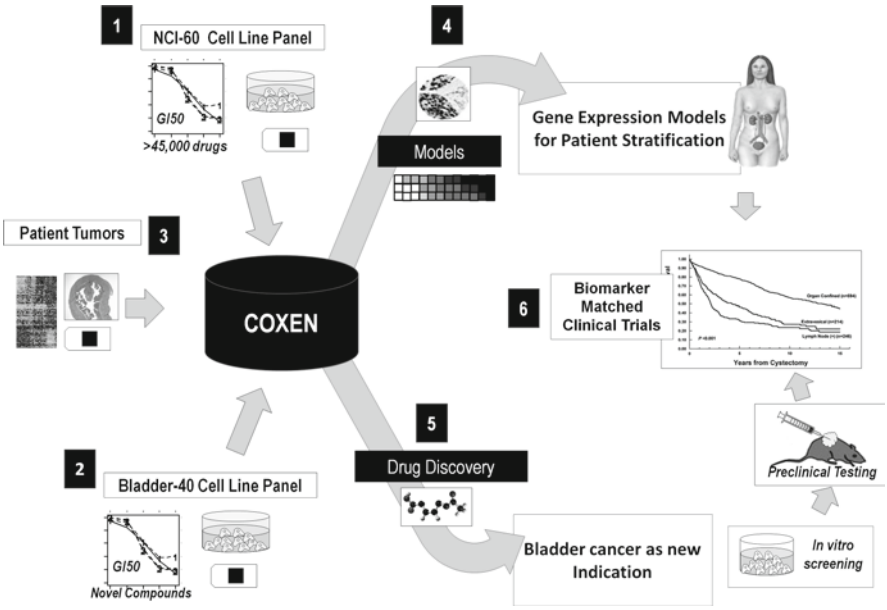

Fig. 9.2 A strategy for prediction of drug efficacy in clinical trials**.** Recent reports have for the first time used gene expression models derived from cell lines to predict therapeutic efficacy in clinical trials (Potti et al. 2006; Lee et al. 2007). One of these, the COXEN algorithm (Crunkhorn 2007), has retrospectively predicted the outcome of several clinical trials and is adaptable to drug discovery (Lee et al. 2007). Input data include: (1) in vitro activity (GI_{50s}) and gene expression data from the NCI-60 cell line screen (Shoemaker 2006) or (2) other tumor-specific activity and gene expression data. Then, (3) gene expression data for the tumor type in question is used to find subsets of sensitivity related genes from cell lines that maintain concordant expression patterns in vivo for use in developing (4) gene expression models predictive of clinical trial outcome. Concordant gene expression signatures may also be used to discover in a new tumor type (the Bladder-40) signatures of sensitivity to the >40,000 compounds tested in the NCI-60 panel, with the potential to (5) discover as yet untested compounds with high activity to evaluate in a preclinical setting. Either way, the goal is to move to (6) biomarker-linked randomized control trials, which can prospectively validate signatures for subsequent implementation in patient care

on shared gene expression profiles of drug sensitivity or resistance for cell lines in vitro and tumors in vivo.

Two recent reports have overcome these limitations (Potti et al. 2006; Lee et al. 2007). Building on our aforementioned studies using gene expression signatures to predict the sensitivity of a panel of 40 bladder cancer cell lines to doublet combinations of chemotherapeutic drugs in bladder cancer, we developed an algorithm called Co-expression Extrapolation (COXEN)(Lee et al. 2007). COXEN, which compares microarray gene expression profiles between cell lines and patient tumors and uses only genes that maintain concordant expression (correlations to other genes, without a priori reference to drug sensitivity, maintaining independence of learning and test sets) to generate signatures predictive of sensitivity or resistance and avoiding genes that are only specific to cell line culture. Initially, we reported

this algorithm's use to predict correctly the outcome of two breast cancer trials where microarray analyses were performed on tumorsand reported the discovery of an entire new class of compounds, the cytotoxic imidazoacridinones for bladder cancer, including NSC-637993, which we discovered to be effective at submicromolar levels.

More recently, we have validated the ability of this approach to predict the outcome of other clinical trials, including a new report showing that such signatures are able to predict the outcome of an additional seven different clinical trials involving ~500 patients with three types of cancers (including bladder cancer), from widely different geographic regions, and including the Takata et al. neoadjuvant chemotherapy patients and Als et al. chemotherapy patients discussed above (Williams et al. Cancer Research, 2009). Figure 9.2 summarizes the COXEN strategy. This and other advanced bioinformatic predictive and discovery strategies hold great promise for the ability to a priori identify and stratify patients for maximal benefit and avoidance of untoward toxicity with standard of care therapies (Als et al. 2007), while allowing rational trial design to reduce the necessary size and cost of trials of novel agents (Simon 2008).

9.6 Concluding Remarks

Though molecular nomograms for prognosis or therapeutic prediction are not yet in routine use for human bladder cancer, the above reports speak of the promise and utility of their development, and one can easily envisage a day in the near future when their application could be a part of routine diagnostic workup and management (Zieger 2008). In fact, several of these studies specifically address the conundrum that we believe explains why many biomarkers have not yet fruitfully entered the clinic for a variety of cancer histologies: the need for interinstitutionally validated and standardized assays providing clinically actionable data. While in bladder cancer, implementation of nomograms based on such markers has lagged behind other diseases, the studies and strategies discussed in this chapter show that their time is nearing.

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Chapter 10
Practical Approaches to the Management of Superficial Bladder Cancer

David A. Levy and J. Stephen Jones

Abstract Bladder cancer has the highest recurrence rate of any malignancy. Although most patients with bladder cancer can be treated with organ-sparing therapy, many experience either recurrence or progression, creating an imperative need for accurate and diligent surveillance. Therefore, the foci of this chapter include the management of noninvasive bladder cancer and the strategies for surveillance of tumor recurrence and progression.

In this chapter, we review; the epidemiology, etiology, pathophysiology, clinical and diagnostic evaluations, available molecular markers for disease as well as the current American Urological Association (AUA) Guidelines Panel Recommendations, and therapies for superficial and recurrent bladder cancer.

10.1 Introduction

Bladder cancer has the highest recurrence rate of any malignancy. Although most patients with bladder cancer can be treated with organ-sparing therapy, many experience either recurrence or progression, creating an imperative need for accurate and diligent surveillance. Therefore, the foci of this chapter include the management of noninvasive bladder cancer and the strategies for surveillance of tumor recurrence and progression.

10.2 Incidence

The 2008 American Cancer Society statistics predict that 68,810 new cases of bladder cancer will be diagnosed annually in the United States (American Cancer Society 2008), correlating to an incidence of 37.3 per 100,000 men and

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9.4 per 100,000 women. The prevalence of the disease is higher for white males (40.6 per 100,000) than African American males (20.4 per 100,000) and white females (10.0 per 100,000) compared to African American females (7.7 per 100,000) (Ries et al. 2003). Bladder cancer is typically diagnosed in older individuals with over 90% of diagnoses occurring in individuals over the age of 55 years, but the disease can occur in younger individuals too including children (Morrison and Cole 1976). The annual mortality rate for the disease approximates 14,100 and given the higher prevalence in males than females (51,230 cases in males and 17,580 cases in females), the disease-specific mortality rates are expectantly higher for males (9,950) than females (4,150) (American Cancer Society 2008).

The vast majority of patients diagnosed with bladder cancer present with noninvasive disease.

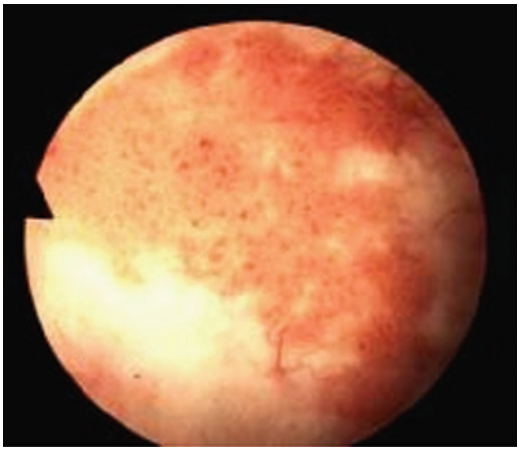
10.3 History of Cystoscopy

Nitze's cystoscope allowed endoscopic visualization of bladder tumors, but Dittel first published the finding in 1885, a year before the inventor documented his results. Beer described the first endoscopic destruction of a bladder tumor soon thereafter, using the Oudin high-frequency current. Keyes subsequently used the more efficient d'Arsonval current, followed by Kidd's diathermy electrode with its "massive ball electrode." By the 1920s, the Stern-McCarthy resectoscope allowed tissue removal without destruction, permitting pathological examination of the specimen (Media File 10.1).

The history of posttreatment surveillance for bladder cancer recurrence is not well documented, but the practice of monitoring tumors with serial endoscopy appears to have arisen with the emergence of cystoscopy in the twentieth century. The recommendation for cystoscopic tumor surveillance every 3 months dates back to at least 1936, apparently based on the fact that substantial numbers of patients with bladder cancer experience tumor recurrence. Early recommendations for initial monthly surveillance have largely been abandoned.

The origin of the traditional timing beginning 3 months following bladder tumor removal is not clear. One theory is that it may have arisen as the timeframe believed to be required for healing after tumor resection. Cystoscopic findings prior to this time would have been difficult to interpret because of numerous factors, including incomplete healing and edema, among others. Nevertheless, the authors have queried colleagues known for subspecialization in bladder cancer and have failed to determine the origin of this practice.

Bladder cancer surveillance standards arose based primarily on expert opinion rather than as an evidence-based standard. The AUA recommends surveillance every 3–6 months for 3 years and at least yearly thereafter (AUA Education and



Media File 10.1 The classic appearance of carcinoma in situ (*CIS*) as a flat, velvety patch. However, using special staining techniques such as 5-aminolevulinic acid, it has been shown that significant areas of carcinoma in situ are easily overlooked by conventional cystoscopy (Courtesy of Abbott and Vysis, Inc.)

Research 2007). The US National Comprehensive Cancer Network has made similar recommendations (Scher et al. 1998).

10.4 Problem

According to the US National Cancer Institute, bladder cancer affects approximately 500,000 people in America. Because most still have an intact bladder, the number of patients under surveillance approaches this figure.

Although the incidence of bladder cancer is approximately 50% that of prostate cancer, the annual expenditures are almost twofold higher for bladder cancer due to the chronic nature of the disease and the need for long-term surveillance. According to the Agency for Health Care Policy and Research of the US Public Health Service, annual expenditures for bladder cancer are $2.2 billion versus $1.4 billion for prostate cancer, suggesting that a close assessment of surveillance techniques and standards is appropriate.

The term ‘superficial bladder cancer’ has been used extensively over the years and should be discouraged. The term implies a harmless nature, which is misleading in many instances. Because it was used to describe the disparate disorders of low-grade papillary bladder cancer as well as the markedly more aggressive form, carcinoma in situ (CIS) and high-grade papillary disease, the World Health Organization (WHO) has recommended it be abandoned. In its place, the term -nonmuscle-invasive bladder cancer should be used and qualified with the appropriate American Joint Committee on Cancer stage (i.e., Ta, T1, Tis). Seventy percent of noninvasive bladder cancers occur as stage Ta, 20% occur as T1, and 10% occur as Tis.

10.5 Etiology

The etiology of bladder cancer is multifactorial and includes environmental as well as molecular factors. Exposure to environmental carcinogens including certain chemical agents, aromatic amines, aniline dyes, pelvic radiation, oxazaphosphorine, chemotherapeutic agents (e.g., cyclophosphamide, iphosphamide), and occupational exposure in the auto, plumbing, leather and apparel, and rubber industries have all been associated with increased risk of bladder cancer (Cole et al. 1972). Additionally, persons who work with organic chemicals and dyes, such as beauticians, dry cleaners, painters, paper production workers, rope and twine industry workers, dental workers, physicians, and barbers, have been reported to have an increased risk. Occupational exposure is presumed to be the cause of bladder cancer up to 25% of the cases (Cole et al. 1972).

Tobacco use is by far the most common cause of bladder cancer in the United States. The likelihood of bladder cancer in smokers is four times that of nonsmokers. A possible correlation between carcinogen excretion in the urine and cumulative urothelial exposure to the carcinogens may be a factor in the development of bladder tumors. In many underdeveloped countries, particularly in the Middle East, *Schistosoma haematobium* infection causes most cases of squamous cell carcinoma. Tobacco abuse in these countries may be changing the ratio as more patients develop transitional cell carcinoma as a consequence of smoking.

10.6 Pathophysiology

As with all cancers, bladder cancer is associated with oxidative DNA genetic changes in the host cells, leading to abnormal and potentially uncontrolled growth.

Transitional cell carcinoma is the most common histological type of bladder cancer in developed countries, accounting for approximately 90% of cases. It is the only cell type commonly associated with successful organ-sparing therapy (except the rare urachal carcinoma, which may be removed with partial cystectomy of the dome and urachal remnant). With a high recurrence rate following local therapy, these patients constitute the surveillance population on whom this chapter is focused.

Squamous cell carcinoma is the second most common cell type associated with bladder cancer in developed countries. Many of these patients are thought to develop the disease due to chronic irritation from indwelling catheters, bladder stones, and, possibly, infections.

Many urothelial tumors are primarily composed of transitional cell carcinoma but contain small areas of squamous differentiation, squamous cell carcinoma, or adenocarcinoma.

Adenocarcinoma of the bladder is rare and is often associated with malignant degeneration of a persistent urachal remnant. Other rare forms of bladder cancer include leiomyosarcoma, rhabdosarcoma, carcinosarcoma, lymphoma, and small cell carcinoma. Except lymphoma, which may be effectively treated with chemotherapy or radiation, these tumors are associated with a poor prognosis.

Stage and grade are particularly important to the likelihood of cancer recurrence and progression in persons with bladder cancer who are treated with local therapy. Importantly, the current WHO terminology recommends tumors be divided into the categories of high grade and low grade on the discernible clinical and chromosomal differences, and hence the grades 1–3 are becoming less used worldwide.

Nevertheless, using the American Joint Committee on Cancer staging system combined with grade, tumors may be classified using a T-G system of labeling. For example, a Ta tumor that is grade 2 (intermediate differentiation) is described as Ta-G2. The extremes are Ta-G1 (low stage, low grade) to T1-G3 (invading lamina propria, high grade), with correspondingly favorable or unfavorable prognoses.

An anomaly to the above concept is in the case of CIS. CIS is defined as flat, high-grade, noninvasive cancer. Although some are tempted to consider CIS a premalignant condition, in reality, it is an aggressive form of cancer that is detected prior to invasion. Therefore, correspondingly aggressive management and surveillance are warranted. Likewise, the opportunity to affect CIS-associated mortality is significant because this type of cancer may respond to conservative therapy. However, if left untreated, CIS eventually becomes invasive and progresses. In addition, a move is developing toward classifying such cancers as either high grade or low grade instead of as multiple levels, which has been used in the past. Regardless, the correlation between stage and grade is significant.

10.7 Genetic Pathophysiology

Bladder cancer is associated with oxidative DNA genetic changes in the host cells. The DNA alterations can be inherited or acquired. Genetic instability may result in abnormal oncogenic activity, which results in altered protein expression and subsequent tumor growth (Theodurescu 2004). The *P53* tumor-suppressor gene and band 9p21, a locus known to be the site of a significant tumor-suppressor gene, are two of the most common and significant missing or mutated gene/gene sites in many patients with bladder cancer. In addition, tumor-suppressor genes *P15* and *P16* on chromosome 9, the *RB* tumor-suppressor gene, the *erb*-b2 oncogene, and the p21-*ras*, c-*myc*, and c-*jun* genes may be mutated. Aneuploidy of chromosomes 3, 7, and 17 is also present in many patients with bladder cancer and may be readily detected using fluorescent in situ hybridization (FISH).

10.8 Clinical

The most common presenting symptom in patients with bladder cancer is hematuria. Most often it is microscopic in nature but it can be intermittent or total gross hematuria on presentation. In the absence of infection or known stone pathology, a normal centrifuged urine sample should contain less than three red blood cells per high-powered field. If microscopic examination reveals greater than three red blood cells per high power field from two of three properly collected urine specimens, a diagnosis of hematuria is made, and cystoscopic examination as well as an evaluation of the upper urinary tract is indicated even in the asymptomatic patient, based on AUA Guidelines for hematuria (Smith et al. 1999).

Occasionally, cases of bladder cancer are identified because of irritative voiding symptoms. Urgency, frequency, nocturia, and/or urge incontinence is typical. CIS is especially likely to cause such symptoms; therefore, patients presenting with unexplained or refractory irritative symptoms should be considered for cystoscopy and cytological examination of urine. The threshold for doing so should be especially low in persons who smoke and in other persons considered to be at risk.

Physical examination findings are otherwise uncommon with localized bladder cancer. Rarely, a mass is palpable during abdominal, pelvic, rectal, or bimanual examination. A bimanual examination may be considered part of the staging of such lesions.

10.9 Indications

Among most patients with bladder cancer who present with noninvasive disease, most remain clinically indolent with even a modicum of urologic intervention. However, some progress, and most experience recurrence at least once in the follow-up period, creating the need for accurate tumor surveillance.

According to the National Comprehensive Cancer Network, the probability of recurrence following local therapy is at least 50% for all stages and grades, whereas 70–90% of patients with T1-G3 tumors experience recurrence. The traditional surveillance protocol involves the following schedule for cystoscopy after removal of the typical noninvasive tumor:

- From 0 to 2 years – Every 3 months
- From 2 to 4 years – Every 6 months
- From 5 years and beyond – Every year

This sequence traditionally begins following the removal of a bladder tumor; however, adherence to this timeframe varies considerably (Schrag et al. 2003). Detection of tumor recurrence at any point in the surveillance sequence results in a restarting of the surveillance schedule (i.e., every 3 months) following appropriate treatment of the recurrent lesion.

Although most relapses occur within the first 5 years, late recurrence can occur at any time; therefore, lifetime surveillance is considered the standard.

While the benefit is not clearly demonstrated, many urologists obtain cytology results at the time of cystoscopy on most, if not all, of the occasions. The role for this and other bladder cancer detection tests are discussed below.

10.10 Relevant Anatomy

Cystourethroscopy is familiar to all urologists. Findings from flexible cystoscopy are as accurate as those from rigid cystoscopy, and flexible cystoscopy is tolerated much better in male patients. Women experience similar levels of discomfort with rigid or flexible cystoscopy.

Viscous lidocaine may decrease the discomfort for men, but lubrication appears to be more important for patient tolerance. The use of lidocaine has shown little benefit in women in randomized controlled trials.

The entire lower urinary tract urothelium should be inspected endoscopically, noting changes in the urethra and in the bladder. Obstructing benign prostatic hyperplasia should be noted because it may suggest an increased risk of perioperative urinary retention.

10.11 Contraindications

Acute infection

Intolerance of pain: Although rare, this may necessitate the use of general anesthesia in the operating room.

Urethral stricture: This could indicate the presence of carcinoma, and a biopsy should be performed if carcinoma is even remotely possible or if the overlying urothelium appears abnormal.

Lidocaine or latex sensitivity: These should be considered, and, if present, lidocaine and/or latex should be avoided.

10.12 Workup

10.12.1 Lab Studies

Dipstick and microscopic examination of the urine allows detection of hematuria or infection. Hematuria suggests the likelihood of bladder cancer recurrence.

Infection should delay instrumentation because of the risk of sepsis and because of the concern that inflammatory changes might further complicate evaluation of the urothelium.

Conventional urine cytology has been deemed the criterion standard to detect tumor markers (Brown 2000), but its role has been questioned. According to a

recent review, the value of conventional cytology appears to have diminished in the last decade. The cause for this decline is unclear and does not appear to be a change in the criteria for determining malignancy through cytological examination because specificity did not change appreciably during the interval. The authors theorized that a decrease in the specialization of cytopathologists reading urine specimens might be responsible. Irrespective of the cause, the sensitivity of cytology in the published literature has diminished, as is shown in the following data reported by Halling and associates in their review of the literature. The sensitivity of conventional cytology for grade 1 tumors was 37% before 1990 but only 11% afterward. Similarly, the sensitivity for grade 2 tumors fell from 75% to 31% (Halling et al. 2000).

Of concern is that, although it is widely believed that cytology might miss low-grade tumors but is the criterion standard for high-grade tumors, this has apparently changed. Based on the Halling et al. review of the literature, cytology found 94% of grade 3 tumors in the earlier era, but, after 1990, cytology reportedly detected only 60% of even high-grade tumors (Halling et al. 2000). Fortunately, although the sensitivity of cytology has clearly declined, the specificity remains high and approaches 100%. Therefore, a positive cytology result should be regarded as a true positive; aggressive investigation for occult disease in both the lower and upper urinary tract should ensue.

A host of newer tumor markers and molecular diagnostic indicators has been used, as follows:

DNA ploidy and image analysis
Chromosomal aneuploidy or polysomy
Bladder tumor–associated antigen/analytes
Nuclear matrix proteins (Parekattil et al. 2003)
ImmunoCyt
Hyaluronic acid and hyaluronidase
Fibrin/fibrinogen degradation product
Telomerase (Halling et al. 2002)
Microsatellite instability
Phenotypic antigens, including Lewis X, M344, DD23, and T138Ag
Growth factors and receptors, including epidermal growth factor receptor, autocrine motility factor, and basic fibroblast growth factor
Oncogene and tumor-suppressor genes
Survivin antibody
Molecular cytology (FISH)

10.12.2 Imaging Studies

The bladder urothelium is not well visualized with routine imaging studies, including CT scanning, MRI, or pelvic ultrasonography. Small tumors are easily missed

on images produced by these modalities, and suggestive findings are commonly related to incomplete bladder filling leaving an irregular area that appears as a filling defect.

Cystography is useful to evaluate for trauma or to help detect ureteral reflux, but the contrast obliterates visualization of small tumors, obviating its value in the evaluation of malignancy.

CIS is not visible on images from any available radiographic study.

Upper urinary tract surveillance is as follows: Transitional cell carcinoma is a field change disease, meaning that the entire field of transitional cells is prone to the DNA changes leading to cancer. Therefore, the entire urothelium should be monitored, especially in high-risk individuals. Because of the association of upper tract transitional cell carcinoma with bladder cancer, the upper tract urothelium should be evaluated radiographically at least upon initial presentation. No standard has been set for the frequency of upper tract surveillance imaging, but most patients present with hematuria and therefore, undergo imaging during the original investigation, regardless of bladder cancer status. Following treatment of a lower urinary tract tumor, the frequency of upper tract imaging is still not standardized. However, many authors recommend annual urography, especially in high-risk patients.

Patients at high risk should undergo intermittent evaluations during the surveillance period. Patients with positive urine cytology results or positive findings from FISH or other bladder tumor indicators who manifest no evidence of a bladder tumor to explain the positive test result should undergo repeat upper tract evaluation.

Excretory urography (i.e., intravenous pyelography), retrograde pyelography, and ureterorenoscopy can help detect upper tract synchronous or metachronous tumors. The accuracy of these tests increases in the order listed, with radiographic imaging missing up to three-fourths of small upper tract tumors in some series if read by a radiologist instead of the urologist present in the operating room during retrograde contrast injection. Office -based cystourethroscopy has a role in some patients with upper tract transitional cell carcinoma treated with nephron-sparing surgery.

CT urography using digital reconstruction of CT images to create a view of the ureters is a promising technology that potentially can blend the advantages of CT (i.e., speed, visualization of renal parenchyma, visualization of nonurological structures) with the advantages of excretory or retrograde urography (i.e., visualization of the upper tract lumen). A few centers currently have the ability to perform this modality, and evaluations are being conducted to assess its role.

Some centers also use CT intravenous pyelography. Kidney-ureter-bladder (KUB) imaging is performed, followed by a noncontrast CT scan of the abdomen and pelvis. Contrast is then injected intravenously, followed by vascular and excretion-phase CT abdominopelvic imaging. The patient then undergoes anteroposterior and oblique abdominal radiography and postvoid radiography.

10.12.3 Other Tests

Sensitivity and specificity of commonly available tumor markers: A recent review reported these sensitivities and specificities for commonly available tumor markers (Lokeshwar and Soloway 2001).

BTAstat yields a sensitivity of 57–83% and a specificity of 46–73%.

Nuclear matrix proteins yield a sensitivity of 47–100% and a specificity of 60–70%.

Immunocyt yields a sensitivity of 86% and a specificity of 79%.

Accu-Dx yields a sensitivity of 52–81% and a specificity of 75–90%.

FISH molecular cytology: Detection of specific DNA alterations known to be associated with bladder cancer is possible using multitarget FISH. DNA probes (stains) hybridize with abnormal chromosomal sites and may be visualized using fluorescence microscopy.

According to several recently published articles, molecular diagnostic testing has improved the previously noted bladder tumor markers. FISH (molecular cytology) has significantly greater sensitivity than conventional cytology while maintaining the known high specificity of cytology (Jones 2006).

The DNA probes chosen for available FISH testing are based on the highest-yielding combination of chromosomal abnormalities. Three of these are centromeric enumeration probes, which allow rapid determination of aneuploidy of chromosomes 3, 7, and 17, the most commonly related to bladder cancer. The fourth probe is used to label the 9p21 locus, known to be the site of a significant tumor-suppressor gene. Loss of this tumor-suppressor gene is also related to cancer recurrence and progression.

Among patients with bladder cancer in whom cytology results were negative, atypical, and suggestive, FISH detected 60%, 89%, and 100%, respectively, allowing identification of cancer in most patients in whom cytology failed to detect cancer recurrence.

Data from the Mayo Clinic indicate that a conversion of FISH results from positive to negative during bacillus Calmette-Guérin (BCG) therapy indicates a response, whereas a failure to convert indicates a high likelihood of cancer persistence following BCG therapy (Kipp et al. 2005).

10.13 Diagnostic Procedures

Cystourethroscopy is performed in all patients undergoing bladder cancer surveillance, in accordance with published regimens. However, only 40% of the patients actually adhere to such recommendations. Failure to undergo standard surveillance has been due to numerous issues. Advanced age and lower-risk tumors are associated with a failure to follow guidelines, as are lower economic status and urban dwelling.

10.14 AUA Guidelines Panel Recommendations

In 1999, the AUA issued evidence-based guidelines for the management of noninvasive bladder cancer. In December 2007, a revision of the guidelines was published based on a comprehensive review of the available literature, as well as individual Panel member experience (Hall et al. 2007). Thus, some of the published recommendations are not evidence-based but a product of practitioner experience. The Panel determined 5 index-patient scenarios for which recommendations could be formulated.

The Panel defined standard guideline statements for which (1) the health outcomes of the alternative interventions are sufficiently well known to permit meaningful decisions and (2) there is virtual unanimity about which intervention is preferred. They defined recommendation guidelines for which (1) the health outcomes of the alternative interventions are sufficiently well known to permit meaningful decisions and (2) an appreciable but not unanimous majority agrees on which intervention is preferred. Finally, they defined option guidelines for which (1) the health outcomes of the interventions are not sufficiently well known to permit meaningful decisions or (2) preferences are unknown or equivocal. Options can exist because of insufficient evidence or because patient preferences are divided and may or should influence choices made.

10.15 Treatment Guidelines Statements

10.15.1 For All Index Patients

Standard: The physician discusses treatment options with the patient, including advantages and disadvantages and adverse effects of intravesical treatment, especially in regard to each particular agent.

10.15.2 Index Patient No. 1

This is a patient who presents with an abnormal growth of the urothelium prior to the establishment of a cancer diagnosis.

Standard: A biopsy specimen is obtained for pathologic analysis. Under most circumstances, all visible tumors should be completely eradicated. If bladder cancer is confirmed, surveillance cystoscopy should be implemented; however, no ideal interval or duration for surveillance cystoscopy has been determined.

Option: An initial single dose of intravesical chemotherapy may be administered immediately following tumor resection. (Immediate use of an intravesical agent is an option rather than a standard because of the lack of pathologic confirmation of

disease at the time of resection, as well as potential side effects, costs, and patient preference. Additionally, intravesical agents are not of benefit in the setting of muscle-invasive disease).

10.15.3 Index Patient No. 2

This is a patient with small-volume low-grade Ta bladder cancer.

Recommendation: An initial single dose of intravesical chemotherapy may be administered immediately postoperatively. (The published guidelines explain that single-dose mitomycin C resulted in 17% fewer recurrences than tumor resection alone when all risk groups were considered, but the risk of recurrence and disease progression in individuals with low-grade Ta disease is "relatively low... and there is no evidence that multiple adjuvant instillations of either BCG or chemotherapy have additional benefit in patients at initial diagnosis of Ta Grade 1 bladder cancer").

10.15.4 Index Patient No. 3

This is a patient with multifocal and/or large-volume, histologically confirmed, low-grade Ta disease or a patient with recurrent low-grade Ta bladder cancer.

Recommendation: An induction course of intravesical therapy with BCG or mitomycin C is recommended with the goal being prevention or delay of recurrence. (The published guidelines reported a decreased probability of recurrence with either BCG or mitomycin C compared with tumor resection alone. Additionally, the report indicated no statistical advantage of either agent with respect to the incidence of tumor recurrence).

Option: Maintenance BCG or mitomycin C may be considered. (The guidelines listed that, while maintenance BCG or mitomycin C is more effective in decreasing recurrence than induction alone, one must consider side effects, discomfort, lack of a uniform dosing schedule, and cost factors that may outweigh the benefits of this approach).

10.15.5 Index Patient No. 4

This is a patient with initial histologically confirmed high-grade Ta, T1, and/or Tis bladder cancer.

Standard: For patients with lamina propria invasion (T1) but without muscularis propria in the specimen, repeat resections should be performed prior to additional intravesical therapy.

Recommendation: An induction course of BCG followed by maintenance therapy is recommended as treatment in these patients. In high-risk patients, BCG has proven superior to mitomycin C in terms of disease-free intervals, regardless of maintenance therapy. No significant data indicate any benefit in terms of disease progression.

Option: Cystectomy should be considered for initial therapy in select patients. No data indicate an advantage of intravesical therapy with regard to disease progression; thus, clinical factors, including tumor size, multifocality, grade, presence of CIS, angiolymphatic invasion, and prostatic urethral involvement are all factors to be considered in this option.

10.15.6 Index Patient No. 5

This is a patient with high-grade Ta, T1, and/or CIS bladder cancer that has recurred after prior intravesical therapy.

Standard: In patients with lamina propria invasion (T1) but without muscularis propria in the specimen, repeat resections should be performed prior to additional intravesical therapy.

Recommendation: Cystectomy should be considered as a therapeutic alternative in these patients. (High-risk individuals in whom initial intravesical therapy fails are at high risk of progression to invasive disease, and definitive therapy may be in their best interest).

Option: Further intravesical therapy may be considered for these patients. (Repeat intravesical therapy may be indicated in individuals with late recurrence after previous complete response to an intravesical agent. Data are insufficient regarding the role of combined agent therapy regimens).

10.16 Medical Therapy

Chemotherapy for bladder cancer is covered in detail elsewhere in this text. In brief, patients with low-grade, low-stage disease may receive expectant treatment or may benefit from BCG or other intravesical therapies. In contrast, patients with T1-G3 disease or CIS are advised to undergo BCG therapy or chemotherapy because of the substantial risk of disease progression, although notably BCG is the only one of those options that is shown to reduce progression, and that is only when maintenance therapy is used.

Smoking cessation decreases the risk of tumor recurrence and progression and improves overall health. Increased water intake has been advocated because it may help dilute carcinogens and decrease their exposure to the urothelium. Conclusive benefit has not been shown. Multivitamin or vitamin A supplementation has also been advocated, but data do not fully support this practice.

10.17 Surgical Therapy

10.17.1 Transurethral Resection of Bladder Tumor

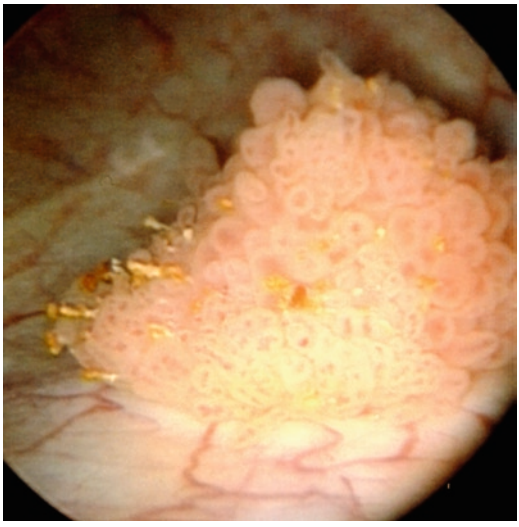
Complete eradication of tumor is the first step. Most tumors are papillary and are easily removed by endoscopically transecting their narrow stalk or base. Following this, biopsy of the base is performed to ensure complete removal and the absence of invasion. Muscle tissue (or fat) must be present in the base biopsy specimen to ensure accurate staging. Without this, accurate staging cannot be ensured (Media File 10.2).

Medium and large tumors are resected piecemeal prior to transection of the stalk in order to ensure that large segments do not remain that might be too large to evacuate through the resectoscope.

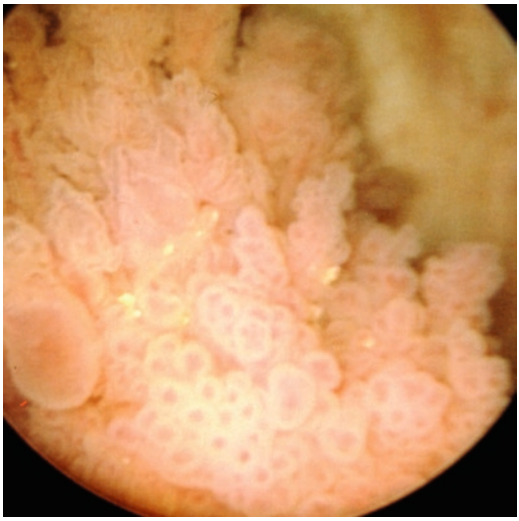
10.17.2 Cystectomy

This is rarely indicated for noninvasive disease. Exceptions are patients with (1) tumor bulk so substantial that complete eradication of tumor is not feasible endoscopically and (2) CIS or T1-G3 tumor persistence despite adequate intravesical management (Media File 10.3).

Patients with T1-G3 cancer in association with diffuse CIS are at especially high risk of progression, and they might be treated with early cystectomy based on a decision made by the physician and patient.



Media File 10.2 Sessile papillary tumors such as this one are typically of low stage and grade (Ta-G1). DAL, MD Glickman Urological and Kidney Institute



Media File 10.3 Sessile lesions as shown usually invade muscle, although occasionally a tumor is detected at the T1-G3 stage prior to muscle invasion. DAL, MD Glickman Urological and Kidney Institute

10.17.3 Random Biopsies

The role of random bladder biopsies is controversial. The minimal benefit of identifying unsuspected CIS must be weighed against the risk of increasing tumor implantation plus the risk of additional bleeding or bladder perforation. However, CIS is often not visible and may be underdiagnosed without bladder biopsies of normal-appearing urothelium. Nevertheless, in the absence of normal urine cytology, random biopsy will be positive in only about 10% of patients.

CIS may not be reasonably removed in total because of its diffuse nature. Therefore, the diagnosis is established and adjuvant therapy is instituted. Obvious areas of CIS may also be fulgurated, but the benefits of this have yet to be proven.

Resection and management of invasive bladder cancer is also covered elsewhere in this text.

10.18 Preoperative Details

Patients scheduled for cystoscopy or anesthetic cystoscopy with transurethral resection of bladder tumor (or bladder biopsies) should ideally have sterile urine documented prior to instrumentation. This is usually presumed by a microscopic urinalysis showing no bacteria or WBCs. A urine culture is ideal but not always feasible for surveillance cystoscopy.

The risk of urinary tract infection with instrumentation is approximately 1%. Therefore, the authors recommend a single dose of oral antibiotic for patients undergoing cystoscopy and a dose of intravenous antibiotics (i.e., cefazolin, gentamicin) for patients in the operating room. Allergies may prompt the use of alternative antibiotic regimens. This is consistent with AUA Best Practice Guidelines.

Some patients need additional antibiotics based on a history of valvular heart disease. The American Heart Association guidelines recommend prophylaxis in these patients to prevent endocarditis. Administer 2 g of ampicillin intravenously or intramuscularly at least 30 min before the procedure (or 2 g of amoxicillin orally at least 1 h before the procedure) in moderate-risk patients. Vancomycin at 1 g intravenously over 1–2 h completed at least 30 min before the procedure may be substituted in patients allergic to penicillin. High-risk patients also receive 120 mg of gentamicin parenterally 30 min before the procedure, and they receive a second dose of ampicillin or amoxicillin 6 h later.

Patients with prosthetics may merit additional antibiotics based on the clinical scenario.

10.19 Intraoperative Details

10.19.1 Transurethral Resection of Bladder Tumor

General or regional anesthesia can be used and indications for such are in part based upon tumor location. Procedure-related morbidity is potentially lower for lateral wall tumors resected with full muscle relaxation, which cannot be achieved with regional anesthesia alone.

Smaller and more friable tumors may be removed at least partially by knocking off fragments with the cutting loop of the resectoscope without the electricity turned on. This sometimes allows partial removal with less risk of bladder perforation.

Pulling the cutting loop away from the tumor is generally much safer than pushing it toward the tumor. Lifting the tumor away from the surrounding normal bladder tissue using the cutting loop is also advisable.

Continuous-irrigation resectoscopes concern some surgeons regarding fluid absorption. However, continuous infusion lessens the bladder wall movement that occurs during filling and emptying and thereby may decrease the risk of bladder perforation. Overfilling also stretches and thins the detrusor, which is another risk factor.

Transurethral resection syndrome due to fluid absorption is uncommon unless the tumor being resected is particularly large. If this is a concern, glycine prevents hemolysis, but not dilutional hyponatremia.

Overuse of cautery at the base of the tumor increases cautery artifact, which can complicate pathological determination of muscle invasion status.

In select patients, office-based fulguration of small tumors allows control of low-risk lesions without incurring the cost and inefficiencies of the operating room (Donat et al. 2004).

10.20 Surveillance Cystoscopy

Patient comfort with surveillance cystoscopy should be paramount. As noted, intraurethral lidocaine may be beneficial in men, but not significantly so in women. Adequate lubrication, gentle technique, and facilitation of patient relaxation are the most effective measures to allow tolerance of the procedure.

Cystoscopy is an embarrassing procedure to the patient. Exposure and handling of the genitalia must be performed with respect. The patient remains exposed only as long as is necessary to complete the evaluation.

Men are most easily evaluated with a flexible cystoscope. Modern versions have superior optics and allow easy visualization of the entire bladder. Miniaturization of the instruments also allows for biopsy and fulguration through the flexible cystoscope (Media File 10.4).

The latest development in surveillance involves advances that integrate video chip technology at the end of flexible cystoscopes, as with the Endo-EYE from Olympus America Inc (see Media File 10.5). (Olympus and Endo-EYE are registered trademarks of the Olympus Corporation, Olympus America Inc, or their affiliated entities).

10.20.1 Cystoscopy Techniques for Men

Different techniques are described, including “painting” the bladder with multiple passes in and out. The authors prefer a “sweeping” technique when using fiberoptic scopes.

The scope is advanced through the urethra under direct visualization, asking the patient to relax his “bottom” while passing through the external urinary sphincter.

Media File 10.4 Flexible cystoscopes such as this one facilitate endoscopic tumor surveillance with minimal morbidity and excellent visualization of the urothelium (Courtesy of Olympus America, Inc.)

Media File 10.5 The latest development in surveillance involves advances that integrate video chip technology on to the end of flexible cystoscopes (Courtesy of Olympus America, Inc.)

Immediately upon entering the bladder, the scope is advanced to its greatest depth while using the thumb to retroflex it against the bladder dome.

Irrigation is turned off to minimize bladder overdistension. This is more comfortable for the patient and minimizes the amount of mucosal surface area that must be inspected. Irrigation may be restarted if distention is inadequate or if debris or blood impairs visualization.

Secondary deflection from the scope angling off the detrusor easily allows a bird'seye view of the bladder base, which is the most common site of primary occurrences. In this position with slight left and right rotations, more than half the bladder is visible. If a lesion is identified, the scope may be advanced closer for a magnified view.

The scope is then pulled back to the bladder neck and directed to view the floor by thumb control. Once the floor is fully visible, the scope is swept toward the patient's right side.

Minor movement in and out of the bladder neck (taking care to not drag the scope over the bladder neck mucosa) allows complete visualization as the scope is swept 270°.

When the scope reaches the 3-o'clock position (patient's left side), the surgeon's wrist cannot complete the circle so the scope is brought back to the base of the bladder and the procedure is repeated in reverse.

Any suggestive areas are inspected carefully, confirming the health of the mucosa or noting any lesions that require further investigation or intervention.

In contrast to the sweeping technique, which is most effective with fiberoptic scopes, the vastly superior optics of modern digital chip technology scopes allow visualization of the entire bladder in most patients by moving minimally in either direction when the tip of the scope sits just inside the bladder neck. After seeing the upper half or more of the bladder, the scope may be advanced into the retroflexed

position and can easily visualize the basilar (or trigonal) half or more of the bladder. This offers more completeness of the examination and is better tolerated by the patient.

10.20.2 Cystoscopy Techniques for Women

Female cystoscopy can be performed as described for men, using the flexible cystoscope, or, because of the relatively straight female urethra, using a rigid cystoscope (Media File 10.6).

In actuality, the female urethra dips slightly down; therefore, placement of the rigid scope is more comfortable if the scope (with an obturator in place to create a smooth tip) is pointed dorsally as it enters the urethra and then redirected ventrally prior to entering bladder neck, following the natural, slightly upward curve of the urethra.

In order to remove the obturator without spilling urine, the scope may be placed gently against the back wall of the bladder while switching the obturator for a lens, although the surgeon must be careful to avoid causing irritation that could mimic CIS. Note that the trigone is the most sensitive part of the bladder, so placing the scope against the back wall instead of the trigone is requisite.

Once the 70° lens is in place, the bladder may be swept similarly by angling the surgeon's end of the scope away from the side of interest. In doing so, the scope (eyepiece) essentially ends up creating a conical motion, with the tip or pivot point being the urethra. The 30° lens is then able to visualize the entire bladder with effort, but the 70° lens can easily visualize the entire bladder unless the bladder is overfilled.

Use of a video camera system is helpful for education of both residents and patients and allows documentation of findings. Monitoring is not usually required for patients undergoing office-based cystourethroscopy. Some urologists also believe their diagnostic accuracy is improved with the magnification and optics of the camera system.

Digital chip endoscopy may be performed as easily in women as it is in men. See cystoscopy techniques for men for a description.

Media File 10.6 Rigid cystoscopes such as this one allow biopsy collection via in-office fulguration of small tumors. Such fulguration may be performed using electrocautery or laser (Courtesy of Olympus America, Inc.)

10.21 Postoperative Details

Patients may prefer to re-dress themselves prior to discussing the findings or allowing family members to enter the examination room.

10.21.1 Follow-up

Patients must understand that minor hematuria or dysuria is normal and expected following instrumentation. Irritative symptoms may be treated with phenazopyridine. Less commonly, anticholinergic agents may help relieve detrusor contractions.

Obstructive symptoms in men usually resolve within a few hours, but urinary retention occasionally occurs. Alpha-blocking agents may be useful if administered prior to complete retention. Patients with preexisting obstructive symptoms may be given preemptive treatment.

10.21.2 Complications

Complications of surveillance cystoscopy may include: urinary tract infection, dysuria, hematuria, urethral stricture, and pain.

In the patient with spinal cord pathology above the T6 level, autonomic dysreflexia can occur as a response to bladder distention, leading to potentially life-threatening hypertension. This may be prevented with nifedipine or terazosin in some cases, but careful monitoring is requisite. Any signs of headache, tremors, or hypertension should lead to immediate cessation of the procedure and emptying of the distended bladder. For this reason, most patients with spinal cord injury should probably undergo cystoscopy in the operating room with anesthesia monitoring.

Complications of transurethral resection of bladder tumor include: bladder perforation, ureteral obstruction, hematuria, urinary retention, transurethral resection syndrome, urinary tract infection, dysuria, hematuria, urethral stricture, and pain (Media file 10.7).

Media File 10.7 Resection of all visible tumors is possible using modern resectoscopes (Courtesy of Olympus America, Inc.)

10.22 Outcomes and Prognosis

The prognosis of bladder cancer is primarily related to tumor grade and stage. In addition, multiplicity or rapid recurrence following an initial transurethral resection of bladder tumor is associated with greater recurrence rates (although early recurrence may also be related to incomplete initial resection or failure to detect a satellite lesion).

Estimates vary, but low-risk cancers (i.e., Ta-G1) recur in at least half of all patients and progress in approximately 5%. In contrast, high-risk cancers carry a correspondingly worse prognosis. T1-G3 cancers usually recur, and they progress in approximately half of all patients. T1-G3 cancers found in association with CIS frequently progress and these patients may be considered for immediate cystectomy.

10.23 Future and Controversies

Although many urologists believe that cystoscopy is infallible in their hands (or eyes), some concerning findings challenge this opinion.

Instilling 5-aminolevulinic acid into the bladder a few hours prior to cystoscopy allows accumulation in malignant sites, which are often not visible during white-light (normal) cystoscopy. When illuminated with a light ranging from 375 to 445 nm, up to one-fourth of small malignant areas may be missed during routine cystoscopy. This has been proven by biopsy studies.

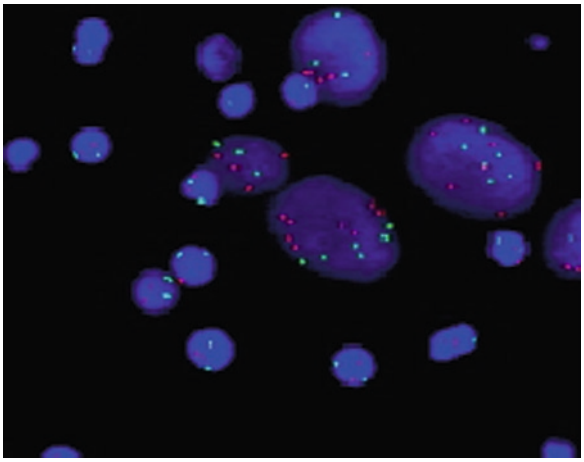
Of concern is that these areas may more likely to harbor higher-grade cancer than those identified otherwise. CIS is missed by conventional cystoscopy 22% of the time.

Halling et al. (2000) similarly found a significant number of cancers that cystoscopy failed to definitively identify . This draws into question whether early recurrences are truly recurrent cancer or simply incompletely removed cancer.

Artificial neural networks using various tumor markers similar to those described by Parekattil et al.(2003) may be more cost-effective for detecting recurrence and progression than the current screening protocol of cystoscopy and conventional cytology at predetermined intervals. Using more effective markers may allow scheduling of cystoscopy on a more logical and targeted schedule than is currently the default.

Nonlinear surveillance strategies have been shown in at least one model to actually decrease the time to detecting tumor recurrence while optimizing the utilization of resources (Kent et al. 1991).

A fresh look at surveillance strategies is in order based on the lack of evidence on which current standards are based and on new findings regarding the ability to predict cancer recurrence using neural networks, prediction models, and improved diagnostic tests, including molecular diagnostic evaluations. As this process progresses, the conventional surveillance protocol will likely change. Using tumor stage and grade in conjunction with improved surveillance methods, resources may be focused on patients at risk of recurrence and progression.



Media File 10.8 Photograph in which fluorescence in situ hybridization centromere staining identifies aneuploidy of chromosome 3. Multiple instances of overexpression of the chromosome (note the multiple red dots, which identify centromeres of this chromosome) prove aneuploidy

Specific concepts likely to help focus surveillance in the near future include improved endoscopic techniques that can identify otherwise imperceptible malignancy and molecular diagnostic tests that can identify malignant change prior to anatomic transformation. However, until such time, cystoscopy in conjunction with some form of cytology (either conventional or molecular cytology, i.e., FISH (Media file 10.8)) is likely to remain the mainstay in surveillance at currently accepted intervals.

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Chapter 11
Clinical Management of Low Grade Bladder Tumors

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Abstract Low-grade urothelial neoplasms have different behavior and better outcome than higher grade tumors. Noninvasive tumors are the most common bladder cancers. Seventy percent are low grade. The incidence is low below the age of 50 but thereafter it steadily grows. The World Health Organization (WHO) 2004 histological classification and risk factors are described. Cystoscopy is the method by which most papillary tumors are detected. Macroscopically, low-grade lesion is nicely papillary. Urinary cytology is negative in the majority but is useful at first diagnosis to predict high-grade tumors. Outpatient fulguration is increasingly popular in recurrent, low-grade tumors. Upper tract exploration at initial diagnosis is performed not only for the detection of upper tract tumors, but also for exploration of hematuria. A complete transurethral resection (TUR) is essential for the prognosis of the patient. Random biopsies are inappropriate. Although TUR eradicates Ta tumors completely they will recur very often. The high variability in the 3-month recurrence rate indicates that TUR is incomplete or provokes recurrences. It is therefore necessary to consider adjuvant therapy in all patients. A single immediate instillation of a chemotherapy agent significantly reduces the recurrence rate and is advocated as standard treatment. The need for more instillations is related to prognostic factors. The most important are multiplicity, recurrence at 3 months, previous recurrence rate, and size of the tumors (>3 cm). Bacille Calmette-Guérin (BCG) should not be used as first-line treatment but can be used in second line. Evolution to higher grade and stage does occur in less than 20% patients over years. Evolution to progression is rare. The EUA guidelines consider the first control at 3 months after TUR advisable as it picks up incomplete resections but the next control can be delayed 9 months. The highest chance of recurrence is in the first 2 years, less in the third year, and few thereafter.

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11.1 Introduction

The low-grade urothelial neoplasms have a clearly different behavior and better outcome than higher grade tumors. Therefore, they merit to be treated separately. While they rarely evolve to a fatal cancer, recurrence of these neoplasms remains a major problem, requiring adjuvant therapies after transurethral resection (TUR) and long-term, but different, follow-up. We can move from using the word *cancer* when describing these tumors, avoiding the psychologic impact of this word on patients. The term *urothelial neoplasms* better describes the matter.

The term noninvasive is used here to refer to Ta disease. The term superficial is vague, and may include both Ta and T1 tumors.

Noninvasive tumors are the most common presentation of urinary bladder cancer and constitute almost half of all newly-diagnosed patients. In a population-based study from western Sweden, 53% of patients with a first diagnosis of bladder carcinoma had papillary stage Ta disease. Seventy percent of these were low-grade carcinomas (Holmäng et al. 2001). The incidence is low below the age of 50 but thereafter steadily grows in frequency.

11.2 Histology

The histological grade, refers to the appearance of the cancer cells under the microscope. In 1998, a new classification of noninvasive urothelial tumors was proposed on the WHO and International Society of Urological Pathology (ISUP) consensus meeting. The new classification system for grading urothelial neoplasms has been published by the WHOin 2004 (Sauter et al. 2004). The objective of this new version was to avoid the overdiagnosis of cancer and to create better criteria for the different grades. The new system has not been as universally accepted as the recent TNM classification and all data of the literature were based on the older 1973 classification: therefore, the use of both classifications is still advocated.

In the new WHO 2004 classification, grade 1 tumors were reclassified either as papillary urothelial neoplasms of low malignant potential (PUNLMP) or true low-grade tumors. The grade 2 tumors are reclassified either to low-grade or high-grade tumors according to specific cytologic and architectural criteria. The terminology used in the new grading system parallels that used in urinary cytology. A website illustrating examples of various grades was developed: www.pathologyj.hv.edu/bladder.The cells in PUNLMP look very much like normal bladder cells and are slowly growing and unlikely to progress. Nevertheless, PUNLMPs still have a high tendency, although lower than for grade 1 tumors, to recur. The differences in recurrence and progression rates justify the distinct classification of PUNLMPs and grade 1 tumors (Holmäng et al. 2001; Fujii et al. 2003).

11.3 Risk Factors

Many of the etiological factors for the development of bladder tumors are known and the urologist should be aware of the types of occupational exposures that may occur to urothelial carcinogens. Aromatic amines were the first to be recognized. At-risk groups include workers in the following industries: printing, iron and aluminum processing, industrial painting, gas and tar manufacturing (level of evidence).

Another prominent risk factor is cigarette smoking, which triples the risk of developing bladder cancer (Zeegers et al. 2000; Bjerregaard et al. 2006). Cigarettes are responsible for up to 50% of bladder tumors.

Urologists should encourage patients to stop smoking as smoking cessation has proven beneficial also for bladder cancer (Strope and Montie 2008).

11.4 Diagnosis

11.4.1 Symptoms

The vast majority of the papillary tumors are detected by the presence of hematuria. Sometimes it may be detected on evaluation for a recurrent urinary tract infection or incidentally by ultrasound, which is often performed as an exploration for hematuria. Bladder irritation in the absence of infection may be a symptom of bladder carcinoma but not of a low grade Ta lesion.

11.4.2 Imaging

Large tumors may be seen as filling defects in the bladder. Intravenous urography (IVU) is also used to detect filling defects in the calyces, renal pelvis and ureters, and hydronephrosis, which may indicate the presence of a ureteral tumor. The necessity to perform routine IVP once a bladder tumor has been detected is now questioned because of the low incidence of significant findings obtained with this method (Goessl et al. 1997; Herranz-Amo et al. 1999). The incidence of upper urinary tract tumors is very low in low-grade tumors, but increases in T1 high-grade tumors (Holmang et al. 1998).

In many centers, CT urography is used as an alternative to conventional IVU (Nolte-Ersting and Cowan 2006). Especially in invasive tumors of the upper tract, it gives more information than IVU.

Ultrasonography is used with increasing frequency as the initial tool to assess the urinary tract in hematuria. This is not only because it avoids the use of contrast agents, but also because sensitive transducers have improved imaging of the upper

urinary tract and bladder. It permits characterization of renal masses, detection of hydronephrosis, and visualization of intraluminal filling defects in the bladder.

11.4.3 Cystoscopy Is the Method by Which Most of the Papillary Tumors Are Detected

In general, cystoscopy is initially performed in the office, using flexible instruments. If a bladder tumor has been visualized in earlier imaging studies, a diagnostic cystoscopy can be omitted since the patient will undergo transurethral resection. A careful description of the finding is necessary. It should include the site, size, number and appearance (papillary or solid) of the tumors as well as a description of mucosal abnormalities. Macroscopically, low-grade lesion is nicely papillary.

11.4.4 Urinary Cytology

Urinary cytology will be negative in the majority of low-grade tumors but is useful at first diagnosis as it predicts the presence of high-grade tumors.

The role of urinary markers is not yet clearly defined and have not found their way to daily clinical practice.

11.4.5 Accuracy of Cystoscopy in Defining Low Grade Papillary Tumors

Outpatient fulguration has become increasingly popular in the management of recurrent, low-grade papillary tumors of the bladder. In order to justify this as definitive treatment of such lesions, the urologist must be able to accurately distinguish the cystoscopic features of low-grade, noninvasive from high-grade, potentially invasive tumors.

Herr (2001) correlated the cystoscopic appearance of recurrent superficial papillary bladder tumors with histology after TUR in 150 consecutive patients. Papillary lesions were classified as TaG1, TaG3, or T1G3 based on their cystoscopic appearance. Tumors classified as TaG1 were less than 0.5 cm and had individually discrete papillary fronds of mucosa surrounding a clearly visible fibrovascular core. Tumors with fused or less discrete papillary fronds that occurred in clusters or were greater than 0.5 cm in diameter were usually graded TaG3. Lesions that appeared papillonodular or solid were classified as T1. These latter tumors were all larger than 0.5 cm. Voided urine cytology was also obtained in each case. Of 84 tumors regarded as TaG1 at cystoscopy, 93% (78/84) proved to be low-grade papillary tumors,

histologically. Seventy-two patients had a cystoscopic TaG1 tumor and negative urine cytology. Of these, 98% (71/72) were TaG1, histologically. Of the 84 papillary tumors that appeared to be low grade, 6 of 84 (7%) were high grade, and 2 of 84 (2%) were confirmed on biopsy to be invasive. Only 3 of 66 (4.5%) tumors considered to be high grade at cystoscopy proved to be low grade, histologically.

In a similar study from the same institution, Herr et al. (2002) evaluated the correlation between cystoscopic appearance and histopathology in 125 patients with 144 recurrent papillary tumors. Consistent with their previous findings, 90 of 97 tumors (93%) considered TaG1 at cystoscopy were confirmed on biopsy to be low-grade papillary lesions. Of the 86 TaG1 tumors associated with negative urine cytology, 85 (99%) proved to be low-grade papillary tumors, histologically. Only 1 of 97 (1%) tumors deemed TaG1 proved to be invasive by biopsy. Of lesions believed to be high grade or invasive, only 6 of 47 (13%) were overgraded by cystoscopic appearance. The authors concluded that since the overwhelming majority of tumors, which appear to be low grade on cystoscopy prove to be low grade on biopsy, these lesions may be safely managed with fulguration alone, especially in the setting of negative urine cytology.

Oosterlinck et al., in an European Organization for Research and Treatment of Cancer (EORTC), multi-institutional study, reported that 5.6% of 501 tumors believed to be noninfiltrating at cystoscopy were understaged compared to histopathology (Oosterlinck et al. 1993a); Tumors appearing superficial that were less than 3 cm in diameter were correctly staged in 96% of the cases. No data were available regarding the accuracy of cystoscopy in predicting tumor grade. In this study, the investigators were asked to include patients before histology was available. The number of patients not included because of doubtful interpretation was not reported.

Other authors reported the potential inaccuracies of cystoscopic evaluation of bladder tumors. Cina et al. (2001) reported that 7 of 13 (54%) papillary lesions confirmed to be high grade by biopsy were believed to be low grade based on their cystoscopic appearance. Of 37 lesions believed to be low grade on cystoscopy, 7 of 37 (19%) proved to be high grade by biopsy. Of seven invasive lesions, three were believed to be noninvasive at cystoscopy. Of nine lesions considered high grade at cystoscopy, 33% were low grade by histologic examination. The authors concluded that grade and stage of papillary neoplasms could not be accurately predicted by cystoscopic appearance alone.

The contradiction between the two first and the last papers can be explained by a different selection of cases. In Herr's study, only recurrences of Ta or T1 tumors with previously known histology were examined. Oosterlinck's study mainly investigated small, new solitary tumors, while in Cina's study results of TUR specimens without any preselection were studied.

In summary: By experienced eyes, and in combination with negative urine cytology, low-grade papillary neoplasms may be identified accurately in over 90% of the cases. Because of the potential for undergrading lesions larger than 0.5 cm in diameter, multiple lesions (>5), those which are papillonodular, and those associated with positive urine cytology should be evaluated further.

11.4.6 Upper Urinary Tract Exploration in Low Grade Bladder Tumors

The need for upper urinary tract imaging when the initial diagnosis of bladder tumors is made is questionable (Goessl et al. 1997; Herranz-Amo et al. 1999). An upper tract exploration at initial diagnosis is performed not only for the detection of upper urinary tract tumors, but also for further exploration of hematuria.

In patients with bladder cancer in general, the incidence of upper urinary tract tumors at the time of diagnosis is low and very rare in low-grade Ta tumors (Yousem et al. 1988).

The incidence of metachronous upper urinary tract tumor after bladder tumor in general ranges from 0.7% to 5.9% (Oldbring et al. 1989; Palou et al. 1992; Solsona et al. 1997; Amar and Das 1985; De Torres et al. 1987; Rabbani et al. 2001), largely dependent on the grade of the tumor and the presence of CIS (Solsona et al. 1997). More specifically, during follow-up the incidence of upper urinary tract tumor for patients with no bladder CIS drops to 0.9% for low-risk patients (Solsona et al. 1997; Millan et al. 2000a).

In patients with low-grade bladder tumors, the low incidence of upper urinary tract tumors, the need for a prolonged follow-up, the potential minimal prognostic impact of a delay in diagnosis, and the high cost of repeated CT scans do not support routine upper urinary tract assessment during follow-up. This was also the conclusion of the guidelines panel of the European Association of Urology (EAU) on upper urinary tract tumors (Babjuk et al. 2008).

11.5 Primary Treatment of Low Grade Ta Bladder Tumors

11.5.1 TUR of Bladder Tumors

The goal of the TUR in TaT1 bladder tumors is to make the correct diagnosis and remove all visible lesions.

The strategy of resection depends on the size of the lesion. Small tumors (less than 1 cm) can be resected en block, where the specimen contains the complete tumor plus a part of the underlying bladder wall. Larger tumors have to be resected separately in fractions, which include the exophytic part of the tumor, the underlying bladder wall with the detrusor muscle and the edges of the resection area. The specimens from different fractions must be referred to the pathologist in separate containers to enable him to make a correct diagnosis. Cauterization has to be avoided as much as possible during the resection to prevent tissue destruction (Babjuk et al. 2008).

A complete and correct TUR is essential for the prognosis of the patient (Brausi et al. 2002).

11.5.2 Need for Random Biopsies and Fluorescence Cystoscopy

A low-grade superficial bladder tumor is expected when urinary cytology does not show malignant cells, when no suspicious red zones suggest CIS, and when the tumor has a nice papillary structure. In these conditions, random biopsies are inappropriate and do not provide any additional information relevant for the treatment and the prognosis of superficial bladder cancer (van der Meijden et al. 1999; Witjes et al. 1992). The number of abnormalities detected in these cases will be very low (van der Meijden et al. 1999). Publications emphasizing the need for random biopsies particularly report the detection of CIS (May et al. 2003), which will be detected by a positive cytology. Identifying an additional small but invisible Ta or T1 lesion does not alter treatment or prognosis in a substantial way and as such the application of fluorescence TUR is not indicated.

Any traumatized bladder mucosa may become an implantation site for floating tumor cells. Although there is no clinical evidence for this phenomenon, there are animal experimental data demonstrating this (Soloway and Masters 1980; Pan et al. 1989). Among possible explanations for the high rate of early recurrences of superficial bladder cancer is the theory of implantation of cells shed during TUR.

11.5.3 Second Resection

In clinical trials, controlling the number of early visible recurrences within 4 weeks of primary resection, the detection of residual tumors is very low (May et al. 2003). The recurrence rate at 3 months also remains low in this best prognosis group of superficial bladder cancer. As such, it seems unrealistic to expect that the second resection will reveal substantial findings that will change treatment and outcome for the patient in this particular group of superficial bladder tumors. Therefore, a second resection is not indicated in low-grade tumors.

11.5.3.1 One Early Post TUR Chemo-Instillation

Although a state-of-the-art TUR by itself could eradicate a TaT1 tumor completely, these tumors will recur in a high percentage of cases and progress to muscle-invasive bladder cancer in a limited number of cases. The high variability in the 3-month recurrence rate indicates that TUR is incomplete or provokes recurrences in a considerable percentage of patients. It is therefore necessary to consider adjuvant therapy in all patients.

The instillation of a chemotherapeutic drug immediately after TUR is an old idea, which was initially tested in the 1970s. It was based on the fact that chemotherapy would be able to destroy floating tumor cells and prevent implantation at any traumatized surface of the bladder. These first clinical trials suggested a reduction in the

rate of tumor recurrence when a perioperative instillation was given. Subsequent randomized studies have shown impressive improvements of the recurrence-free rate (May et al. 2003; Rajala et al. 1999; Rajala et al. 2002; Okamura et al. 2002; Tolley et al. 1988, 1996; Ali-el-Dein et al. 1977). Sylvester et al. (2004) included 7 randomized trials with recurrence information on 1,476 patients in their meta-analysis. Based on a median follow-up of 3.4 years and a maximum of 14.5 years, 267 of 728 patients (36.7%) receiving one postoperative instillation of epirubicin, mitomycin C (MMC), thiotepa, or pirarubicin experienced a recurrence as compared to 362 of 748 patients (48.4%) with TUR alone (odds ratio [OR] 0.61, $P<0.0001$). This meta-analysis has shown that one immediate instillation of chemotherapy after TUR decreases the relative risk of recurrence by 39% in patients with Ta or T1 bladder cancer.

Although the majority of the patients included in these randomized trials had a single tumor, it was found that both patients with a single tumor (OR 0.61) and those with multiple tumors (OR 0.44) benefited from a single instillation. However, after one instillation, 65.2% of the patients with multiple tumors had a recurrence compared to 35.8% of the patients with a single tumor, showing that one instillation alone is suboptimal treatment in patients with multiple tumors. In a trial excluded from the meta-analysis because some patients received additional instillations before recurrence, Zincke et al.(1983) also found that patients with multiple tumors benefited from an immediate instillation of thiotepa or doxorubicin. Defining risk groups according to whether the tumor was single or multiple and as per the result of the first follow-up cystoscopy, Tolley et al.(1988,1996) found that the benefit of MMC in both treatment groups combined was similar in low-, medium-, and high-risk cases. Other subgroup analyses could not be done in the meta-analysis due to the absence of individual patient data, but the two other studies in which they were performed suggest that the treatment is beneficial across all categories of patients.

With the possible exception of the one study with thiotepa in which no difference was found, the meta-analysis suggests that no large differences exist with regard to efficacy between the different chemotherapeutic agents. The low concentration of thiotepa used may be responsible for the lack of efficacy.

Looking at initial recurrences only, 11.7 TURs were saved for every 100 patients treated. Thus, the number needed to treat to prevent one recurrence is $1/0.117 = 8.5$. Since the cost of a TUR, anesthesia, and hospitalization is probably more than 8.5 times the cost of one instillation in most countries, a single instillation should be cost-effective. However, if and when one takes into account that the instillation is given before pathology is known, it will be applied to bladder lesions, which will not profit by the treatment. In some studies, this was up to 20% of the patients, making it less cost-effective (Berrum-Svennung et al. 2008).

11.5.3.2 Recent Clinical Trials

EAU Guidelines and, to a lesser extent, the AUA Guidelines recommend one immediate instillation after TUR in all patients (Witjes et al. 1992).Nevertheless, three recently published studies questioned the benefit of an immediate instillation, at

least in some subgroups of patients (Berrum-Svennung et al. 2008; Gudjónsson et al. 2009; Cai et al. 2008).

In a study of 307 patients with 1–3 papillary tumors, Berrum-Svennung et al. (2008) confirmed the results of the meta-analysis and found that a single instillation with Epirubicin significantly delayed the time to first recurrence during the 2-year study period ($p=0.02$). They found that an immediate instillation only prevented recurrences between 1 and 5 mm with no reduction in recurrences greater than 5 mm. As these small recurrences could be easily fulgurated using local anesthesia, the actual benefit of an immediate instillation was brought into doubt. About one half of the patients with a first recurrence were treated as outpatients and there was no difference between the treatment groups for duration of time spent in the hospital for overnight surgery.

Note that in patients with a strict follow-up schedule, most recurrences should be small and the bigger tumors may be those which are not sensitive to the drug used. A reduction in the overall recurrence rate, even in small recurrences, may permit a much less aggressive follow-up schedule and will spare them the psychological stress of knowing that they have recurred (Oosterlinck et al. 2008).

In addition, Berrum-Svennung et al. question the benefit in multiple tumors, which are subsequently treated with additional instillations, even though they did not specifically look at this in their study.

Gudjonsson et al.(2009), in a study with 219 low- to intermediate-risk patients, also found that a single instillation with Epirubicin significantly delayed the time to first recurrence during a follow-up period extending up to 6 years ($p=0.016$). Although they found that an immediate instillation prevented recurrence in the low-risk patients (approximately 50% of the patients), they claimed that there was little or no benefit in patients at intermediate or high risk of recurrence. In this study, which is underpowered to make reliable subgroup analyses, the follow-up extended out to 6 years, potentially diluting the size of any treatment effect occurring within the first 2 years of follow-up. In 117 patients with multiple tumors, there was some separation in the recurrence curves starting at 6 months which disappeared at 2 years. The number of patients with intermediate- to high-risk tumors was too small to draw definitive conclusions in this subgroup.

The meta-analysis suggested a benefit in patients with multiple tumors based on concordant results in two studies, but the number of patients with multiple tumors ($n=111$) was too small to draw definitive conclusions.

Cai et al. (2008) studied the effect of an immediate instillation in 161 high-risk patients scheduled to receive 3 years of treatment with BCG.There were no statistically significant differences in time to first recurrence between the two treatment groups ($p=0.095$) but the trend was in favor of the early post-TUR instillation. The study was largely underpowered to detect realistic differences (Sylvester et al. 2009).

Summary: The three trials mentioned above do not allow us to draw any definitive conclusions beyond those given in the meta-analysis and should not be used as a justification not to give an immediate instillation in certain subgroups of patients such as in those patients who are at intermediate or high risk of recurrence. The real question in intermediate- and high-risk patients is not whether an

immediate instillation provides benefit, but rather, what is the role of an immediate instillation in patients who will receive additional intravesical instillations?

This was assessed in a recent systematic review. Based on limited data from five studies in mixed patient populations, the results suggest that one immediate instillation may still be necessary if further chemotherapy is given during only 6 months; however, it might not be necessary if given during 12 months. One immediate instillation of Epirubicin might not be less effective than a delayed course of multiple Epirubicin instillations in low- to intermediate-risk patients (Sylvester et al. 2008). Obviously there are economic, convenience, and quality-of-life reasons to limit the number of instillations to as few as possible.

Until there is sufficient evidence to the contrary, we believe that an immediate instillation should still be recommended in all patients with nonmuscle-invasive bladder tumors.

11.5.3.3 Working Mechanism

The effect of one instillation may be explained either by the chemoresection of tumor left behind after an incomplete TUR or by destroying circulating tumor cells that could implant at the site of resection. Incomplete TUR may be an issue even in patients with solitary tumors as seen by the large variation between institutions in the recurrence rate at the first follow-up cystoscopy after TUR (Brausi et al. 2002). Oosterlinck et al. found that 10 patients out of 242 had residual tumors 1 month after TUR, only one of which was in the instillation arm (Oosterlinck et al. 1993b). Masters et al. (1999) found a 44% complete response rate in a marker lesion 3 months after one instillation of epirubicin. Thus, one instillation can, in fact, eradicate tumor left behind after TUR.

Several animal experiments also support the hypothesis of tumor implantation at traumatized areas in the bladder (Soloway and Masters 1980; Pan et al. 1989).

As suggested by Solsona et al.(1999), the effect of one instillation appears to occur early, mainly during the first 2 years, with a possible dilution of the treatment effect with longer follow-up. Investigating the percentage of patients who experience recurrence rather than considering the time to first recurrence may, in fact, underestimate the size of the treatment effect. Looking to the Kaplan-Meyer curves of recurrence in the study of Gudjonsson et al. (2009) demonstrates that the effect is restricted to the first 2 years and dilutes thereafter. It has been calculated from the data of five randomized trials (Hinotsu et al. 1999) that the reduction of recurrence lasts for a period of approximately 500 days.

11.5.3.4 Timing of the Instillation

In all studies, the instillation was given within 24 h, generally either immediately after TUR or within 6 h after. Kaasinen et al.(2002) found a doubling in the risk of recurrence if the first of 5 weekly MMC instillations was not given on the same day

as the TUR in patients with frequent recurrences. In two EORTC trials where patients received nine instillations of epirubicin or MMC over 6 months, starting treatment on the day of TUR was more effective than starting 7–15 days later in patients who did not receive further maintenance after 6 months (Bouffioux et al. 1995). There is thus some evidence that the instillation should be given on the same day as the TUR and that this may be critical for the efficacy. In one study, in which the instillation was given the day after TUR, no advantage could be demonstrated (Hendricksen et al. 2008).

11.5.3.5 Toxicity and Precautions

Despite the fact that no serious adverse effects have been mentioned in the trials published on immediate adjuvant chemotherapy instillations, several reports have appeared on severe and prolonged complications due to extravasation of the drug after an early intravesical instillation. Doherty at al. reported the local effects of an immediate instillation of chemotherapy (mainly epirubicin) in cystectomy specimens (Hendricksen et al. 2008). It was associated with a more extensive necrosis of the bladder wall and fat necrosis of extravesical tissue than the usual muscle necrosis seen after TUR alone. An area of thin muscularis propria may undergo necrosis resulting in secondary perforation. None of the 12 patients described by Doherty et al., however, reported local symptoms. In contrast, the effect of extravasation after intravenously administered chemotherapeutic drugs is well-documented. It induces long-lasting necrosis, provoking pain with a low tendency of healing. Recently, several cases of severe and long-lasting complications due to extravasation of MMC have been reported (Doherty et al. 1999). A distal ureteral stenosis has also been described that was probably due to intravesical MMC (Nieuwenhuijzen et al. 2003). There is certainly an underreporting of these complications as not every urologist who has seen such a complication is eager to report such an event. In any case, urologists should be aware of the potential risk of extravasation of chemotherapeutic drugs and its consequences.

If there is a possibility of perforation after an extended TUR, an immediate instillation should not be given. In case of the possibility of intraperitoneal leakage or significant resorption from the extravesical space, it seems advisable not to use a dose which is greater than the dose, which is acceptable as one single intravenous injection. Indeed, one case of myelosuppression has been described when 80 mg of MMC was retained for 2 h after TUR of a large tumor (Oehlschlager et al. 2003). Clear instructions to the nursing for controlling the free flow of the bladder catheter at the end of the instillation should be given.

Nevertheless, it is clear from the review of the literature that one immediate instillation after TUR is an adjuvant treatment that adds hardly any morbidity to the operation itself. Nearly all patients already have a catheter after TUR and, if local regional anesthesia is used, patients will not experience any additional discomfort. The reluctance to use this treatment strategy should be reconsidered since the potential benefits clearly outweigh the possible risks and costs.

11.5.3.6 Further Intravesical Chemotherapy

A single immediate instillation of a chemotherapy agent significantly reduces the recurrence rate in patients included in a low-risk group and this might be considered as the standard treatment for these patients, but the question is whether this approach is enough for patients at higher risk of recurrence or whether patients need further adjuvant intravesical chemotherapy.

In the meta-analysis from Sylvester et al.,(2004) patients with multiple tumors had a recurrence rate significantly lower than that of the control group but significantly higher than patients with solitary tumors. In consequence, the need for more than one early instillation might be related to prognostic factors for recurrence (see further).

As the progression risk is very limited in these patients, one option is to start second instillation therapy after the first recurrence. Using these prognostic factors, patients at high risk for recurrence can be identified and therapy could be applied as follows: (a) one single instillation for patients with good prognostic factors and (b) further instillations for patients with prognostic factors associated with a high recurrence rate.

Dose, schedule, duration, and choice of drug are subject of another chapter in this book. After a systematic review by Sylvester et al. (2008) of results of randomized clinical trials it is still not possible to give clear recommendations based on evidence.

11.5.3.7 The Role of BCG

Overall, in a systematic literature overview, BCG significantly reduces the recurrence rate compared to chemotherapy (Tawkif et al. 1986; Huncharek and Kupelnick 2003; Shelley et al. 2003). However, concerning the low-risk group, this evidence is not clear. Concerning low- to intermediate-risk groups BCG was not superior to MMC unless maintenance was applied (Shelley et al. 2003). In all series, BCG was significantly more toxic than intravesical chemotherapy. In consequence, in patients with low-grade tumors at high risk of recurrence, BCG should not be used as first-line treatment but can be used as a second-line therapy. See also the chapter in this book dedicated to this subject.

11.6 Prognostic Factors

The classic way to categorize patients with TaT1 tumors is to divide them into risk groups based on prognostic factors derived from multivariate analyses. Using such a technique, it was proposed to divide patients into low-risk, intermediate-risk, and high-risk groups (Böhle et al. 2003). When using these risk groups, however, no difference is usually made between the risk of recurrence and progression.

Low-grade tumors have a very low tendency for progression but may be highly recurrent. Holmäng et al.(2001) have evaluated recurrence and progression patterns of patients with TaG1 lesions to whom intravesical therapy was rarely administered. They noticed 71% recurrences at 5 years follow-up of TaG1 bladder tumors. The recurrence rate was much higher in patients with multiple tumors. Even patients with PUNLMP, despite the low malignant potential of these tumors, have a high and long-lasting chance for recurrence (Fujii et al. 2003).

In order to separately predict the short-term and long-term risks of both recurrence and progression in individual patients, the EORTC developed a scoring system and risk tables (Sylvester et al. 2006). The basis for these tables is the EORTC database, which provided individual patient data for 2,596 patients diagnosed with TaT1 tumors who were randomized in seven EORTC trials. Patients were treated according to the protocols, which did not contain the second TUR and maintenance BCG therapy.

The scoring system is based on the six most significant clinical and pathological factors, which were already previously identified as important for prognosis:

- Number of tumors
- Tumor size
- Prior recurrence rate
- T category
- Presence of CIS
- Tumour grade

Tables 11.1 and 11. 2 provide a patient's risk of recurrence and progression based on their most important clinical and tumor characteristics. Using these tables, the urologist can discuss with a patient his prognosis and offer different treatment options.

11.6.1 Follow-up

Patients with low-grade tumors should be followed, although early detection of recurrence is not as important as it is for superficial bladder tumors with a higher grade. As elucidated above, the most important factors are multiplicity, recurrence at 3 months, previous recurrence rate, and size of the tumors (greater than 3 cm).

Evolution to a higher grade can occur. Leblanc et al.(1999) noted 21 progressions to G2 or G3 and 3 to CIS in a total of 152 TaG1 tumors with a follow-up to 6–241 months (mean 76 months). Thus, 17% evolved to a higher grade and of them 5 developed muscle-invasive disease (3.3%). Borhan et al. (2003) found an evolution to a higher grade in 179 TaG1 tumors in 29 cases (16.3%). Only five progressed to T1 and three to muscle-invasive disease. Holmäng et al. (2001) saw a 2.4% progression rate to muscle-invasive disease.

In conclusion, evolution to higher grade and stage in TaG1 tumors does occur, but in less than 20% of the cases over years. Evolution to progression is a rare event.

Table 11.1 Weighting used to calculate recurrence and progression scores

Factor	Recurrence	Progression
Number of tumors		
Single	0	0
2–7	3	3
≥8	6	3
Tumor diameter		
<3 cm	0	0
≥3 cm	3	3
Prior recurrence rate		
Primary	0	0
≤1 Recurrence/year	2	2
>1 Recurrence/year	4	2
Category		
Ta	0	0
T1	1	4
CIS		
No	0	0
Yes	1	6
Grade (1973 WHO)		
G1	0	0
G2	1	0
G3	2	5
Total score	0–17	0–23

CIS carcinoma in situ, *rec/yr* recurrence per year

Table 11.2 Probability of recurrence and progression according to score (electronic calculators for Tables 3 and 4 are available at http://www.eortc.be/tools/bladdercalculato/)

Recurrence score	Probability of recurrence at 1 year (95% CI)	Probability of recurrence at 5 years (95% CI)	Recurrence Risk Group
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%)	
10–17	61% (55, 67%)	78% (73, 84%)	High risk
Progression score	Probability of progression at 1 year (95% CI)	Probability of progression at 5 years (95% CI)	Progression risk group
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%)	

11.6.2 Frequency of Cystoscopy

Many authors suggested to diminish the heavy schedules of cystoscopy used in the past and to adapt them to the risk factors mentioned above. However, there are rare data which calculate the risk at any given surveillance schedule. Based on their experience in 120 papillary G1 and G2 tumors, Oge et al. (2000) proposed to

postpone cystoscopy for another 9 months in those cases where the first cystoscopy at 3 months is negative. This is also the recommendation of the EUA guidelines. The expert panel considered the first control at 3 months after TUR still advisable as it picks up the incomplete resections and remains an important prognostic factor.

11.6.3 Duration of Follow-up

Looking to all the Kaplan-Meyer curves in clinician studies on nonmuscle-invasive bladder tumors, one notices that the highest chance of recurrence is in the first 2 years, less in the third year, and fewer/year thereafter. All studies in which patients were followed over a long period mention recurrence even after several years of follow-up with no disease. A risk of recurrence remains lifelong, but most experts propose to stop cystoscopic surveillance when it remains negative for 5 years.

11.6.4 Active Surveillance

Several small series of active surveillance of recurrences of selected low-risk tumors have been published in recent years (Gofrit et al. 2006; Pruthi et al. 2008; Hernández et al. 2009). It is based on the slow growth of these tumors and the low chance of progression, which allows the delay of treatment (Soloway et al. 2003). The tumors should be small recurrences (less than 1 cm) of low-grade tumors and urinary cytology must be normal. The editorial comments following these articles clearly demonstrate that the experts consider this track should be followed (Oosterlinck 2006).

11.6.4.1 Office Fulguration of Recurrent Low Grade Tumors

In an attempt to minimize the need for TUR and reduce the cost of hospitalization and anesthesia, several authors have investigated the feasibility and efficacy of outpatient fulguration of cystoscopically-appearing low grade, noninvasive, recurrent papillary tumors of the bladder.

The feasibility of fulguration or ablation of bladder tumors via flexible cystoscopy has been confirmed in multiple studies. German et al.(1992), using topical local anesthetic gel, found that only 2 of 17 (12%) patients described the procedure as painful. Dryhurst et al. further refined the anesthetic technique by instilling a 60 mL solution of lidocaine retained in the bladder for 20 min prior to fulguration (Dryhurst and Fowler 2001). Syed et al. (2001) described the use of the Holmium: Yag laser for tumor ablation via a flexible cystoscope under local anesthesia. In that study, 83% of patients scored their pain as 2 or less out of 10 on a visual analog scale.

Wedderburn et al.(1999) evaluated the efficacy and tolerance of outpatient fulguration in 103 patients with recurrent low-grade tumors. Median follow-up was 21 months. Half of the patients (52/103) had no recurrence following fulguration. Discomfort was mild or negligible for 80% of the patients.

Herr and coworkers were among the first to publish on office fulguration (Donat et al. 2004). His group presented a prospective analysis of 267 consecutive patients with a history of urothelial carcinoma of the bladder who underwent routine surveillance cystoscopy over a 4-year period (Herr et al. 2007). These included 238 (89%) with an initial tumor of stage T1 or less and 25 (9%) who presented with a muscle-invasive tumor. Patients with low grade-appearing and fewer than 5 recurrences, all of which were less than 0.5 cm in diameter, were considered for fulguration. Patients who had positive urine cytology underwent formal biopsy. Of the 267 patients, 103 underwent office fulguration at least once during the study period, although only 74 of 123 (60%) were managed by fulguration alone. Patients who underwent fulguration were at no greater risk of disease progression or cancer death than those who never underwent fulguration and were managed by TUR of tumor alone.

Their publication on 215 low-grade bladder tumors only, confirmed the earlier experiences. With a median follow-up of 8 years, only one TUR was necessary every 3 years and one fulguration/2 years; There were only 17 patients (8%) who progressed in grade or stage. The authors concluded that office fulguration is appropriate for selected patients with recurrent low-grade superficial urothelial carcinoma of the bladder.

11.7 Summary

In summary, there is sparse prospective data regarding the efficacy of fulguration alone in the management of recurrent bladder lesions. However, an extensive experience from Memorial Sloan-Kettering suggests that in selected patients with less than five small (<0.5 cm) low-grade-appearing recurrent tumors and negative urine cytology, office fulguration alone is safe and effective. All authors concur that office fulguration alone is inappropriate for initial management of a bladder lesion.
Papilloma
Papillary neoplasm low malignant potential (PUNLMP)
Low-grade carcinoma, grade 1
High-grade carcinoma, grade 3

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Chapter 12
Treatment of Low-Grade Bladder Tumors

Satya Allaparthi and K.C. Balaji

Abstract Low-grade bladder tumors have relatively low risk of recurrence and progression compared to high-grade tumors; management options include surveillance, transurethral resection, intravesical chemotherapy, photodynamic therapy, and laser ablation. Role of radical or partial cystectomy is limited; however, it may be used in patients with large-volume tumors, intractable bleeding, and high-grade recurrence refractory to conservative treatment. This chapter is focused on various treatment options for low-grade bladder tumors.

12.1 Introduction

Recent 2004 World Health Organization (WHO)/International Society of Urological Pathology (ISUP) classification of bladder tumors basically showed that grade 1 (G1) (WHO 1973 classification) tumors should be subdivided into Papillary urothelial neoplasm of low malignant potential (PUNLMP) and low-grade carcinomas, whereas most grade 2 (G2) and all grade 3 (G3) cases are defined as high-grade carcinomas. Grade 2 tumors, whose morphology borders with that of grade 1, become low-grade carcinomas as well. However, some controversies followed the introduction of the 1998 WHO/ISUP classification of bladder tumors (Epstein et al. 1998; Bostwick and Mikuz 2002; Busch and Algaba 2002), mainly because of lack of validation, reproducibility, and translation studies. The key point of the latest classification is a description of the categories that has been expanded in the current version to improve their recognition. One group (papillary urothelial neoplasm of low malignant potential) with particularly good prognosis does not carry the label of "cancer"; it avoids use of ambiguous grading such as grade 1/2 or 2/3 (according to 1973 WHO classification). The group of non-invasive high-grade carcinoma is

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large enough to contain virtually all those tumors that have biological properties (and a high level of genetic instability) similar to those seen in invasive urothelial carcinoma (Lopez-Beltran and Montironi 2004).

12.2 Treatment of Low-Grade Bladder Tumors

As the risk of recurrence and progression is relatively low compared to high-grade tumors, low-grade tumors are generally managed by surveillance, transurethral resection for recurrent tumors, photodynamic therapy, or laser ablation. The natural untreated history of low-grade bladder tumors clearly shows a substantial risk of recurrent disease in 3 years, ranging from 35% to 40% in the lowest-risk tumors to 70–80% in the highest-risk tumors. The risk of tumor progression to muscle-invasive disease varies depending on the risk category of the patient, but is, on average, 10% in 3 years. The 5-year cancer-specific survival is somewhat under 90% (Schrier et al. 2004). However, in rare cases that are not amenable to above ablative procedures, alternate treatment options such as, partial or radical cystectomy and radiation therapy can be used.

12.2.1 Transurethral Resection of Bladder Tumor

The gold standard for the initial diagnosis and treatment of superficial bladder cancer is Transurethral resection of bladder tumor (TURBT) (Babjuk et al. 2008; Nieder et al. 2005).This procedure allows an adequate histological examination of the tumor for diagnosis and staging. It establishes the diagnosis and allows pathologic analysis of the resected tumor specimen for tumor grade and depth of bladder invasion (Holzbeierlein and Smith 2000). Appropriate resection techniques should be used, as the quality of the initial TURBT may have substantial influence on tumor recurrence. Recent reports indicate that TUR is not complete in many cases, even when the surgeon believes that all visible lesions have been removed. Kolozsy found residual tumor in 12.7% of pTa and in 36.2% of pT1 tumors when performing systematic biopsies after TUR (Kolozsy 1991). Klan et al. in a similar study found residual tumor in 43.5% of pT1 tumors (Klan et al. 1991). In a prospective study, Vogeli et al. found a recurrence in 37% of pTa and 43% of pT1 tumors 4–6 weeks after TUR. Mersdorf et al. in high-grade, multiple tumors reported a recurrence rate of 45% and 58% in pTa and pT1 tumors, respectively, 6–8 weeks after the first TUR.

12.2.2 Procedure

TURBT is performed under regional or general anesthesia to remove all visible tumors, to provide specimens for pathologic examination, and to determine

stage and grade. Bimanual examination of the bladder should be performed under anesthesia before prepping and draping, unless the tumor is clearly small and should be repeated after resection. Any fixation or persistence of a palpable mass after resection suggests locally advanced disease. A 30° lens placed through a resectoscope sheath is used for resection, because deflection allows visualization of the loop placed at this location. Continuous irrigation can lessen bladder wall movement and thinning of the detrusor through overdistention (Vogelzang et al. 2006). Friable, low-grade tumors can often be removed without the use of electrical energy, with nonpowered cutting loop which will break off most segments.

To minimize the chance of bladder perforation, piecemeal resection is to be performed delaying transection of any stalk until most of the tumor is resected in order to maintain counter traction. Lifting the tumor edge away from detrusor lessens the chance of perforation (Holzbeierlein and Smith 2000). Higher-grade, more solid tumors and the base of all tumors require the use of cutting current; cautery yields hemostasis once all tumors are resected. Cold cup biopsy can be obtained from base of the tumor and sent to pathology separately to determine the presence of muscle invasion and Elliks evacuator can be used to gather the specimen.

12.2.3 Complications of TURBT

Minor bleeding and irritative symptoms are common side effects in the immediate postoperative period. Five percent of cases have major complications of uncontrolled hematuria and clinical bladder perforation.The vast majority of perforations are extraperitoneal, but intraperitoneal rupture is possible when resecting tumors at the dome (Collado et al. 2000). The risk of tumor seeding due to perforation is not widely reported but appears to be low (Balbay et al. 2005).

Anecdotal reports have identified extravesical recurrences after perforation, theoretically due to seeding (Mydlo et al. 1999). It has been suggested that the risk of tumor seeding is higher in patients who undergo open repair, but this may be related to patient selection because only serious intraperitoneal perforations are likely to be managed in this manner (Skolarikos et al. 2005).

Management of extraperitoneal perforation is usually possible by prolonged urethral catheter drainage. Intraperitoneal perforation is less likely to close spontaneously and often requires open or laparoscopic surgical repair. Decisions for surgical correction should be made based on the extent of the perforation and the clinical status of the patient. TUR syndrome from fluid absorption is uncommon but reported in literature. Ureteral orifice resection during TURBT is a known complication. Scarring is minimal and obstruction unlikely if pure cutting current is used. Upper tract imaging can determine presence or absence of obstruction. Balloon dilation of the orifice or endoscopic incision can correct the situation, but failure to respond may require reimplantation (Rees 1969; Posta et al. 1980).

12.3 Repeat TURBT

A single TUR may not remove all tumors, particularly invasive cancer, even when performed by experienced urologists (Zurkirchen et al. 2004). Repeat TUR performed within several days to several weeks of the original resection showed residual tumor at the site of the initial resection at least 40% of the time (Klan et al. 1991). It was found that second TURBT performed within 6 weeks of the initial resection detected residual tumor in 26–83% of cases and corrected clinical staging errors in 50% of those cases (Miladi et al. 2003).

Complete tumor removal is not always possible, whether due to excessive tumor volume, anatomic inaccessibility, medical instability requiring premature cessation, or risk of perforation. A restaging TUR found residual tumor in a substantial proportion (43–76%) of patients presenting with high-grade Ta or T1 bladder tumors including invasive cancer in 24–38% of patients (Herr 2005).

In up to 25% of specimens repeat TURBT demonstrates worse prognostic findings in the evaluation of T1 tumors (Schwaibold et al. 2006). This is more likely if no muscle is identified on initial pathology, which can occur in almost 50% of cases. The Vanderbilt University group reported that 64% of T1 lesions were at risk of understaging when muscle was absent compared with 30% when muscle was present in the specimen. It was noted in a German observational study that survival was 63% in patients who underwent a second TURBT versus 40% for those who did not (Grimm et al. 2003). Within 6 weeks of initial resection, 37% of patients with initial T1 bladder tumors had persistent tumors on second resection (Zurkirchen et al. 2004). Schips et al. prospectively evaluated the findings at first and second TURBT for patients with high-grade T1 bladder tumors and also found residual disease in >50% of patients (Schips et al. 2002). Multifocality and tumor grade increased the risk of finding residual tumor on second TURBT. Although 76% of patients with a solitary T1 lesion at first TURBT had a negative second TURBT, only 53% of those with multifocal T1 lesions had a negative repeat TURBT. Moreover, 73% of those with papillary-appearing T1 lesions at first resection had a negative repeat TURBT compared with only 47% of those with solid-appearing T1 lesions (Schips et al. 2002).

Repeat resection is also helpful in the setting of a second opinion unless clear evidence of muscle invasion is identified on the initial resection, or if the outside pathology slides are not available for review. Consensus is that patients with pT1 and high-grade Ta tumors merit repeat resection (Nieder et al. 2005). There is no consensus on timing of repeat TURBT, but most authors recommend 1–4 weeks after the initial resection (Nieder et al. 2005). Biopsying the base and margins of each tumor site is an alternative strategy to restaging TUR as a separate procedure during the first procedure with the patient under the same anesthesia, and submit such tissue separately, which in essence recapitulates a second TUR.

Key Points

1. The risk of upstaging on second TURBT is ≥30% if muscle is present in the specimen and even higher if muscle is not present.

2. The risk of residual tumor on second TURBT is also significant.
3. Even for solitary, papillary-appearing tumors, the risk is 24–27%.
4. It is recommended that a second TURBT be considered for all patients with high-grade Ta or any T1 urothelial carcinoma.
5. A standard of universal repeat resection for high-grade or T1 tumors should be recommended in an attempt to prevent understaging and possible progression to metastatic disease.
6. No evidence on the timing of a second TURBT is available; the consensus opinion is that this should be performed within 2–6 weeks after the initial resection.

12.4 Role of "Random" Bladder and Prostatic Additional Biopsies

Biopsies of any suspicious areas are an important part of a complete evaluation. Based on the understanding that Carcinoma In Situ (CIS) can exist in normal-appearing urothelium, some authors advocated the use of random biopsies to identify CIS in otherwise normal-appearing mucosa. When cytology is positive or when exophytic tumor is of nonpapillary appearance, cold cup biopsies from normal-looking mucosa should be performed. When abnormal areas of urothelium are seen, it is advised to take "cold cup" biopsies or biopsies with a resection loop. Material obtained by random or directed biopsies must be sent for pathological assessment in separate containers.

Cold-cup biopsies may not provide as much information regarding muscular invasion but provide tissue sampling without cautery artifact (Soloway et al. 1978). May et al. performed random biopsies in high-risk patients and found that the results were positive in 12.4% and altered treatment in 7%, including 14 of 1,033 patients in whom the only positive tissue was in the random biopsy, not the primary resected tumor (May et al. 2003). Swinn et.al reported that even when velvety red patches were sampled, only 11.9% of biopsies were positive.

A European Organization for Research and Treatment of Cancer (EORTC) retrospective review found that 10% of random biopsies were positive (3.5% CIS) and concluded that such biopsies were not warranted (van der Meijden et al. 1999). The current consensus is that random biopsies are not indicated in low-risk patients, that is, those with low-grade papillary tumors and negative cytology.

The involvement of the prostatic urethra and ducts in male patients with TaT1 bladder tumors has been reported. Although the exact risk is not known, it seems to be higher if tumor is located on the trigone or bladder neck, in the presence of bladder CIS and in multiple tumors.

If neobladder creation is anticipated, prostatic urethral biopsy may be performed for high-risk disease (Holzbeierlein and Smith 2000). The theoretical risk that biopsies provide an exposed bed to aid tumor implantation must be weighed against the information obtained from cold cup and urethral biopsies (Mufti and Singh 1992; Yamada et al. 1996). TUR of the prostate (TURP) and TURBT of a low-grade bladder tumor may be performed at the same setting, but resection

of a high-grade bladder tumor should not be done coincident to TURP to avoid tumor seeding and possible intravasation of tumor cells that are likely to metastasize (Tsivian et al. 2003).

12.5 Laser Treatment

Laser treatment is an option that allows effective ablation of most superficial tumors. It is best suited for the treatment of recurrent superficial disease, because pathologic analysis of the initial lesion is usually desirable, requiring electrosurgical resection.

Laser coagulation allows minimally invasive ablation of tumors up to 2.5 cm in size. The neodymium:yttrium-aluminum-garnet (Nd:YAG) laser has the best properties for use in bladder cancer.

12.5.1 Advantages

1. Minimal bleeding, ability to use with flexible cystoscopes, and potential for less postoperative irritative symptoms. No risk of obturator reflex. Small lesions can be treated easily using intravesical anesthesia.

Because there is no tissue available for pathologic inspection, the optimal candidate for laser therapy is the patient with recurrent, low-grade lesions whose biology is already known.

The largest clinical experience has been with the neodymium:yttrium-aluminum-garnet (Nd:Yag) laser with an end-fire noncontact fiber (Smith 1986a, b). The most efficient delivery appears to be an end-fire noncontact fiber with a 5–15-degree angle of divergence, which allows variable penetration depth of up to 5 mm (Smith 1986a, b; Holzbeierlein and Smith 2000). The Nd:Yag laser primarily causes thermal coagulation with a penetration depth of 3–5 mm. Hofstetter et al. developed the energy parameters used most often for the treatment of bladder tumors. A power output of 35 W for 3 s did not exceed a temperature of 60 C on the outer surface of the bladder. Thus this energy level was considered to be optimal for bladder cancer treatment and safe in preventing laser injury to adjacent organs (Hofstetter et al. 1981).

Lesions can be coagulated until nonviable through protein denaturation using a straight or 90-degree noncontact “free beam” laser using power output of up to 60 W. Limiting energy to 35 W precludes exceeding 60°C on the outer bladder wall, minimizing the risk of perforation unless higher energy is needed for a very large tumor (Hofstetter et al. 1981).

Lasers of other wavelengths have also been used. Argon, potassium titanyl phosphate, and the holmium yttrium-aluminum-garnet laser have all been used in the treatment of superficial TCC (Smith 1992; Johnson 1994). Reports suggest

lower recurrence rates using laser compared with TURBT, but this remains inconclusive (Smith et al. 1983; Malloy and Wein 1984; Beisland and Seland 1986; Beer et al. 1989).

Holmium:yttrium-aluminum-garnet (YAG), argon, and potassium titanyl phosphate (KTP) lasers ablate tissues by cutting (vaporization) and thus have limited applicability due to lack of deep coagulation (Jahnson et al. 1991; Holzbeierlein and Smith 2000).The carbon dioxide laser is completely absorbed by fluid and so is not appropriate for use in the treatment of bladder tumor.

12.5.2 Complications

The most significant complication of laser therapy is forward scatter of laser energy to adjacent structures, resulting in perforation of a hollow, viscous organ such as overlying bowel. This is rare but most commonly occurs with the neodymium:YAG laser because of its deeper tissue penetration than with holmium:YAG and KTP lasers (Smith 1986a, b).

12.6 Photodynamic Therapy

Principle: Photodynamic therapy relies on the photosensitization of cancerous cells with subsequent administration of light therapy. Hematoporphyrin preparations, derived from degradation of hemoglobin, are the commonly used photosensitizers. These porphyrin-based photosensitizers were first used to detect tumors by use of fluorescence. Treatment of superficial bladder cancer with photodynamic therapy was first described in 1975 (Kelly and Snell 1976).

A hemoporphyrin photo sensitizer was given intravenously with subsequent activation by mercury light illumination of the bladder. The early use of this therapy for bladder cancer was confined to individual tumor treatment by focal illumination. Now it is more commonly used with whole bladder illumination, not only to treat recognized tumors, but also to identify areas of the bladder mucosa that may represent cytoscopically unrecognized carcinoma.

Photodynamic therapy is often used in patients whose conditions do not respond to standard intravesical therapies, and in these patients, response rates at 3 months have ranged between 57% and 100% (Walther 2000). Effective photosensitization and cell death requires oxygen in addition to the sensitizer and the light source. After excitation by light exposure, the photosensitizer reacts with oxygen to form cytotoxic free radicals (Mitchell et al. 1985).

Photofrin (porfimer sodium), a porphyrin mixture of dihematoporphyrin ethers and esters, is the most commonly used sensitizer in bladder cancer. (Walther 2000) This agent is administered intravenously 2–3 days before light administration to allow its clearance from normal tissue and to maximize its concentration in the tumor. The depth of light penetration varies inversely to its wavelength.

(Walther 2000) Red light (630 nm) is often used because it is absorbed by the lowest excitation band of the porphyrins.

Red light will also penetrate tissue up to 1 cm and is not absorbed as readily by normal hemoglobin in the bladder wall. Green light (540 nm) is better than red light for the treatment of carcinoma in situ because there is higher energy delivery and less tissue penetration.

12.6.1 Procedure

After intravenous photosensitization, the bladder is illuminated by laser light with an optical fiber that can be passed through a standard cystoscope and placed in a central location in the bladder. The light therapy, administered as whole-bladder treatment, usually takes between 12 and 20 min and requires general anesthesia in the operating room to limit patient movement and to ensure uniform light delivery to the bladder urothelium. A Foley catheter is usually placed temporarily after light administration. In patients with carcinoma in situ, which is often refractory to standard intravesical therapy, an overall response rate of 66% has been reported (Walther 2000). Patients with papillary tumors have an overall response rate of 54%, with an estimated median time to recurrence of 25–48 months (Walther 2000).

12.6.2 Complications

Most patients have significant irritative bladder symptoms associated with microscopic hematuria that usually peaks on the second posttreatment day (Walther 2000). Although photodynamic therapy appears to be effective, its widespread use has been limited by its toxicity. Dermal sensitivity resulting in sunburn has been reported in 19% of patients. Decreased bladder volume (at least 50%) occurs in 16% of patients, and debilitating bladder fibrosis can also occur, leading to cystectomy. (Walther 2000) Current research on photodynamic therapy is focused on the development of more tumor-selective photosensitizers that might be associated with less dermal and bladder toxicity.

12.7 Radical or Partial Cystectomy

While most patients with low-grade tumors can be managed by ablative methods, large-volume or multifocal tumors, extremely symptomatic tumors unmanageable by ablative methods alone can benefit from partial or radical cystectomy.

12.7.1 Indications for Radical Cystectomy in Patients with Low-Grade Bladder Tumors

Among patients with low-grade noninvasive tumors treated with initial intravesical therapy, progression rates as high as 53% have been observed, with death from bladder cancer occurring in as many as one-third of those followed for up to 15 years (Cookson et al. 1997). Some patients with low-grade nonmuscle-invasive disease harbor potentially lethal tumors justifying aggressive surgical intervention when clinically indicated. The indications for radical cystectomy among patients with low-grade noninvasive disease include high-grade disease recurrence after TUR and intravesical therapy. Patients with concomitant Carcinoma In Situ (CIS) who are refractory to conservative intravesical therapy including salvage therapy should also be offered the option of radical cystectomy. Despite the term low-grade bladder tumors, up to 50% of patients with presumed low-grade bladder tumor nonmuscle-invasive disease who undergo cystectomy will actually be found to have muscle-invasive disease. Considering that, up to 15% would already have micrometastases (Chang and Cookson 2005). Some patients may choose cystectomy as an initial treatment option, which must be balanced against the potential risks, benefits, and impact on quality of life.

12.7.2 Outcome of Radical Cystectomy in Low-Grade Noninvasive Bladder Tumors

In a recent series of T1 tumors treated with cystectomy, the estimated 10-year disease-free survival rate for pathologic T1 tumors was 92% (Bianco et al. 2004). This rate was compared with a 64% 10-year disease-free survival among those with muscle invasion at the time of cystectomy. In a large cohort from a study by Stein et al. with long-term follow-up, the 10-year recurrence-free survival rate among patients who had radical cystectomy with pathologic T1 tumors was 78% (Stein et al. 2001).The risk of progression must be weighed against the risk, morbidity, and impact on quality of life for cystectomy. Thus, a reasonable goal might be, as termed by “timely” cystectomy for patients at risk (Chang and Cookson 2005).

12.7.3 Partial Cystectomy

Partial cystectomy is particularly an attractive option in a select group of these patients because it preserves the bladder without compromising overall outcome of the patient. In well-selected patients, partial cystectomy can yield favorable results (Brannan et al. 1978) with a survival rate of 58%. An ideal patient for partial cystectomy is one who

has a normally functioning bladder with good capacity, a first-time tumor recurrence with a solitary tumor, and a tumor location in an area that allows a 1–2-cm margin of resection, such as at the dome.

12.8 Radiation Therapy

While RT is rarely indicated for low-grade noninvasive tumors, RT has a role in palliative management of symptomatic patients with large-volume tumors. In particular, external beam RT has been shown to be effective in the management of patients with intractable hematuria and patients who are unfit or not amenable to surgical management. Radiation therapy in the treatment of nonmuscle-invasive bladder cancer is generally restricted to those individuals who refuse cystectomy after the failure of intravesical therapy or who are unsuitable for major surgery (van der Werf-Messing 1984). A complete response to radiation therapy and TUR is attainable in 50–75% of patients, but the additional benefit of radiation to TUR remains unclear (Jahnson et al. 1991; De Neve et al. 1992). Five-year response rates are 44–60%. There is no significant effect on CIS. Due to reports that up to 50% of patients will develop progression and a high likelihood of death (Dunst et al., 1994; Rodel et al., 2006), there is a minimal role for radiation therapy other than for palliative purposes in this population.

12.9 Predicting Recurrence and Progression in Low-Grade Tumors

The dual risks of recurrence and progression mandate an adequate surveillance program for patients with low-grade bladder tumors. Although a variety of different protocols exist, there is consensus that surveillance needs to be most intense initially, when the recurrence risk is greatest. Furthermore, recurrences and progression may be seen even after a long disease-free interval, thus necessitating life-long follow-up (Leblanc et al. 1999). On the other hand, for low-risk lesions, such as grade 1 Ta tumors with no associated CIS, the surveillance interval may safely be prolonged (Olsen and Genster 1995) and, in the absence of recurrences during follow-up, ultimately discontinued (Haukaas et al. 1999).

The classic way to categorize patients with TaT1 tumors is to divide them into risk groups based on prognostic factors derived from multivariate analyses. Using such a technique, it was proposed to divide patients into low-risk, intermediate-risk, and high-risk groups. A simple scoring system was derived based on six clinical and pathological factors: number of tumors, tumor size, prior recurrence rate, T category, carcinoma in situ, and grade. The probabilities of recurrence and progression at 1 year ranged from 15% to 61% and from less than 1–17%, respectively.

At 5 years, the probabilities of recurrence and progression ranged from 31% to 78% and from less than 1–45%, respectively (Sylvester et al. 2006).

When using these risk groups, however, no difference is usually made between the risk of recurrence and progression. Although prognostic factors may indicate a high risk for recurrence, the risk of progression may still be low and other tumors may have a high risk of both recurrence and progression.

12.10 Surveillance

There is currently no agreement on the recommended follow-up schedules for low-, intermediate-, and high-risk disease. The International Bladder Cancer Group (IBCG) has proposed the following schedule which is based on the EAU recommendations for follow-up, with minor modifications (Babjuk et al. 2008). Update March 2008. Arnhem, the Netherlands: European Association of Urology; 2008. http://www.uroweb.org/fileadmin/tx_eauguidelines/TaT1%20Bladder%20Cancer.pdf:

- Low-risk disease:
- (a) Surveillance cystoscopy at 3 months for 1 year. (b) If negative, subsequent cystoscopies are advised at 9 months and then yearly for a minimum of 5 years. (c) No upper tract investigations are required.
- High-risk disease:
- (a) Cystoscopy and cytology at 3 months. (b) If negative, subsequent cystoscopies and cytology assessments should be repeated every 3 months for a period of 2 years, every 4 months in the third year, every 6 months thereafter until 5 years, and annually thereafter. (c) Annual upper urinary tract imaging should also be considered.
- Intermediate-risk disease: Follow-up schedule should be between that for low- and high-risk disease and should be adapted according to individual patient factors.

12.10.1 Cystoscopic Surveillance

Office-based cystoscopy is a rapid, relatively painless way of visual access to the urothelium. A velvety red mucosal patch is classically described as Carcinoma in situ (CIS) although the reliability of such findings has been questioned. With the emergence of tumor markers and the development of newer endoscopic technology, including fluorescent cystoscopy, the role of cystoscopy as a "gold standard" in cancer detection has come under scrutiny (Kriegmair et al. 1996; Filbeck et al. 1999). It allows identification of the site and characteristics of most tumors in office-based setting. Cystoscopy has high positive predictive value because most lesions believed to be malignant are proven so pathologically. The endoscopic appearance

cannot reliably predict tumor stage or grade, although sessile morphology and/or the presence of necrosis suggest high-grade disease likely to be invasive (Mitropoulos et al. 2005).

Flexible fiberoptic cystoscopes are almost as sensitive as rigid and are markedly more comfortable for men (Meyhoff et al. 1988), although there is no clear advantage to their use in women because of the short, straight female urethra. Newer digital chip cystoscopes offer similar tolerability but better visualization due to clarity and magnification on video monitors (Quayle et al. 2005). Flexible cystoscopy has essentially replaced rigid cystoscopy for surveillance in men in North America and may do so in women.

Adherence to surveillance recommendations is often suboptimal, as illustrated in a series of 6,717 patients aged 65 or older with nonmuscle-invasive bladder cancer identified in the Surveillance, Epidemiology and End Results (SEER)-Medicare linked database of the National Cancer Institute (Schrag et al. 2003). During the five contiguous 6-month intervals following initial diagnosis, only 40% of patients had an examination during all five periods, while 18% were examined during two or fewer of these periods. Among the factors that were independently associated with a lower intensity of surveillance were age $\geq$ 75 years, nonwhite, favorable tumor histology (i.e., well-differentiated versus poorly differentiated), and a high degree of comorbidity.

Several authors recommend termination of surveillance at 5 or more years for low-risk patients (Haukaas et al. 1999). However, the actual cost of surveillance cystoscopy is responsible for only 13% of the expenditures for bladder cancer care in one study, so the financial opportunity may be limited for such efforts (Hedelin et al. 2002). In addition, the risk of recurrence and potential for progression exists beyond this period. Reports of late recurrences of high-grade cancer years after the original tumor temper some authors' enthusiasm with terminating surveillance at any point (Thompson et al. 1993; Morris et al. 1995; Leblanc et al. 1999; Zieger et al. 2000).

Tumor recurrence on initial 3-month cystoscopy and number of tumors on initial resection (single or multiple) provide the most predictive information with regard to recurrence in several studies. Absence of recurrence on the 3-month surveillance cystoscopy in patients with TaG1 tumors is associated with recurrence rates so low that annual cystoscopy appears safe even at that point (beginning 12 months after the initial resection (Fitzpatrick et al. 1986; Olsen and Genster 1995)). Finally, patients with a negative cystoscopy and a negative UroVysion assay are at very low risk of recurrence in the following 6 months, creating opportunity to individualize the surveillance schedule (Sarosdy et al. 2002).

12.10.2 Fluorescence Cystoscopy

Photodynamic diagnosis (PDD) offers the ability to augment detection of neoplastic tissue through fluorescent enhancement. The use of white light may lead to missing

lesions that are present but not visible. Fluorescence cystoscopy is performed using violet light after intravesical instillation of a photosensitizer or its precursor, usually 5-aminolevulinic acid (5-ALA) or hexyl aminolevulinate (HAL). It has been confirmed that fluorescence-guided biopsy and resection are more sensitive than conventional procedures in detecting malignant tumor, particularly CIS (Schmidbauer et al. 2004). False positivity, however, can be induced by inflammation and recent TUR or intravesical instillation. The benefit of fluorescence-guided TUR for recurrence-free survival was shown in several small randomized clinical trials (Denzinger et al. 2007), but its definitive value in improving the outcome of patients for progression rates or survival remains to be proven and the additional costs of the equipment should be considered.

12.11 Discussion and Conclusion

Clinical management of low-grade bladder tumors involves TURBT, laser treatment, photodynamic therapy, partial or radical cystectomy, and radiotherapy. Initial treatment advice is given based on these risk groups. Initial treatment is a complete TUR with biopsies, if there is an indication such as abnormal cytology. The additional value of random biopsies has been studied extensively and considered to be low (van der Meijden et al. 1999). In case of an incomplete resection, or any doubts about the completeness, especially in case of T1 tumors when conservative treatment is planned, a second TUR within 2–4 weeks after the first one should be considered.

In the EAU guideline, for example, patients are differentiated into low-risk (single Ta, G1, ≤3-cm diameter), high-risk (T1, G3, multifocal or highly recurrent, CIS), and intermediate-risk (all other tumors, Ta-1, G1-2, multifocal, >3-cm diameter) (Oosterlinck et al. 2002). According to the EAU guidelines, surveillance was based on low-risk disease and high-risk disease with cystoscopy and urine cytology. Office-based flexible cystoscopy was considered cost effective procedure in continuous surveillance of these patients.

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Chapter 13
Intravesical Chemotherapy

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Abstract The rationale for intravesical therapy is to maximize the exposure of tumors located in the bladder to therapeutic agents while limiting the systemic exposure. According to the American Urological Association (AUA), intravesical chemotherapy is recommended as a single immediate instillation after a transurethral resection of bladder tumor (TURBT) and also as 6–12-weekly prophylactic courses for intermediate risk tumors. We discuss the indications and practical aspects of administration of intravesical chemotherapy. The properties and side effects of various intravesical agents are also dealt with. Newer methods improving the efficacy of the intravesical drugs are also detailed.

13.1 Introduction

Urinary bladder being an easily accessible organ is well suited for topical therapy. Hence it is not surprising that intravesical chemotherapy has been extensively studied and utilized. The rationale for intravesical therapy is to maximize the exposure of tumors located in the bladder to therapeutic agents while limiting the systemic exposure. An understanding of pharmacology of the chemotherapeutic agents and drug delivery is of great importance in selecting the appropriate agent.

13.2 Goals and Principles of Intravesical Chemotherapy

The objective of intravesical chemotherapy and immunotherapy are to eradicate residual disease, prevent tumor recurrence and progression. An ideal intravesical agent should have minimal systemic absorption and maximum efficacy (Lamm and Torti 1996). The absorption and effectiveness of the drug is determined by physiochemical properties of the drug, physiological variables in urine and tissue pharmacokinetics (Gasion and Cruz 2006; Wientjes et al. 1993). The absorption

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and efficacy of the intravesical chemotherapeutic agents can be modified by increasing the dose of the drug, decreasing dosing volume, increasing the contact time, decreasing urine production, maximizing bladder emptying and altering the pH (Wientjes et al. 1993).

13.3 Indications for Intravesical Chemotherapy

According to American Urological Association (AUA), European association of Urologists (EAU), and Société Internationale d'Urologie (SIU) guidelines, intravesical chemotherapy is recommended as single immediate instillation after a transurethral resection of bladder tumor (TURBT) and also as 6–12 weekly prophylactic course for intermediate risk tumors (Babjuk et al. 2008; Hall et al. 2007; Sternberg et al. 2007).

13.3.1 Single Perioperative Instillation

Both the EAU and AUA guidelines advocate the use of an immediate, single instillation of intravesical chemotherapy following TURBT (Babjuk et al. 2008; Hall et al. 2007; Nieder et al. 2005a; Oosterlinck et al. 2005; Sylvester et al. 2004) Fig. 13.1. The EORTC (European organization of research and treatment of cancer) meta-analysis found no significant differences in efficacy among the

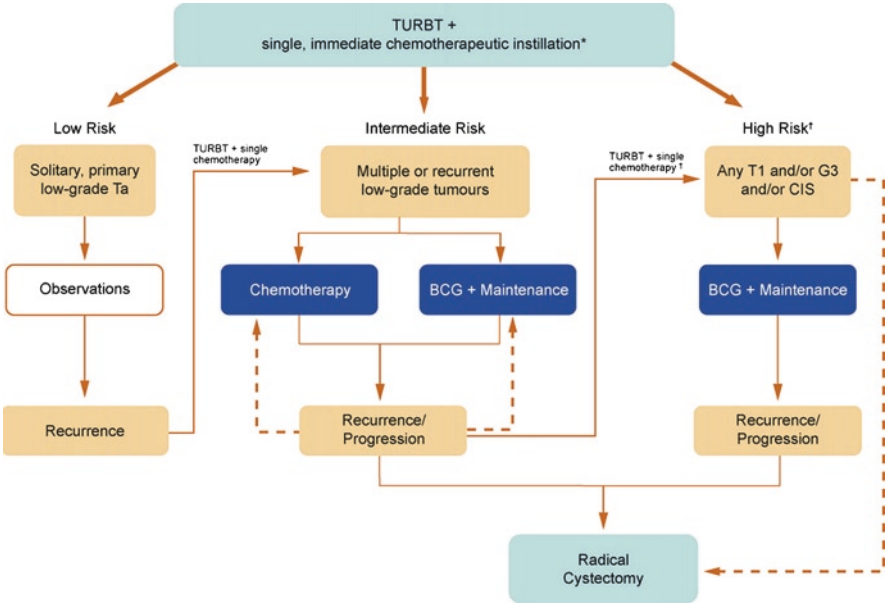

Fig. 13.1 Reproduced from European Urology Supplement (Lamm et al. 2008)

chemotherapeutic agents studied. Therefore, choice of agent is left to the physician (Sylvester et al. 2005a).

The time period within which the installation is completed is very important. In all the studies included in the EORTC meta-analysis, the instillation was administered within 24 h (Sylvester et al. 2005b). Kaasinen et al. found that the risk of recurrence doubled if the instillation was not given within 24 h of TURBT (Kaasinen et al. 2002). However, an immediate, single instillation of chemotherapy should be refrained in cases of suspected intra- or extraperitoneal perforation of the bladder (Kaasinen et al. 2002). The benefit of an immediate single instillation of chemotherapy has not been proven in high grade disease.

13.3.2 Induction Cycle

The EAU and AUA guidelines suggest that intravesical chemotherapy or BCG (Bacillus Calmette Gu'erin) should be offered to patients with intermediate-risk disease following complete TURBT and a single, immediate instillation of chemotherapy (Babjuk et al. 2008; Hall et al. 2007; Nieder et al. 2005a; Oosterlinck et al. 2005). A meta-analysis conducted by the EORTC and the Medical Research Council found that adjuvant chemotherapy after TURBT significantly improves disease-free survival compared to TURBT alone (Pawinski et al. 1996). Review of controlled trials showed a mean decrease in tumor recurrence by 14% (Lamm et al. 1995). However there is no evidence that adjuvant chemotherapy delays progression.

13.3.3 Maintenance Therapy

An EORTC randomized study demonstrated that 1 year of monthly maintenance and 6 months of monthly maintenance chemotherapy had similar efficacy in reducing recurrence rate when the first instillation was given immediately after TURBT (Bouffioux et al. 1995). A review of clinical trials on intravesical chemotherapeutic instillations for NMIBC (non-muscle invasive bladder cancer) suggested that a short intensive schedule of instillations within the first 3–4 months following an immediate instillation is as effective as longer-term treatment schedules (Witjes and Hendricksen 2008). The authors suggested that use of long-term instillations for 1 year should only be considered when an immediate instillation has not been performed (Witjes and Hendricksen 2008).

13.4 Sylvester Risk Assessment

Sylvester et al. have developed a prediction tool using data from seven EORTC randomized clinical trials conducted between 1979 and 1989 (Sylvester et al. 2006). In this risk assessment tool, risk factors were given a score separately

for recurrence and progression (Sylvester et al. 2006). The sum of all the risk factor scores is calculated separately for recurrence and progression. These final scores predict the probability of recurrence and progression at 1 and 5 years (Tables 13.1 and 13.2).

13.5 Practical Aspects of Intravesical Therapy

Strategies to establish a perioperative plan for intravesical chemotherapy

1. Meet with pharmacy and nursing personnel to discuss plans and verify the drug availability.
2. Include immediate perioperative chemotherapy on the operative schedule to alert staff.
3. Call pharmacy before or early into case to verify need for drug.
4. Set up a closed system to minimize nursing contact with chemotherapeutic agent (O'Donnell 2005).

Table 13.1 Weighting used to calculate recurrence and progression scores

Factor	Recurrence	Progression
Number of tumors		
Single	0	0
2–7	3	3
≥8	6	3
Tumor diameter		
<3 cm	0	0
≥3 cm	3	3
Prior recurrence rate (recurrence/year)		
Primary	0	0
≤1	2	2
>1	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
Total score	0–17	0–23

CIS carcinoma in situ

Table 13.2 Probability of recurrence and progression according to total score

Recurrence score	Probability of recurrence				Recurrence risk group
	At 1 year		At 5 years		
	%	(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)	
10–17	61	(55–67)	78	(73–84)	High risk
Progression score	Probability of progression				Progression risk group
	At 1 year		At 5 years		
	%	(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	

Method of administration

1. Place a three-way catheter in the OR attached to an irrigant fluid, which is left turned off.
2. Administer the chemotherapy agent through the main catheter port, clamp with hemostat and attach to a drainage bag. The system is thus closed.
3. Staff should be notified to unclamp after 1 h.
4. Run 1 L of saline through the irrigant port over next 30–60 min.
5. Remove and discard the Foley along with urinary drainage bag into biohazard container (O'Donnell 2005).

13.6 Complications of Intravesical Chemotherapy

13.6.1 Cystitis

Chemical cystitis is a frequently encountered side effect of intravesical chemotherapy, occurring in as many as 56% of doxorubicin-treated patients, 41% of mitomycin C (MMC)-treated patients, and approximately one-third of epirubicin-treated subjects (Thrasher and Crawford 1992; van der Meijden et al. 2001). Suggested treatments include oxybutynin, phenazopyridine, or propantheline bromide.

13.6.2 Hematuria

Hematuria is seen in up to 40% of patients treated with intravesical chemotherapy (Witjes and Hendricksen 2008; Lundholm et al. 1996). It occurs concomitantly

with cystitis, more common seen with extensive TURBT. In this scenario, a urine culture is necessary to exclude bacterial cystitis and the instillations are deferred until the urine is clear. In the case of persistent hematuria a cystoscopy should be performed to rule out residual disease.

13.6.3 Contracted Bladder

A contracted bladder due to extravasation of one of the intravesical therapeutic agents is a serious complication. This is usually associated with multiple TURBTs and maintenance instillations. Management includes withholding intravesical therapy and rarely even cystectomy. Cystoprostatectomy with orthotopic neobladder reconstruction may be the optimal solution to alleviate severe lower urinary tract symptoms and to remove the risk of subsequent urothelial malignancy (Nieder et al. 2005b).

13.6.4 Contact Dermatitis

Contact dermatitis has been reported in up to 10% of patients treated with intravesical MMC and often leads to eczema-like desquamation of the skin on the palms, soles, perineum, chest, and face (Nieder et al. 2005a; Thrasher and Crawford 1992). Careful cleansing of the hands after drug-handling and cleansing of the genitals and perineum after voiding may help prevent contact dermatitis associated with intravesical MMC (Nieder et al. 2005a). Management of this adverse event requires cessation of therapy. The use of topical steroid creams usually relieves the symptoms.

13.6.5 Bladder Calcifications

Bladder wall calcifications can occasionally result following administration of intravesical mitomycin C (Sylvester et al. 2005b; Herr 1997). They rarely cause symptoms.

13.6.6 Myelosuppression

Myelosuppression is very rarely noted in patients treated with mitomycin C and may result from the use of high-concentration instillations in a recently traumatized bladder (Thrasher and Crawford 1992; Issell et al. 1984; Zein et al. 1986). The management of myelosuppression involves cessation of intravesical chemotherapy and close monitoring of the white blood cell count.

13.7 Chemotherapeutic Agents

13.7.1 Mitomycin

Mechanism of action
Mitomycin C is a 334-kDa (kilo Dalton) alkylating agent that inhibits DNA synthesis. MMC has an intracellular effect resulting in the production of an alkylating agent. The mode of action is poorly understood.

Dosage
The dose varies between 20 and 80 mg per instillation. It is most commonly given as 40 mg in 40 mL of saline or sterile water administered weekly for 8 weeks followed by monthly instillations for 1 year.

Efficacy
MMC is primarily administered as a single perioperative instillation and less frequently given weekly for 6–8 weeks after a TURBT. Data from the EORTC metanalysis of 23 studies have confirmed that the average net benefit for single perioperative MMC is about 14% at 1–3 years and 7% at 7 years (Pawinski et al. 1996). Lamm et al. performed a meta-analysis of five controlled trials and reported that the recurrence rate was reduced by 15% (Lamm 1992). The advantage of MMC was 15% (52% recurrences in the control groups versus 37% in the MMC group) (Lamm 1992). A long-term effect on recurrence and disease progression was not demonstrated (Lamm 1992). In an EORTC marker lesion study (30,864), the complete response rate for the marker lesion after eight instillations with 80 mg of MMC was 50% (Bouffioux et al. 1992). The 6 and 9 weekly instillation when compared with 6 weekly BCG-RIVM had similar disease-free percentage for pTa, pT1, and CIS (Witjes et al. 1998; Witjes et al. 1993). A metanalysis of nine trials with a median follow up of 26 months found similar recurrence rate for BCG (7.67%) and MMC (9.44%) (Bartoletti et al. 2005). Huncharek and Kupelnik reported a meta-analysis of 2,427 patients, examining the endpoint of progression in eight clinical trials, and found no clear advantage for BCG over intravesical chemotherapy (Huncharek and Kupelnick 2004).

Maintenance therapy
Huland et al. compared 3-year MMC instillation therapy (42 instillations of 20 mg) to no intravesical therapy in a randomized trial after complete TUR and found a recurrence rate as low as 10.2% when compared with a control group 51% (Huland et al. 1990). Recently, a study showed that long-term maintenance with MMC was associated with a significant reduction in recurrence rates compared to short-course therapy (Richie 1992). Mallstrom et al. found that maintenance BCG was superior in preventing recurrence compared to maintenance MMC, although no difference was found for progression and survival (Malmstrom et al. 1999).

Improving efficacy
Recently, there have been suggestions that the efficacy of MMC can be improved by altering the delivery methods. This can be achieved by eliminating residual

urine volume, overnight fasting, using sodium bicarbonate to alkalinize the urine thereby reducing drug degradation, and increasing concentration to 40 mg in 20 mL (Au et al. 2001). Addition of local microwave therapy to MMC, 20 mg/50 mL reduced the recurrence rates from 57% to 17% in a multicenter trial. Electromotive intravesical MMC appears to improve drug delivery into bladder tissue and reduces recurrence rates from 58% to 31% (Di Stasi et al. 2003).

Side effects
The side effects are attributed to local toxicity and typically occur after several instillations (de Groot and Conemans 1991). The most common side effects are frequency, chemical cystitis, and allergic skin reactions due to contact dermatitis (Smith et al. 1999).

Guide lines In patients at low risk of tumor recurrence and progression immediate instillation of single dose of chemotherapy is recommended as the adjuvant treatment. In patients at intermediate or high risk of recurrence, one immediate instillation of chemotherapy can be followed by further instillations of chemotherapy or a minimum of 1 year of BCG (Babjuk et al. 2008; Hall et al. 2007).

Conclusion
The response rates of MMC seem higher than other chemotherapeutic drugs. Superiority of BCG over MMC for intermediate risk is not well established. MMC maintenance therapy is controversial.

13.7.2 Adriamycin

Adriamycin (Doxorubicin, ADM) is a 580-kDa anthracycline antibiotic that acts by binding DNA base pairs, inhibiting topoisomerase II, and inhibiting protein synthesis. The response rates of up to 56% have been reported when ADM was used as treatment for papillary tumors, while for CIS the response was only 34% (Bouffioux et al. 1992; Lamm et al. 1991). The most frequent side effect of ADM is chemical cystitis, seen in 25–30% of the patients (Thrasher and Crawford 1992). Rare side effects are allergic reactions (0.3%), gastrointestinal side effects (1.7%) and fever (0.8%).

13.7.3 Epirubicin

Epirubicin (EPI) exerts a similar antitumor action as ADM (Onrust et al. 1999). With a molecular weight of 544 kDa, its absorption is very limited (Tsushima et al. 1998). The local adverse effects of epirubicin are confined to chemical cystitis which occurs in about 3% of patients (Torelli et al. 2001). Most studies have shown that perioperative epirubicin reduces the recurrence rate by 13–27% (Liu et al. 2002; Rajala et al. 2002; Okamura et al. 1994). Maintenance therapy has shown benefit in some studies, however most of them showed no significant ben-

efit (de Groot and Conemans 1991; Smith et al. 1999; Eto et al. 1994; Kondo et al. 1999; Torelli et al. 2001; Okamura et al. 1998).

13.7.4 Valrubicin

Valrubicin (AD32) is a *N*-trifluoroacetyl, 14-valerate derivative of the anthracycline ADM (Onrust and Lamb 1999) Valrubicin is the only drug approved by the US Food and Drug Administration for BCG refractory CIS, in patients who refuse surgery or are medically unfit to undergo surgery. The initial reported complete response rate was 21%, however only 8% of patients remained disease free at the last evaluation (Steinberg et al. 2000). In a prospective phase II marker lesion study, 40 patients with TCC underwent a deliberately incomplete TUR leaving a tumor <1 cm in diameter in the bladder. 54% had a complete response (Newling et al. 2001). The most commonly reported adverse effects were dysuria (77%), hematuria (59%), and urgency/frequency (23%) (Patterson et al. 2000).

13.7.5 Gemcitabine

Gemcitabine is a novel deoxycytidine analog with a broad spectrum antitumor activity. It has a molecular weight of 299 kDa and after intracellular activation, the active metabolite is incorporated into DNA, resulting in DNA synthesis inhibition (Gontero et al. 2008). The molecular weight of gemcitabine is lower than that of commonly used intravesical chemotherapeutic agents such as MMC (389 kDa) and doxorubicin (589 kDa). This will enable better penetration into the bladder mucosa. However, it is also large enough to avoid significant systemic absorption in an intact bladder (Gontero et al. 2008).

Dosage
Two thousand milligrams of gemcitabine in 50 or 100 mL normal saline, administered intravesically for up to 2 h and additional doses once a week for 6 week has been well tolerated (Laufer et al. 2003).

Efficacy
Intravesical gemcitabine has been tested in several phase I studies (Laufer et al. 2003; Dalbagni et al. 2002). Phase II studies have assessed the activity of intravesical gemcitabine on a marker lesion in intermediate-risk Ta/T1 bladder cancer, showing complete response in up to 60% of cases (Gontero et al. 2004). A favorable profile in prophylaxis was confirmed in another phase 2, single-arm, multicentric Italian experience (Bartoletti et al. 2005). Attempts have been made to test the activity of intravesical gemcitabine in high-risk NMIBC, achieving unexpected complete responses in carcinoma in situ refractory to Bacillus Calmette-Gue´rin. Initial activity was substantial; 50% of the patients achieved a CR, and 23% demonstrated a partial response. Initial trials have also documented "clinically relevant" responses in prophylaxis (Dalbagni et al. 2006) (Mohanty

et al. 2008). Thirty-four patients with low- to intermediate-risk solitary or multiple lesions less than 2 cm received 4 weekly instillations of gemcitabine 2,000 mg in a neoadjuvant setting (Maffezzini et al. 2007).

Side effects
Mild transient urgency is seen in 12–26% and rarely leucopenia (32).

Guidelines
Although early results are promising, the limited patient population indicates the need for additional phase II and randomized phase III trials (Babjuk et al. 2008; Hall et al. 2007).

Conclusion
The current level of evidence indicates that gemcitabine possesses clinical activity, but further confirmation is awaited from additional phase III trials.

13.7.6 Interferon

Interferons are natural glycoproteins that mediate host immune responses such as the stimulation of phagocytes, inhibition of nucleotide synthesis, upregulation of tumor antigens, cytokine release, enhanced natural killer cell activity, and activation of T and B lymphocyte (Naito et al. 1991). Among the subtypes, interferon-α has been the most extensively studied. Its efficacy is dose dependent (Belldegrun et al. 1998; Torti et al. 1988). Interferon as a solitary agent is more expensive and less effective than BCG or intravesical chemotherapy in eradicating residual disease, preventing recurrence of papillary disease, and treating CIS (20–43% complete response). As a prophylactic agent, interferon alone demonstrated recurrence rates that were generally inferior to those of BCG alone (Glashan 1990; Kalble et al. 1994), although it can occasionally be effective in patients who have failed BCG with 15–20% complete response.

Interferon-α has also been studied in combination with either chemotherapy or BCG (Bercovich et al. 1995; Stricker et al. 1996). However, there are no data to demonstrate superior efficacy of BCG with interferon compared with BCG alone as initial treatment, and BCG remains standard therapy for frontline management of high-risk disease.

13.7.7 Apaziquone

Eoquin (EO9) (Spectrum Pharmaceuticals Inc., Irvine, CA, USA) is a novel indole-quinone derivative of MMC. The enzyme deoxythmidine-diaphorase, which is found in 40% of bladder tumors, activates EO9. The normal bladder tissue lacks this enzyme and hence does not activate E09 thus decreasing toxicity (38) (Li et al. 2001). van der Heijden et al. [40] performed a phase 2 marker lesion study on patients with Ta–T1 G1–G2 NMIBC undergoing TURBT, with 6 weekly 4 mg/40 mL EO9 and a complete response of 67% (van der Heijden et al. 2006) (Table 13.3).

Table 13.3 Comparisons between intravesical agents (Adapted from Connor et al. 2005, 2005)

Agent	MW	Peri-op use	Risk group	Cystitis (%)	Other toxicity	Dropout (%)	Concentration/ dosage	Cost*
Doxorubicin (Adriamycin)	580	Yes	Low-intermediate	20–40	Fever, allergy, contracted bladder, 5%	2–16	50 mg/50 mL	$36
Epirubicin	580	Yes	Low-intermediate	10–30	Contracted bladder rare	3–6	50 mg/50 mL	$595
Thiotepa	189	Yes	Low-intermediate	10–30	Myelosuppression 8–19%	2–11	30 mg/30 mL	$80
MMC	334	Yes	Low-intermediate	30–40	Rash 8–19%, contracted bladder 5%	2–14	40 mg/20–40 mL	$130
BCG	N/A	No	Intermediate-high	60–80%	Serious infection, 5%	5–10	1 vial/50 mL	$150
Interferon	23,000	No	Salvage	<5%	Flu-like symptoms 20%	Rare	50–100 MU/50 mL	$670–$1,340
Gemcitabine	300	Yes	Salvage	Mild	Occasional nausea	<10	1–2 g/50–100 mL	$540–$1,080

13.8 Combination Chemo-immunotherapy

The use of sequential chemo and immunotherapy utilizes the different working mechanism with potentiation of antitumor effect. Increase in fibronectin by chemical cystitis may have a synergistic effect on the BCG adherence to bladder wall (Mosher 1984; Kavoussi et al. 1990). An EORTC marker lesion study (30,897) with sequential MMC and BCG (four times vs six times), for low stage, low-grade recurrent bladder tumors, indicated a complete response rate of 69% (Maffezzini et al. 1996). The Finn bladder group conducted two studies comparing MMC with an alternating MMC/BCG schedule in patients with CIS or rapidly recurring papillary tumors (Rintala et al. 1996; Rintala et al. 1995). There was a significant difference in CIS group while the efficacy for papillary tumors was equal in both arms (Rintala et al. 1996).

13.9 Device-Assisted Therapy and Newer Approaches

Current research on intravesical chemotherapy focuses on methods to improve the efficacy. This can be accomplished by using chemical and physical methods to enhance urothelial permeability. Chemical methods include use of permeation enhancers (e.g., dimethyl sulfoxide), agents that disturb the cellular tight junctions (e.g., Chitosan) and substances that degrade the extracellular matrix (e.g., hyaluronidase) (Chen et al. 2003; Grabnar et al. 2003; Hashimoto et al. 1992; Kuppermann et al. 2005; See and Xia 1992).

Several physical methods used to disrupt the urothelium are under investigation, this includes electromotive therapy (iontophoresis/electrophoresis) and hyperthermia (Brausi et al. 1998; Di Stasi et al. 1999). The Synergo® (Medical enterprises, Ltd., Amsterdam) system induces bladder wall hyperthermia around 42–43 8C 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 (Horn et al. 1985; Maier and Baumgartner 1986). It has higher side-effects, although these are moderate and transient. Van der Heijden et al. reported the use of thermochemotherapy in a prophylactic manner in 90 patients with intermediate- and high-risk NMIBC [44] (Brausi et al. 1998). Of the total number of patients, 14.3% and 24.6% experienced a recurrence after 1 and 2 years follow-up, respectively (Brausi et al. 1998). In 41 patients in whom BCG failed, the recurrence rates were 23% and 41%, respectively (Brausi et al. 1998). Witjes et al. recently presented a multicenter study in which 57 patients (40 BCG failures, 29 with concomitant papillary tumors) with CIS were treated with 6–8 weekly and 4–6 monthly sessions of thermochemotherapy with an excellent response rate of 94% (Di Stasi et al. 1999). Electromotive drug administration (EMDA) is based on the principle of temporarily enhancing penetration of drugs through the urothelial barrier of the bladder by creating an electrical gradient between the bladder wall and the bladder

contents. Colombo et al. compared 4 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, 66%, 40%, and 27.7%, respectively (Eto et al. 1994; Di Stasi et al. 2003). Di Stasi et al. compared MMC only, MMC combined with EMDA, and BCG in 108 high-risk patients and obtained complete responses in, 31%, 58%, and 64%, respectively, after 6 months of follow-up (Di Stasi et al. 1997). Although side effects of EMDA were higher than MMC, it was far lower than BCG (Di Stasi et al. 1997).

In an effort to prolong the contact time of a drug with the tumors, a number of methods have been used. This includes sustained-retention delivery platforms like bioadhesive microspheres or hydrogel systems, solution-state thermosensitive polymer that transforms into hydrogel matrix at body temperature, and a slow-release gelatin-doxorubicin complex (Le Visage et al. 2004; Tyagi et al. 2004; Ye et al. 2001). Magnetic targeting is achieved by placing a magnet externally on the skin covering a predetermined site in the bladder (typically where tumors reside) to localize drug containing magnetic particles in tumors (Leakakos et al. 2003). Some authors have developed different assays to determine in vitro drug sensitivity and selecting the drug accordingly to improve the effectiveness of intravesical chemotherapy (Burgues et al. 2007).

Gene therapy restores cell growth by correcting gene defects, high selectivity for tumor cells with mutated genes and avoidance of emergence of chemo resistance. Gene therapy targets include p53, interferon, basic fibroblast growth factor, and interleukin-8 (Fodor et al. 2005; Connor et al. 2005; Inoue et al. 2001; Inoue et al. 2000; Werthman et al. 1996). Other experimental approaches include small interfering RNA (siRNA), which silence the expression of the targeted gene in a sequence-specific manner at the mRNA level (Schiffelers et al. 2004; Verma et al. 2003; Xia et al. 2002). Treatment with survivin- or telomerase-targeted siRNA down regulates the expression of the corresponding protein and suppresses the growth of bladder tumor cells (Hou et al. 2006; Ning et al. 2004; Zou et al. 2006).

13.10 Conclusion

Intravesical chemotherapy is an effective treatment for patients with superficial bladder cancer. Adjuvant intravesical chemotherapy does influence the recurrence rate however it has no impact on progression and survival.

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Chapter 14
Intravesical Immunotherapy: BCG

John H. Bishay, Eugene S. Park, and George P. Hemstreet

Abstract Since its introduction by Morales et al. in 1976, intravesical Mycobacterium bovis bacillus Calmette-Guérin (BCG) has served as a key, adjuvant therapy in the treatment of non-muscle invasive bladder cancer. Despite decades of use, the exact mechanism of action of BCG remains unclear; nevertheless, multiple studies have continued to document its efficacy in the treatment of non-muscle invasive bladder cancer. Mechanistic investigations have elucidated many processes of the immunotherapeutic pathways responsible, but our understanding needs to be further studied to establish a more efficient means to decrease recurrence and progression. In the USA alone, urologists prefer the use of BCG to intravesical chemotherapy to a ratio of 2:1. Furthermore, the American Urologic Association, in its most recent published guidelines on the management of non-muscle invasive bladder cancer, has recommended BCG be the first-line treatment for carcinoma in situ because of its proven track record. BCG, a live-attenuated strain of bacteria, is fraught with potential adverse reactions; the weight of treatment success must be carefully balanced against any possible harm to prospective patients. In conjunction with TUR, adjuvant use of BCG will continue to be utilized as more definitive guidelines are created with minimal dose therapy and reduction in side effect profile. This chapter will discuss current mechanistic understanding of BCG immunotherapy, possible markers of BCG treatment prognosis, and the clinical applications for the treatment of initial and recurrent non-muscle invasive bladder cancer.

14.1 Introduction

The multimodality management of genitourinary neoplasms serves as a reflective paradigm for the management of other malignancies. Included is the immunotherapeutic management of bladder cancer with no exception. Local resection of the

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primary neoplasm and control of the field disease/effect is an important and still under appreciated concept as we implement targeted therapy, organ preservation, and prevention in our attempt to reduce health care costs by understanding carcinogenesis rather than treating the genetically unstable neoplasm. Although we have made remarkable progress in defining the human genome and elucidating the complexities of signaling pathways driven by genetic, epigenetic, and endogenous/exogenous environmental factors, only now is the scientific community extensively utilizing biomarkers to comprehend individual risk assessment in terms of the evolving neoplasm. Morales in his historic landmark intravesical instillation of BCG not only represented the first successful nonspecific vaccine for cancer, but has lead to additional research providing insight into the fundamental mechanisms important to immunologic cancer control. Management of bladder cancer has heralded the concept of local therapy with primary resection followed by vaccine therapy as well as chemo and radiation therapy. The ability to locally administer BCG, and to visually, pathologically, immunologically, and biochemically monitor vaccine therapy has provided insight into important immunologic mechanism that may be useful for the vaccine treatment of other neoplasms. This chapter historically reviews the successful individualized therapeutic vaccine management of bladder cancer, the complexities of defining precise therapeutic treatment modalities considering dose scheduling, alterations in immunologic function, and the importance of biomarkers for individual risk assessment, diagnosis, therapeutic targeting, and monitoring and predicting treatment outcomes.

BCG was first developed between 1908 and 1921 by Calmette and Guerin when they engineered a strain of Mycobacterium bovis into a live, attenuated vaccine against tuberculosis (Mahairas et al. 1996). Eight years later, Pearl made the observation that patients with tuberculosis seldom develop malignancy, sparking the hypothesis that the vaccine may harbor antineoplastic activity (Pearl 1929). It was on this postulation that Morales et al. embarked on their landmark study in 1976 where the group successfully demonstrated that intravesical BCG following transurethral resection of bladder tumor (TURBT), decreased disease recurrence (Morales et al. 1976). Encouraged by this initial data, Lamm et al. recorded the first controlled trial confirming the efficacy of intravesical BCG in 1980. In this study, 37 patients were randomized to surgical resection or resection plus intravesical and percutaneous BCG. In the BCG arm, recurrence rate after 1 year was 22% while surgical resection alone had a recurrence rate of 42% ($p = 0.01$) (Lamm et al. 1980). It was further recorded in 1981 that intravesical BCG was efficacious in the treatment of residual disease after tumor resection. Tumor regression was demonstrated in 59% of patients with incomplete resection who then received intravesical and intradermal BCG for 6 weeks (Morales et al. 1981).

Several other investigators have followed suit and demonstrated similar findings in phase III clinical trials. A recent meta-analysis of 25 trials by Han and Pan reported that recurrence occurred in 949 (40.5%) of 2,342 patients who underwent BCG maintenance therapy in comparison to 1,205 (49.7%) of 2,425 patients in the non-BCG group (combined odds ratio (OR) = 0.61; 95% CI 0.46–0.80; $p < 0.0001$)

(Han and Pan 2006). In regard to tumor progression, Bohle and Bock analyzed five trials that demonstrated statistically significant superiority for BCG compared to Mitomycin c (MMC) for the prevention of tumor progression with maintenance therapy (combined OR = 0.66; 95% CI 0.47–0.95; $p = 0.02$; median follow-up of 26 months) (Bohle and Bock 2004). Additional clinical trials have confirmed that BCG is the most effective intravesical therapy for non-muscle-invasive bladder cancer and is superior to intravesical chemotherapy with lower recurrence (Lamm et al. 1991a, b, 1995; Malmstrom et al. 1999; Sylvester et al. 2005) and progression (Herr et al. 1988; Lamm et al. 2000; Sylvester et al. 2002). As a result of these controlled phase III clinical trials, BCG has been shown to be an effective treatment adjunct in non-muscle invasive bladder cancer and in fact, is the treatment of choice for carcinoma in situ.

14.2 Mechanism of Action

The complexities of BCG as a live organism provide it with the ability to stimulate multiple lines of immune responses promoting an antitumor environment. Current investigation continues as multiple in vitro, in vivo murine, and human studies provide a glimpse into the immunological mechanism whereby BCG induces an antitumor response. Following the instillation of BCG, both soluble and cellular immune mediators appear to mediate an antineoplastic response, stimulating both the innate and adaptive immune system. A comprehensive summary of the mechanism of action is illustrated in Fig. 14.1. This immuno-response is supported by studies in athymic, nude mice which provide the first conclusive evidence concerning BCG's ability to stimulate the immune system via a cell-mediated, thymus-dependent response. In contrast to wild-type mice, tumor-bearing athymic mice were unable to mount an immune response and were unresponsive to BCG stimulation. Transfer of BCG-sensitized splenocytes in wild-type snygeneic mouse model adaptively transferred a delayed-type hypersensitivity (DTH) to BCG antigens, providing recipient mice with antitumor activity comparable to intravesical BCG therapy (Ratliff et al. 1987a, b). Subsequent murine studies confirm that the initiation of the antitumor immune response is disengaged with multiple immunodeficient breeds (NK-deficient beige mice, IFN-γ knockout (KO), IL-12 KO, T-cell depletion, Polymorphonuclear neutrophil granulocyte depletion) instilled with BCG (Ratliff et al. 1993; Brandau et al. 2001; Riemensberger et al. 2002; Suttmann et al. 2006). In spite of all the evidence supporting the importance of a functional immune system as a requisite for a therapeutic response, in vitro studies indicate BCG directly induces an antiproliferative effect on tumor cells (Hawkyard et al. 1992; Pryor et al. 1995). However, this antiproliferative mechanism has not been observed in vivo, and may be attributed to different tumor microenvironments in vitro and in vivo or the different time and dose of BCG exposure in cell culture versus intravesical therapy (Brandau and Suttmann 2007).

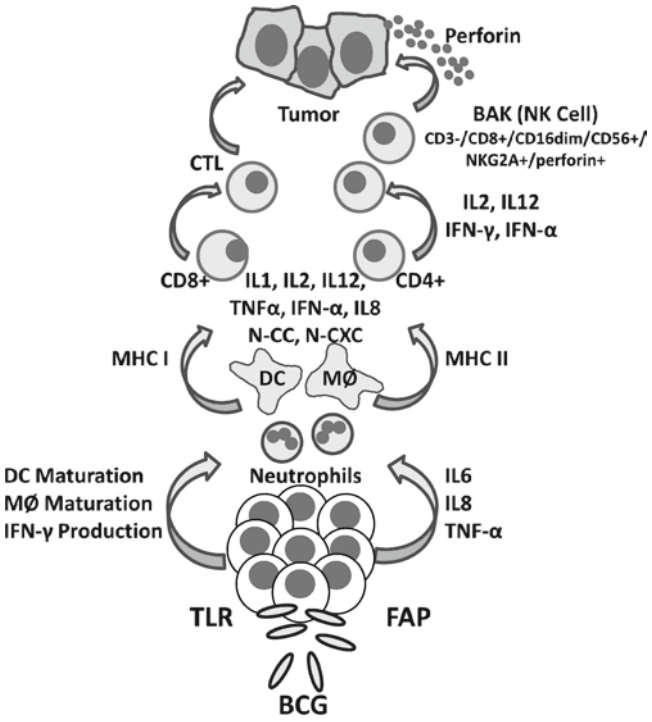

Fig. 14.1 *Mechanism of BCG Immunotherapy.* BCG binds to the urothelium via fibronectin attachment protein (*FAP*) and toll-like receptor (*TLR*) (in vitro studies only). Early after binding, innate immunity induces the production of proinflammatory cytokines (IL6, IL8, TNF-α, IFN-γ) that stimulate the migration of neutrophils, macrophages (MØ), and dendritic cells (*DC*). While inflammation develops secondary to neutrophil granulocyte release, MØ and DCs produce a second group of cytokines (IL1, IL2, IL12, TNF-α, IFN-α, IL8) and chemokines (N-CC, N-CXC) that activate lymphocytes including CD4+, CD8+, and NK cells. Producing TH1 subset of cytokines (IL2, IL12, IFN-γ, IFN-α), cytotoxic T-cells (*CTL*), and BCG-activated killer cells (BAK cells: $CD3^{-}/CD8^{+}/CD16^{dim}/CD56^{+}/NKG2A^{+}/perforin^{+}$ NK cells) destroy tumor cells via cell-mediated cytotoxicity

14.2.1 BCG Attachment

The bladder provides a desirable micro environment for a local immune response following intravesical instillation of BCG. At each clinical treatment, several hundred million bacteria are introduced, but many less adhere and induce a therapeutic response. In fact, most of the mycobacteria are eliminated after the first post-instillation micturation. In response to the remaining adherent mycobacteria (exact number unknown) to both tumor and normal urothelial cells via fibronectin attachment protein (FAP), urothelial cells secrete multiple proinflammatory cytokines. Adherence of BCG is dependent on FAP located on the surface of each mycobacteria, preferentially occurring at electrocauterization

sites, binding to fibronectin (Ratliff et al. 1987a, b; Zhao et al. 2000). BCG attachment is imperative for effective therapy and interruption of adherence results in no antitumor response (Ratliff et al. 1987a, b; Kavoussi et al. 1990). Furthermore, murine studies indicate that FAP has effective antitumor properties that inhibit tumor growth at a level comparable to BCG (Sinn et al. 2008).

14.2.2 Cytokine and Chemokine Production

The induction of cytokine production subsequent to instillation induces an efficient proinflammatory environment. Multiple patient studies have demonstrated the induction of urine cytokines to include tumor necrosis factor-α (TNF-α), granulocyte macrophage colony stimulating-factor (GM-CSF), interferon-γ (IFN-γ), interleukin (IL) 1, IL2, IL5, IL6, IL8, IL10, IL12, and IL18 (Haaff et al. 1986; Bohle et al. 1990a, b; Prescott et al. 1990; de Boer et al. 1992, 1997; Alexandroff et al. 1996; Thalmann et al. 1997, 2000; Jackson et al. 1998; Eto et al. 2005). Accompanying cytokine production, instillation stimulates neutrophils, monocytes, and macrophage infiltration into the bladder wall (Bohle et al. 1990a, b, c; de Boer et al. 1991; Prescott et al. 1992; Suttmann et al. 2003, 2006; Ludwig et al. 2004). The release of granules from neutrophils enhances the antitumor effect, but also causes irritative urinary symptoms described by many patients after BCG therapy (Suttmann et al. 2003; Ludwig et al. 2004). Neutrophils and monocytes also release chemokines important for cell-mediated cytotoxicity (Bohle et al. 1990a, b, c; de Boer et al. 1991; Prescott et al. 1992; Suttmann et al. 2003, 2006; Ludwig et al. 2004). The release of cyotokines and chemokines in this manner induces the influx of lymphocytes to include CD4+ T cells. CD4+ T cells produce cytokines further shifting the production of T helper type-1 cells. A study via Suttmann et al. illustrated that polymorphonuclear neutrophil granulocytes direct the migration of effector cells such as CD4+ T-cells to the bladder through chemokine secretion. This occurs a few hours after instillation of BCG, secondary to the innate immune response (de Boer et al. 1991; Ludwig et al. 2004; Suttmann et al. 2006). Recent research has also pinpointed the production of both N-CC (i.e., macrophage-derived chemokine, monocyte chemoattractant protein, macrophage inflammatory protein, and eotaxin) and N-CXC (i.e., interferon-inducible protein 10) chemokines as potent attractants secreted by mononuclear cells in vitro (Luo et al. 2007).

14.2.3 Infiltrating Lymphocytes

As CD4+ cells continue to infiltrate into the bladder, inflammatory granulomatous structures form within the bladder wall (Lage et al. 1986; Bohle et al. 1990a, b, c; Prescott et al. 1992). These structures are characterized by giant cells and epithelioid granulomas. Furthermore, these infiltrates are surrounded by dense areas of

lymphocytes and eosinophilic granulocytes (Lage et al. 1986). Immunohistochemical studies confirm that the granulomas predominately contain CD4+ T helper cells. It has further been demonstrated that CD4+ infiltrating cells correlate with the increased expression of HLA-DR, CD25, ICAM1, and major histocompatibility complex II on both infiltrating cells and the urothelium (Bohle et al. 1990a, b, c; Prescott et al. 1992). This infiltrative state can last from 3 to 12 months after 6 weeks of therapy, providing the rationale for maintenance therapy. This immune response differs dramatically from nonspecific cystitis, in which early granulocytic infiltration exists with no significant infiltrate of mononuclear cells including lymphocytes and macrophages (Bohle and Brandau 2003).

14.2.4 TH1 Immune Response

The importance of the TH1 immune response has been thoroughly studied. Multiple murine studies indicate that there is a restricted induction of TH1 cytokines maintaining a balance in the TH1/TH2 immune response. An important component of the TH1 response is IFN-γ, which is up-regulated in contrast to IL4 (TH2 cytokine) which is down-regulated (McAveney et al. 1994). In an orthotopic bladder cancer model by Riemensberger et al., BCG therapy was effective in wild-type mice but not knock-out IFN-γ/IL12 mice. Furthermore, BCG therapy was more effective in IL10 knock-out mice, confirming that the TH2 response is not essential for antitumor activity of BCG (Riemensberger et al. 2002). A second study investigating BCG dose and the TH1/TH2 response illustrated that intravesical BCG induced elevated levels of IFN-γ, IL2, and IL-12p40 (TH1 cytokines) and a decrease in IL10 and IL4 (de Boer et al. 2005). In light of these findings, an IFN-γ-secreting recombinant BCG strain was developed, and demonstrated improved survival in an orthotopic bladder cancer model (Arnold et al. 2004). Recent studies have also illustrated the use of IL12 as a treatment option or possible adjuvant therapy with BCG, as intravesical IL12 gene therapy improved survival (Horinaga et al. 2005). In short, the TH1 response is imperative in the cell-mediated cytotoxic effect of BCG.

14.2.5 Natural Killer Cells

Although CD4+ and CD8+ lymphocytes are necessary to stimulate the antitumor response, another subpopulation of lymphocytes known as Natural Killer (NK) cells provide a direct cytotoxic effect on tumor cells (Ratliff et al. 1993; Brandau et al. 2001; Sonoda et al. 2007). Inhibition of NK cells via anti-asialo GM1 and NK1.1 antibody did not abrogate the antitumor response of BCG (Ratliff et al. 1986). NK cells recognize MHC class I molecules through inhibitory NK cell receptors (iNKR) that deliver inhibitory signals and deactivate NK cell cytotoxicity, lysing cells that do not express the self MHC class I molecule such as tumor cells

(Tomita et al. 1990). These subpopulations of lymphocytes are stimulated by TH1 related cytokines including IL2, IL12, IFNα, and IFNγ (Thanhauser et al. 1995; Suttmann et al. 2004). A special subset of NK cells known as BCG-activated killer cell (BAK cell) has been identified, and are denoted as $CD3^{-}/CD8^{+}/CD16^{dim}/CD56^{+}/NKG2A^{+}/perforin^{+}$ phenotype (Brandau et al. 2001; Brandau and Bohle 2001). BAK cells kill tumor cells by degranulating perforin without activation of the Fas Ligand (FasL) pathway (Brandau et al. 2000). Perforin, simply known as pore-forming protein, stimulates cell death by inserting itself into the cell membrane, disrupting cellular homeostais. In contrast, other studies have indicated that other effector mechanisms are responsible for tumor cell eradication, including TNF-family FasL or TNF-related apoptosis-inducing ligand (TRAIL) (Ludwig et al. 2004; Mehmut et al. 2005).

14.2.6 Innate Immune Response

Associated with this cell-mediated response is the stimulation of the innate immune response. During this process, the immune system recognizes pathogen-associated molecular patterns (PAMPs) such as the bacteria's outer cell wall composition of peptidoglycan, mycolic acids, lipomannan, and lipoarabinomannan (Brennan 2003). PAMPs stimulate immune recognition receptors such as toll-like receptors (TLR) (Janeway and Medzhitov 2002; Heine and Ulmer 2005). In vitro studies have delineated these mechanisms, providing evidence that TLR2 and 4 are stimulated by interactions with Mycobacterium cell wall molecules leading to the maturation of dendritic cells, macrophages, and production of IFN-γ (Seya et al. 2001; Fricke et al. 2006). With this host of cell-mediated cytotoxicity and innate recognition, immunotherapy with BCG has become the most effective immunotherapy to date, and will continue to improve as the understanding of tumor-induced immunosuppression continues.

14.2.7 Tumor-Induced Immunosuppression

In response to the excitement of immunotherapy in cancer, many studies have been undertaken to identify tumor-induced immunosuppression in order to improve the success of immunotherapy. The role immunoregulatory cells in bladder cancer are poorly defined. However, it is known that bladder carcinoma induces the infiltration of type-1 regulatory T (Tr1) cells expressing FOXP3, TNFβ, and IL10 (Loskog et al. 2007). These cells differ from natural $CD25^{+}$ Tregs based on their ability to induce a response via tolerogenic cytokines IL-10 and TNFβ (Lizee et al. 2006). Peripheral blood of patients with bladder cancer does display increased numbers of $CD4^{+}CD25^{+}$ T cells as well. These cells suppress the expansion of allogeneic T cells from healthy donors in vitro (Loskog et al. 2007). Further studies are needed

to investigate the immunoregulatory mechanisms in bladder cancer in order to improve the efficacy of BCG therapy (Brandau 2007).

14.2.8 BCG Treatment Prognosis and Biomarkers

Understanding the mechanism whereby BCG induces an antineoplastic response has revealed multiple possible prognostic markers to predict treatment outcome with BCG. Not only will advances in biomarkers improve prognosis, but they will provide a means for clinicians to improve risk assessment, diagnosis, and individualized BCG therapy (Hemstreet et al. 2001). One means of assessing prognosis is measuring induced BCG immunological effects. Urinary IL2 is one promising prognostic marker (Haaff et al. 1986; Fleischmann et al. 1989; de Reijke et al. 1996, 1999; Schamhart et al. 2000). Kaempfer et al. analyzed the induction of IL2 and IFN-γ mRNA in peripheral blood mononuclear cells from bladder cancer patients in remission who had elevated levels of IL2 mRNA in contrast to relapsed patients who had normal levels (Kaempfer et al. 1996). Watanabe et al. also found that IL2 was an independent prognostic cytokine of responder status (Watanabe et al. 2003). IL8 and IL18 have also been identified as candidate prognostic cytokine markers (Thalmann et al. 1997, 2000). A second means of assessing prognosis to BCG is analyzing the effect that the tumor has on the cellular components of the stroma. The number of infiltrating Tumor Associated Macrophages (TAMs) may be useful in predicting the response of BCG immunotherapy (Takayama et al. 2009). In contrast, high number of infiltrating CD83(+) TIDCs or CD68(+) TAMs portend a poor response to BCG immunotherapy (Ayari et al. 2009). Finally, field disease effect modifying the production of cellular proteins may have use in predicting prognosis. For example, quantitative fluorescence image analysis (QFIA) has demonstrated that G-actin was persistently elevated in a group of patients who had a higher rate of recurrence after treatment with BCG (Hemstreet et al. 1999, 2001). Table 14.1 lists a summary of other potential biomarkers that might be used to monitor treatment with BCG.

Pathologic diagnosis remains the ultimate biomarker for clinical decision criteria complemented by routine urinary cytology. However, one of the major confounding variables in the management planning of patients with bladder cancer is precisely defining the extent of local disease in the bladder, prostate, and upper collecting system. In the management of low-grade tumors, there may be concomitant high-grade disease (CIS) elsewhere in the bladder or adjacent collecting system. Thus, combining biopsy with urine or bladder wash cytology biomarkers decreases under-staging biopsy error. The recommendation for staging bladder cancer patients who are scheduled for resection of their primary lesion is to consider random bladder biopsies and biopsy of the prostatic urethra in addition to urethral washes. Biopsies should also include the area adjacent to the primary neoplasm and random biopsies in each region of the bladder focusing on suspicious areas (Matzkin et al. 1991; van der Meijden et al. 1999; Lokeshwar et al. 2005; Mungan et al. 2005). Determining the presence of high- or low-grade bladder

Table 14.1 Biomarkers for BCG

Sample	Biomarker	Sensitivity	Specificity	Status
Urine soluble	Nuclear matrix protein (*NMP 22*) (Lokeshwar and Soloway 2001; Kumar and Kumar 2006; Grossman et al. 2006) *Without hematuria	47–100%	77–87%	FDA approved
	Bladder tumor antigen (BTA)	57–82%	68–93%	FDA approved
	(Ellis et al. 1997; Sarosdy et al. 1997; Wiener et al. 1998; Pode et al. 1999; Thomas et al. 1999; Heicappell et al. 2000) 1. BTA-Stat 2. BTA-TRAK	66–77%	50–75%	FDA approved
	Survivin (Weikert et al. 2005; Kitsukawa et al. 2008; Pu et al. 2008)	69–90%	95–100%	
	Hyaluronan and Hyaluronidase (Simpson, Lokeshwar 2008)			
Urine cellular	ImmunoCyt (Mian et al. 1999; Pfister et al. 2003; Hautmann et al. 2004; Lodde et al. 2004; Messing et al. 2005)	60–100%	75–84%	FDA approved
	UroVysion (Sarosdy et al. 2002; Toma et al. 2004; Laudadio et al. 2005; Yoder et al. 2007)	69–74%	65–95%	FDA approved
	Telomerase (Sanchini et al. 2005)	90%	88%	
	Microsatellites (Frigerio et al. 2007)	93–100% high grade		
Urine and hematologic	TIMP-3 (Hoque et al. 2008)			
	E-cadherin (Durkan et al. 1999; Shi et al. 2008)			
Histologic	Ras signaling pathway (Wu 2005)			
	Phosphatidylinositol 3-kinase (PIK3) (Lopez-Knowles et al. 2006)			
	Tumor-infiltrating lymphocytes (Sharma et al. 2007)			
	Tumor-infiltrating macrophages (Ayari et al. 2009; Takayama et al. 2009)			
	p53 (Malats et al. 2005)			
	microRNAs (Neely et al. 2008)			

cancer in relation to the primary neoplasm is critical for those patients receiving BCG therapy or intravesical chemotherapy. Patients with high-grade disease (CIS, Ta, T1) are candidates for BCG therapy whereas patients with low-grade tumors are usually candidates for intravesical chemotherapy. Cytology results prior to primary resection can provide an important clue for suspecting high-grade disease pre-resection and thus it is an important biomarker in the decision process (Lokeshwar et al. 2005). In the instance where CIS is suspected, or is difficult to discern from inflammation associated with BCG therapy, fluorescence cystoscopy may be useful as a tool to identify CIS or to determine the presence of residual premalignant disease (Jichlinski et al. 2003; Schmidbauer et al. 2004; Hungerhuber et al. 2007). Molecular studies confirm that fluorescent field disease changes are associated with genetic abnormalities on FISH studies of the involved area, confirming the presence of premalignant field disease. Fluorescence-based methods also appear most useful for studying biomarkers on cells in the context of an inflammatory milieu. Thus, one useful role for urinary FISH studies is cytology evaluation during BCG immunotherapy or chemotherapy to monitor the elimination of the cancer as well as the premalignant field. Other markers such as NMP22 is not recommended for detecting cancer associated with BCG secondary to high false-positive results related to hematuria (Stampfer et al. 1998). Recent studies investigating microRNA as a biomarker for bladder cancer show promise because these are stable, small molecules that are readily measured in the urine and are less susceptible to degradation than cellular or soluble proteins released into the urine (Neely et al. 2008).

Additional studies on biomarkers (Table 14.1) before and after BCG vaccine immunotherapy are clearly needed for diagnosis and or risk assessment. Currently there are no biomarker profiles approved by the FDA specifically for prospectively predicting risk or prognosis of treatment of non-muscle invasive bladder cancer. However, there is considerable excitement related to predicting disease progression in individuals who have their primary tumor resected. This is highly relevant to the management of patients with CIS or high-grade T1 disease. Knowing with certainty which individuals are at high risk for progression should be useful in deciding further treatment, increasing the demand for biomarkers that are highly accurate (high specificity and sensitivity).

14.3 Clinical Use of BCG

14.3.1 Indications

Bladder cancer is the fifth most common malignant disease with more than 60,000 new cases diagnosed yearly in the USA and approximately 14,000 deaths annually (Jemal et al. 2008). Worldwide, there are approximately 145,000 cases of bladder cancer deaths making it the 13th most numerous cause of death from cancer (Parkin et al. 2005). Approximately 75–80% of patients initially present with non-muscle

invasive tumors of stages Ta, T1, or carcinoma in situ (CIS). Depending on the stage and grade of bladder cancer, these tumors may have a recurrence and progression rate of up to 78% and 45% at 5 years, respectively, following TURBT (Sylvester et al. 2006). Since 1998, several investigators have published data from meta-analyses combining the results of randomized phase III clinical trials involving intravesical BCG therapy (Sylvester et al. 2002, 2005, 2006; Bohle and Bock 2004; Han and Pan 2006). These studies share three main conclusions. First, adjuvant BCG combined with TURBT decreases the recurrence rate (36–40.5%) of non-muscle invasive bladder cancer when compared to TURBT alone (49–55%) (Han and Pan 2006; Hall et al. 2007). Second, BCG is superior to intravesical chemotherapy in the prevention of disease recurrence (31.9–38.6% vs 46.4–48.5%, respectively) (Bohle and Bock 2004; Sylvester et al. 2005). Only one meta-analysis demonstrated no clear superiority of an induction course of BCG when compared to chemotherapy (Hall et al. 2007). And finally, BCG is the only treatment positively impacting progression of non-muscle invasive cancer. Discrepancies in percentages are most likely the result of various follow-up periods ranging from 12 to 60 months.

As a result of these trials, several evidence-based guidelines (established by the American Urologic Association (AUA), the European Association of Urology (EUA), and the National Comprehensive Cancer Network (NCCN)) have been drafted to recommend indications where BCG therapy is warranted. The three major indications include: (1) adjuvant induction cycle for intermediate risk tumors (low-grade Ta), (2) adjuvant induction cycle plus maintenance therapy for high-risk tumors (high-grade Ta, T1), and (3) Induction cycle plus maintenance therapy for primary treatment of carcinoma in situ (CIS). In a large meta-analysis Hall et al. recently documented treatment indications for intravesical therapy of non-muscle invasive bladder cancer. Results are summarized in Table 14.2 (Hall et al. 2007). Contraindications to intravesical BCG administration include compromised immune status, active tuberculosis, acute urinary tract infection, lower urinary tract disruptions/perforations (intravasation risk), fever of unknown origin, and pregnancy or active nursing (Brandau and Suttmann 2007).

14.3.2 Treatment Schedule and Dosage

Multiple strains of BCG are marketed and are all derived from the initial Pasteur strain. A recent meta-analysis confirmed that the five most commonly used strains; Connaught, Pasteur, Tice, Armand Frappier, and Tokyo do not differ in preventing tumor progression (Sylvester et al. 2002). Other Routes of BCG administrations, such as oral, percutaneous, or intralesional have not been shown to be more efficacious than intravesical immunotherapy alone or in combination (Lamm et al. 1990, 1991a, b; Luftenegger et al. 1996). These different strains represent effective treatment in the management of non-muscle invasive bladder cancer. What remains disputed, though, is the dosage and scheduling of therapy.

Table 14.2 Intravesical treatment indications summary of guideline for the management of nonmuscle invasive bladder cancer: 2007 update (Hall et al. 2007)

Patient presentation	Intravesical treatment	
Abnormal growth on urothelium	Optional: single dose postoperative intravesical chemotherapy.	Optional secondary to uncertain pathology, cost, side effects, patient preference, non-beneficial for muscle invasive tumors. Treatment should be considered in papillary appearing lesions (Ta) with no contraindications.
Small volume, low-grade Ta bladder cancer.	Recommendation: single dose intravesical chemotherapy postoperatively.	Decrease recurrence by 17% vs TURBT alone.
Multifocal and/or large volume, histologically confirmed, low-grade Ta or recurrent low-grade Ta	Recommendation: induction course of intravesical therapy with BCG or MMC	Decrease recurrence by 24% with BCG and 3% with MMC.
	Optional: maintenance BCG or MMC	More effective at decreasing recurrence vs. induction alone; 31% and 18% with BCG and MMC maintenance, respectively.
		Optional secondary to cost, side effects, and low risk of progression in this patient.
Initial histologically confirmed high-grade Ta, T1 and/or CIS bladder cancer	Recommendation: induction course of BCG following repeat resection followed by maintenance therapy.	Decrease recurrence by 34% vs 62% with MMC maintenance. Decrease progression by 5% vs MMC maintenance.
High-grade Ta, T1, and/or CIS bladder cancer which recurred after prior intravesical therapy	Optional: further intravesical therapy.	Evidence that patients will respond to second induction, especially BCG. Patients at high risk of progression should be excluded due to risk of muscle invasion and/or metastasis.

Historically, Morales et al. described their treatment schedule as a 6-weeks induction cycle consisting of weekly intravesical treatments with 80 mg Armand Frappier strain BCG suspended in a 50 mL saline solution ($1–5 \times 10^8$ CFU of mycobacteria per instillation) (Morales et al. 1976). Interestingly enough, Morales admits in his landmark trial that the duration of 6 weeks was arbitrarily chosen because BCG was manufactured and distributed in packages of six vials; he concluded that further studies would likely need to define the optimal dosing schedule for therapy. To this day, this induction schedule remains the most commonly used worldwide, despite randomized, multicenter-controlled study demonstrating it is markedly inferior to the SWOG 3 weeks maintenance schedule (Lamm et al. 2000).

Dosage regimens also remain undefined. Several different strains of BCG exist and each strain has a unique dose. As the benefit of treatment must be weighed carefully against the risk of adverse reactions, several trials and meta-analyses are focusing on the optimization of BCG dosage and delivery schedules. Investigators are actively testing partial dose BCG therapy (1/3, 1/4, 1/6, of the full dose) and have shown evidence that lower doses may be equally effective as full dose therapy (Mack et al. 2001; Martinez-Pineiro et al. 2002, 2005; Ojea et al. 2007) if not superior (Pagano et al. 1995). Multiple maintenance schedules have been used in prospective studies, but regardless of schedule, each demonstrated a reduction in recurrence when compared to BCG induction alone (Table 14.3). These studies consistently demonstrate a reduction in recurrence when compared to induction alone (Hall et al. 2007). Han et al. demonstrated similar results in a subsequent meta-analysis (Han and Pan 2006). The SouthWest Oncology Group (SWOG) conducted a large prospective study investigating toxicity, recurrence, progression, and survival. It demonstrated a significant improvement on recurrence reduction in patients with CIS or high-risk lesions with the addition of a maintenance schedule versus induction alone. Induction included 6 weeks of 120 mg Connaught BCG intravesically and 10^7 colony-forming units percutaneously, weekly for 6 weeks. Patients in the maintenance arm received intravesical and percutaneous administrations of BCG every week for 3 weeks at 3, 6, 12, 18, 24, 30, and 36 months. Median recurrence-free survival was 35.7 months with no maintenance and 76.8 months with maintenance ($p<0.0001$) (Lamm et al. 2000). The EAU guidelines suggest that maintenance for at least a year may be required to obtain an optimal response, but they warn that their review of meta-analyses cannot provide an exact optimal length for maintenance therapy. Ultimately, maintenance is superior to induction alone because the risk of recurrence and progression is life long, especially in high-grade non-muscle invasive bladder cancer (Herr 1997). Evaluation of SWOG 8507 1 year after completion of the 36-months maintenance schedule demonstrated an increase in the rate of recurrence, establishing the need for longer maintenance schedules. Prolonged maintenance schedules will become more utilized with dose reduction and prolongation of the interval between courses, and have even been suggested to last up to 12 years. Although the debate on maintenance schedules

Table 14.3 BCG maintenance schedules

Strain/concentration	Induction: 2–4 weeks after resection	Maintenance
Connaught 81 mg in 50.5 mL (Lamm et al. 2000)	Every week for 6 weeks	Every week for 3 weeks at 3, 6, 12, 24, 30, and 36 months
Connaught 81 mg (Palou et al. 2001)	Every week for 6 weeks	Every week for 6 weeks every 6 months for 2 years
Pasteur 120 mg in 0 mL (Hudson et al. 1987)	Every week for 6 weeks	Every 3 months
Pasteur 120 mg in 50 mL (Badalament et al. 1987)	Every week for 6 weeks	Monthly for 2 years

continues, treatment has provided clinicians with appropriate and efficacious immunotherapy.

14.3.3 Salvage Therapy

Failure of BCG after two courses of treatment should prompt the clinician to seek alternative therapy. The exact role of BCG in patients who have failed or who have had only a partial response to induction and maintenance is under active investigation. Trials are underway testing the drug in novel fashion and in combinations with other therapies (Table 14.4). For example, intravesical BCG plus interferon has been suggested for salvage therapy. This concept was established as trials demonstrated that IFN was tolerable, but not efficacious over BCG alone. Further studies indicated that in patients with CIS, treatment with BCG+IFN had a 60–70% complete response (CR) if never previously treated with BCG or if they failed only 1 prior induction or relapsed more than 1 year from induction (Luciani et al. 2001; Grossman et al. 2008). O'Donnell et al. evaluated the combination of BCG + IFNα2b as salvage therapy in patients with recurrence following intravesical BCG. With this combination, 63% of patients were disease free at 12 months and 53% were disease free at 24 months (O'Donnell et al. 2001). SWOG 8507 found that 64% of patients who had persistent CIS at 3 months went on to CR by 6 months (Lamm et al. 2000). Further analysis of 1,007 patients in a national multicenter phase II trial demonstrated that 45% of patients following recurrence subsequent to BCG alone were disease free at 24 months. This landmark study established significant risk factors for recurrence including stage T1, tumor size >5 cm, prior BCG failure more than once, and multifocality of tumor sites. Patients whose last relapse was more than a year from their last treatment had recurrence rates similar to naïve, BCG-treated patients who responded to treatment (Joudi et al. 2006). Mechanistic studies have demonstrated that upon the second course of induction with BCG, inflammatory cytokines and other markers of immune response peak at approximately 3 weeks in contrast to 6 weeks subsequent to initial exposure (de Boer et al. 1991; de Reijke et al. 1999). It is possible that proceeding with the 4th,

Table 14.4 Salvage therapy agents/modalities

BCG+interferon	BCG+IFNα 50 million units induction+3 weeks reduced does BCG at 3, 9, 15 months	60–70% complete response if never treated with BCG or failed only 1 prior induction or relapsed more than a year from induction
Intravesical gemcitabine	2,000 mg/50 mL 1–2 instillations per week for 6 weeks	Safe, but efficacy unclear
Intravesical valrubicin	800 mg weekly instillations for 6 weeks	Only US FDA approved drug
Cystectomy		Restage refractory tumors to identify progression of disease, and indication for cystectomy

5th, and 6th instillation after peak response has occurred during the second induction course could result in immunosuppression, and subsequent iatrogenic BCG failure.

Chemotherapeutic agents represent another alternative for salvage therapy. The anthracycline Valrubicin has been investigated and approved for intravesical treatment of BCG-refractory CIS of the bladder with good efficacy and acceptable toxicity. This drug is delivered intravesically at a dose of 800 mg weekly for 6 weeks (Patterson et al. 2000; Steinberg et al. 2000). Gemcitabine is another alternative for BCG-refractory bladder cancer. In a phase II clinical trial, 18 of 24 (75%) intermediate-risk and 7 of 16 (43.7%) high-risk patients responded to instillation of gemcitabine (2,000 mg/50 mL) in weekly instillations for 6 weeks (Bartoletti et al. 2005; Dalbagni et al. 2006; Hendricksen and Witjes 2006). Further studies are needed to fully define this agent's role in salvage therapy.

For patients who fail conservative therapy and show evidence of disease progression, an argument can be made to move directly to surgical resection. Radical cystectomy (RC), though usually reserved for muscle invasive bladder cancer, may preemptively arrest the progression of a non-muscle invasive cancer before it has time to invade the muscularis propria (Chang et al. 2003; Hassan et al. 2004; Lee et al. 2006). There have been arguments stating that delay in RC for high-grade T1 bladder cancer in exchange for intravesical therapy has ultimately decreased disease-free survival. Most identify under-staging, and a consequent delay in definitive treatment (Lambert et al. 2007). In response, restaging with subsequent TUR is important for risk stratification to determine response or stage progression. T1 tumors should be restaged 2–6 weeks after initial diagnosis to prevent tumor progression (Herr 2005). Ultimately, the concept of salvage therapy remains investigational at this time. Though it makes sense to proceed from less invasive forms of therapy to more aggressive treatment, the exact timing of these treatments has yet to be defined.

14.3.4 Prostatic and Upper Tract Disease

Comprehending the subtleties of prostatic urothelial carcinoma is a key factor in managing patients with urothelial cell carcinoma (UCC) of the bladder. Patients suffering from mucosal prostatic urothelial carcinoma (PUC) must be differentiated from those with stromal invasion (T4) because BCG is non-efficacious in patients with T4 disease. In patients with mucosal limited disease treated with transurethral resection of the prostate (TURP) and BCG, approximately 86% had a complete response in both the prostate and bladder (Orihuela et al. 1989). In a more recent study, it was demonstrated that TURP followed by BCG immunotherapy eliminates PUC with a 5-year recurrence-free survival rate of 90% however, with bladder and prostatic urethra involvement, the 5-year survival rate was only 30% compared to 89–95% in patients without urethral involvement. Cancer-specific mortality in this group was 25% compared to less than 20% without involvement. These results support the administration of BCG following TURP as

the preferred treatment in patients with PUC (Gofrit et al. 2009). Follow-up biopsy of the prostatic urethra is mandatory at 3-month intervals (Kirkali and Canda 2006), and if positive, cystectomy is indicated secondary to the high rate of bladder cancer mortality in these patients (Taylor et al. 2007).

Similar to the field disease characteristic of prostatic involvement, upper tract carcinoma in situ, while rare, continues to be an important factor in the management of patients with TCC treated with BCG and cystectomy. Monitoring the upper tracks on an annual basis in patients with a previous history of high-grade bladder disease is imperative. In patients with primary or disease associated with bladder cancer, nephroureterectomy remains the primary treatment modality. Several studies indicate BCG for CIS of the upper urinary tract is as effective as nephroureterectomy. The most recent by Kojima et al. evaluated 17 patients, six of which underwent nephroureterectomy and 11 treated with BCG therapy. This study demonstrated that there was no significant difference in 5-year recurrence-free survival or cancer-specific survival between nephroureterectomy vs. BCG with a 67% and 77% recurrence-free survival at 58.3 months median follow-up (Kojima et al. 2006). In contrast, a comprehensive review of 89 patients, 50 of which were treated with adjuvant BCG therapy after resection of upper tract lesion did not demonstrate a reduction in recurrence with follow-up of 61.1 ± 54.8 months (Rastinehad et al. 2009). More recently, BCG in combination with IFNα2b is also being piloted as an adjuvant for upper tract TCC. One study followed ten patients treated with BCG + IFN for a median of 24 months. Eight of the ten patients demonstrated complete response (Katz et al. 2007).

14.3.5 Side Effects/Complications

As with the use of any other pharmacologic agent, BCG's efficacy is limited by its potential adverse reaction profile. It is estimated that 90% of patients will experience one or more side effects. Fortunately, most adverse reactions are not life-threatening and serious side effects are rare, occurring in less than 5% of patients. Complications can be divided into six main subdivisions including bladder contracture, epididymitis/prostatitis/urethral infections, hematuria, lower urinary tract symptoms (LUTS), fever/chills/flu symptoms, and systemic infection. LUTS occurs in 38% of patients undergoing induction and 57% of those undergoing maintenance therapy. Systemic side effects such as fever/chills/flu symptoms occurred in 19% and 22% when comparing induction versus maintenance, respectively. Systemic infections, local infections, and bladder contractures were uncommon at approximately 7%, 4%, and 3% respectively (Hall et al. 2007). Patients with LUTS during early intravesical instillations will continue to have them throughout the treatment schedule (Berry et al. 1996). Side effects in association with BCG occur within the first 6 months of treatment, and continuation of maintenance therapy after this time period does not increase the toxicity associated

with treatment (Bohle et al. 2003; van der Meijden et al. 2003). The most dreaded, and propitiously, the rarest complications are sepsis (0.4%) and mortality secondary to sepsis (Lamm et al. 1992).

The savvy clinician would do well to keep these complications in consideration; only 16% of patients in the SWOG trial completed the 3-year maintenance cycle because of inability to tolerate side effects (Lamm et al. 2000). Another recent study has estimated that perhaps only a third of all patients may be able to tolerate long-term maintenance therapy (van der Meijden et al. 2003). These results have been the rationale behind investigating whether partial dose therapy can allow for better patient tolerability to a potentially lengthy treatment regimen. Preliminary data seem to indicate that partial-dose BCG may be equally effective as full-dose BCG (Mack et al. 2001; Martinez-Pineiro et al. 2002, 2005; Ojea et al. 2007). Another alternative under investigation is to administer 1,800 mg of prulifloxacin, which decreases the adverse effects of BCG, therefore improving patient compliance with BCG induction (Damiano et al. 2009).

A caveat must be made in the attempt to minimize dose-related toxicity. It is possible that the degree of toxicity is directly proportionate to the inflammatory response generated by BCG therapy. Indeed, several investigators have observed that a higher incidence of LUTS has been correlated with lower recurrence rates (Orihuela et al. 1987; Luftenegger et al. 1996; Saint et al. 2001). However, Sylvester et al. demonstrated that even though there is a direct correlation between the incidence of adverse reactions and lower recurrence rates, there does not seem to be a direct cause-and-effect relationship (Sylvester et al. 2003).

14.4 Conclusion

Since its first use 30 years ago, BCG has remained an effective element in the management of noninvasive bladder cancer. During this time, a large number of immunologic and clinical studies have illuminated the importance of cytotoxic effector cell activation in order to achieve an antineoplastic effect. Along with this is the stimulation of innate immune processes. Although we have elucidated these intricate steps in the production of BCG immunotherapy, there is still much to know about its interactions with the immune system. Advances in research will continue to provide us with improvements on immunotherapy. Along with these advancements, improvements in detection including fluorescent cystoscopy and biomarkers will aid in risk assessment, diagnosis, individualized BCG therapy, and predicting/monitoring response to BCG. Multiple clinical trials have provided us with data demonstrating that BCG continues to be the first-line treatment in the management of CIS. As BCG toxicity continues to be one of the largest determining factors in the ability to undergo maintenance, advances in minimal dosing techniques and side effect reduction will provide clinicians with more definitive guidelines for the use of BCG, appropriately weighing potential harm vs. potential treatment outcomes.

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Chapter 15
Cystectomy for Nonmuscle-Invasive Bladder Cancer

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Abstract Nonmuscle-invasive bladder tumors represent a heterogeneous group of cancers that include those that are generally papillary in nature and limited to the mucosa (Ta), those that are high grade, flat, and confined to the epithelium (carcinoma in situ, CIS), and those that invade into the submucosa or lamina propria (T1). For patients with nonmuscle-invasive bladder cancer, the initial treatment is generally a complete cystoscopic transurethral resection of all visible bladder tumors (TURBT). Although most nonmuscle-invasive bladder cancers are safely and effectively treated by transurethral resection (TURBT) and/or intravesical instillation therapies, some forms have a high propensity to invade and progress and a more aggressive therapy is often required. Delaying cystectomy in these patients may lead to decreased disease-specific survival. Therefore, one of the biggest challenges in the management of nonmuscle-invasive bladder cancer is the decision regarding when to abandon conservative therapy and to proceed with radical cystectomy. This chapter discusses the role of radical cystectomy as the initial treatment of nonmuscle-invasive bladder cancer.

15.1 Introduction

Nonmuscle-invasive bladder cancer, given its high tendency to recur, coupled with an ever-present possibility to progress to potentially life-threatening muscle-invasive disease, remains a challenging clinical problem. Approximately, 70% of all new cases of urothelial bladder cancer are classified as nonmuscle-invasive or superficial (Kirkali et al. 2005; Lee and Droller 2000; Heney 1992). Nonmuscle-invasive "superficial" bladder tumors represent a heterogeneous group of cancers that include those that are generally papillary in nature and limited to the mucosa (Ta), those that are high grade, flat,

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and confined to the epithelium (carcinoma in situ, CIS), and those that invade into the submucosa or lamina propria (T1). The most important prognostic factors are histologic stage and grade. Other factors that have been assessed include the presence of multifocal disease, the frequency and time of recurrence, the tumor size, and the presence or absence of CIS. Although these characteristics have a predictive value on a population basis, no parameter has been found to reliably predict the biological behavior of superficial TCC on an individual basis. These short comings have led to great efforts to define the biological potential of these tumors on a molecular level. Unfortunately, the application of molecular markers in the treatment and management of nonmuscle-invasive bladder cancer has not yet been established for clinical practice.

For patients with nonmuscle-invasive bladder cancer, the initial inevitable treatment is a complete cystoscopic transurethral resection of all visible bladder tumors (TURBT). One of the most seemingly easy, but often-difficult initial steps, is to achieve complete tumor resection. Furthermore, to ensure that the tumor has not invaded the bladder muscle, it is the joint responsibility of the surgeon to provide muscle in the specimen and the pathologist who must note and describe the presence or absence of an involvement of the deep muscle layers. Although most nonmuscle-invasive bladder cancers are safely and effectively treated by transurethral resection (TURBT) alone with or without intravesical instillation therapies, some forms have a high propensity to invade and progress with a more aggressive therapy becoming necessary. An estimated 40–80% of nonmuscle-invasive bladder cancers will recur within 6–12 months if managed exclusively with a TURBT without additional therapy, and 10–25% will develop to muscle-invasive, advanced regional, or metastatic disease. One of the biggest challenges in the management of nonmuscle-invasive bladder cancer is the decision when to abandon conservative therapy and to proceed with radical cystectomy. It must be emphasized that bladder preservation in the form of an effective transurethral resection, possibly in combination with intravesical therapy, is clearly preferable to radical surgery in nonmuscle-invasive tumors. However, bladder preservation should not override patient survival and the potential for cure. Failure of conservative therapies, particularly in the presence of high-risk factors, should prompt more aggressive therapy. This decision process of "if" and "when" to proceed with radical cystectomy can be difficult and must be individualized on the basis of the tumor characteristics, patient desires, and quality-of-life considerations. Early radical cystectomy may be advocated in selected high-risk patients with nonmuscle-invasive bladder cancer for the following reasons:

- There remains a significant clinical staging error even with advanced conventional imaging techniques and basic histopathological evaluation.
- Despite the use of intravesical chemotherapy and immunotherapy, there remains the potential for tumor progression and death not to mention the potential of treatment-related side effects.
- After radical cystectomy recurrence-free and overall survival rates are excellent.
- Accurate pathologic evaluation of the primary tumor and regional lymph nodes can help with the decision on the need for a more aggressive adjuvant, e.g., systemic therapy.

- The option of orthotopic lower urinary tract reconstruction, along with nerve-sparing techniques, has improved the quality of life and lessened the negative social impact associated with cystectomy.

Thus, even when being "aggressive" and proceeding with cystectomy in the setting of noninvasive disease, too often, the procedure is performed after the window of curability has closed. On the other hand, a substantial number of patients are at danger to be overtreated when early cystectomy is recommended as an alternative to a conservative therapeutic approach.

15.2 Nonmuscle-Invasive Bladder Cancer: Recurrence and Progression

15.2.1 Factors Predicting Recurrence and Progression in Nonmuscle-Invasive Disease: Value and Limitations

The combination of tumor grade and invasion depth continues to be the most commonly used and helpful predictor of bladder cancer behavior and progression (Stein 2000). Tumor size may influence both recurrence and progression. In patients with a single tumor, the size of the tumor predicted recurrence rates in the first 2 years after initial resection (Table 15.1). Heney et al. (Heney 1992; Heney et al. 1983) reported progression to muscle invasion in 35% of patients with superficial TCC larger than 5 cm compared with only 9% of those with smaller tumors. A solid tumor structure is also more likely to be associated with recurrence and invasion. The number of papillary tumors may also influence recurrence. In the series by

Table 15.1 Stage description and WHO grading

(a) Stage	
Stage	Description
Ta:	Papillary, mucosally confined, basement membrane intact
T1:	Invades lamina propria, muscularis propria not involved
Tis:	"Flat," full-thickness mucosal involvement with high-grade cells, diffuse
(b) 1973 WHO grading	
Grade	Description
G1:	Well differentiated
G2:	Moderately differentiated
G3:	Poorly differentiated

(c) 2004 WHO grading

- Urothelial papiloma
- Papillary urothelial neoplasm of low malignant potential (PUNLMP)
- Low-grade papillary urotheial carcinoma
- High-grade papillary urothelial carcinoma

WHO World Health Organization

Heney et al. (1983) recurrence rates for multiple tumors ranged from 40% to 90%, compared with 18–60% in those with solitary lesions. One of the most useful determinants of ultimate recurrence potential may be the presence or absence of recurrence on the first follow-up cystoscopy, usually performed 3 months after tumor resection. Fitzpatrick et al. (1986) noted that 79% of patients with no recurrence at the time of first cystoscopy had no further recurrence for the remainder of their follow-up period. In those who did have recurrence at 3 months, only 10% had no further recurrence (Fitzpatrick et al. 1986). Therefore, early recurrence of superficial lesions after initial resection may help identify those tumors in need of closer follow-up or earlier aggressive, respectively, more prolonged adjuvant therapy.
The lamina propria, which lies just beneath the bladder mucosa, is rich with blood and lymphatic vessels, channels that allow for hematogenous and lymphatic progression, and metastases. Therefore, the depth of lamina propria invasion may also be an important determinant of the malignant potential of T1 tumors (Angulo et al. 1995; Younes et al. 1990; Cheng et al. 1999). It has been recommended that, when noted, the depth of invasion of the muscularis mucosae should be indicated in the pathology report, realizing that proper identification and description of this layer may not be possible in up to 30–50% of cases.

Furthermore, the accuracy of clinical staging is of particular importance when major differences in treatment that may directly affect patient outcome exist due to often subtle differences in pathologic features. With respect to noninvasive tumors, clinical understaging is particularly problematic, with error rates ranging from 27% to 62% (Freeman et al. 1995; Dutta et al. 2001; Wood et al. 1989; Amling et al. 1994; Stein and Skinner 2003; Pagano et al. 1991; Soloway et al. 1994; Ghoneim et al. 1997; Bianco et al. 2004). This is caused in part by the limitations of radiographic imaging that are known to result in understaging of bladder cancer even in patients with known muscle-invasive disease (Paik et al. 2000; Herr 1996). In addition, the absence of uninvolved muscularis propria in the TUR specimen is a significant cause of understaging. Dutta et al. (2001) demonstrated that of 63 patients with Stage T1 disease, 41% had no muscularis propria in the TUR

Table 15.2 Important factors predicting the likelihood for recurrence or progression in non-muscle invasive bladder cancer

- Cystoscopic findings
- Pathologic factors
- Stage
- Grade
- Presence of carcinoma in situ
- Tumor size
- Tumor number
- Tumor structure
- Biologic markers
- Recurrence at first follow-up cystoscopy
- Treatment response
- BCG failure

specimen and 62% of these were found to have been understaged at cystectomy. In contrast, only 30% with Stage T1 disease in whom muscularis propria was present, the latter being described as unaffected, were understaged. Therefore, the presence of uninvolved muscularis propria significantly reduces, but does not eliminate, the risk of clinical understaging. Understaging was associated with statistically worse disease-specific and recurrence-free survival, supporting earlier reports on negative impact of understaging on survival (Freeman et al. 1995).

Although not without recognizing the prognostic limitations, the combination of clinical findings available from routine pathology report, the latter including grade, stage, and the presence of CIS, represent the most important risk factors for nonmuscle-invasive disease (Millan-Rodriguez et al. 2000) (Table 15.2).

15.2.2 Clinical Understaging

Clinical staging errors in patients with nonmuscle-invasive bladder cancer, either due to an inappropriate TUR by the urologist or a false diagnosis made by the pathologist – to mention the obvious reasons for a staging mistake – have been reported repeatedly between 34% and 62% (Stein 2000). Freeman et al. (1995) reviewed the UCLA series of cystectomies performed for noninvasive bladder cancer. In this series, 34% of patients were pathologically upstaged to muscle-invasive or metastatic disease at the time of cystectomy. Amling et al. (1994) reported a clinical understaging error of 44% in patients with carcinoma in situ and Ta lesions and an understaging rate of 37% in patients with stage T1 bladder tumors. In this series, patients who were clinically understaged had worse cancer-specific survival rates. In contrast to a median survival of 12.2 years for the 28 patients in whom superficial TCC was confirmed during cystectomy, the long-term survival was reduced to 5.7 years for patients with a clinical upstaging following the definite histopathological evaluation of the cystectomy specimen (50 patients with pT2-T4 disease; $p = 0.005$). In a report by Soloway et al. (1994), a 36% clinical understaging rate was seen in patients initially suspected as having superficial disease. Pagano et al. reported an overall staging error rate of 51% in patients with noninvasive T1 and Tis tumors, and clinical understaging occurred in 35% of these patients. Supportingly, Ghoneim et al. reported a pathologic understaging error of 62% in a study including 53 patients with T1 or Ta tumors. Therefore, there is an inherent risk to treat muscle-invasive disease by an inappropriate conservative management in the form of TUR alone with or without instillation therapy.

15.2.3 Stage T1

Stage T1 tumors usually demonstrate an unfavorable natural history. These tumors are usually high grade and, because they have already demonstrated invasive potential, have a significant propensity for progression. As demonstrated, the permanent and not insignificant risk of clinical understaging remains the biggest impediment to

proper and timely treatment. Often, the histopathological reports in Stage T1 tumors make no mention of deep musculature present in the resection samples and thus leave the question of an affection of the deep muscle layer open for interpretation. When the pathologic report reliably indicates the presence or absence of deep musculature in the resection samples, the decision maker has to actively discuss the question with the pathologist to avoid a substantial staging error. T1 tumors are potentially lethal cancers with varying degrees of aggressiveness and progression rates of 30% to 50% (Heney 1992; Heney et al. 1983), which might be even higher with long-term follow-up (Herr 1991, 2005; Pansadoro et al. 1995; Peyromaure et al. 2003; Pham and Soloway 1997; Shahin et al. 2003). As T1 tumors are considered to be nonmuscle-invasive bladder cancers, this is a dangerous misnomer that underestimates the potential to cause significant morbidity and mortality, as they behave in a manner more typical of invasive cancers. However, the decision to perform bladder preservation or early radical cystectomy for clinical T1 tumors is challenging. It is currently one of the most difficult management issues in bladder cancer.

15.2.4 Stage Tis (CIS-Carcinoma In situ)

Carcinoma in situ comprises about 10% of all cases of bladder cancer, with approximately one half diagnosed as an isolated lesion and the other half occurring in association with papillary tumors. These tumors represent a subset of nonmuscle-invasive bladder cancer with a high progression rate and a distinct malignant clinical behavior.

Ever since 1976, Althausen et al. (1976) reported that between 40% and 83% of CIS tumors will ultimately progress to muscle-invasive disease. In a review of the urologic literature on carcinoma in situ in 1992, Lamm et al. (Lamm 1992) reported an average incidence of progression to muscle-invasive disease of 54%. In patients supposed to exclusively reveal CIS up to 20% of those treated with cystectomy were found to contain some elements of microscopic tumor invasion (Farrow et al. 1976). In a series examining patients with high-grade Stage T1 bladder cancer, Masood et al. (2004) demonstrated that the presence of CIS led to an upstaging of lesions in 55% of radical cystectomy specimens versus only 6% in those presenting without CIS. Additionally, CIS increases the risk of developing extravesical occurrences, particularly in the upper tract (Cookson et al. 1997). For patients with carcinoma in situ associated with low-grade papillary TCC, Althausen et al. (1976) reported an 83% incidence of progression to subsequent muscle invasion.

The presence of carcinoma in situ in association with a papillary tumor also predicts the risk for early tumor recurrence. The tumor recurrence rate was as high as 73% if carcinoma in situ or dysplasia were present at the time of initial tumor resection compared with 43% in the absence of these findings (Kiemeney et al. 1994). Diffuse carcinoma in situ that is associated with bladder symptoms has the greatest potential for progression.

One of the troublesome aspects of carcinoma in situ is that it is often multifocal and can be difficult to identify on cystoscopic examination alone. Areas of mucosal dysplasia or carcinoma in situ can be found in normal-appearing urothelium in patients with and without papillary tumors. Carcinoma in situ does not respond to radiotherapy and, if untreated, will often progress to invasive cancer. Intravesical BCG is especially effective in the treatment of carcinoma in situ of the bladder. Before the introduction of intravesical BCG, most patients with carcinoma in situ underwent radical cystectomy. Nowadays, numerous studies show that BCG has an effect in the treatment of this entity and is now the first-line medical instillation treatment for this disease. In 1992, Lamm et al. (Lamm 1992) reviewed studies involving 718 patients. The initial tumor-free rate averaged 72% following BCG instillation for the treatment of bladder carcinoma in situ. In a study from 1985, Brosman et al. (Brosman 1985) suggested that carcinoma in situ can be eradicated in more than 80% of patients using relatively intensive BCG protocols. Mori et al. (1986) in 1986 and Lamm et al. (Mori et al. 1986; Lamm et al. 2000) in 2000 confirmed these good results. In the meantime, about six data analyses have determined the efficiency of BCG instillation therapy for the treatment of superficial TCC with or without CIS. Whereas four studies described a reduced risk for recurrence, two studies postulated an even decreased risk for recurrence for tumor progression following instillation therapy with BCG. However, this issue is still discussed controversially. The only point that can be considered as proven is that to achieve the positive effects that have been associated with BCG therapy a maintenance protocol (>1/year) is necessary. However, in this setting, the potential side effects have to be weighed against the benefit of BCG instillation therapy. Intravesical BCG is the conservative treatment of choice for patients with bladder carcinoma in situ.

15.3 Indications for Radical Cystectomy for Nonmuscle-Invasive Bladder Cancer

15.3.1 High-Grade Nonmuscle-Invasive Disease

Most nonmuscle-invasive bladder cancers, approximately 75%, are stage Ta and confined to the bladder mucosa. Low-grade Ta tumors rarely progress to muscle-invasive disease. However, stage T1 tumors, particularly those of higher grade, and carcinoma in situ represent tumors with much more malignant potential. Immediate radical cystectomy for Ta-T1, high-grade bladder cancer is typically recommended for patients with a long life expectancy, who are at high risk for tumor progression, e.g., in case of multiple and/or large tumors. The rationale for immediate cystectomy rather than BCG therapy is based upon the 50% risk of progression to muscle-invasive disease and the 35% long-term cancer-specific mortality with BCG (Cookson et al. 1997). Addressing the therapeutic benefit of BCG instillation

therapy, it remains to be classified whether this approach reveals an impact on the recurrence rate and also reduces the risk for tumor progression. However, it can be taken for granted that to induce an effect maintenance therapy with BCG over a period of at least 1 year is necessary. On the other hand, recognizing the lifelong risk of developing muscle-invasive tumor recurrence and the resulting need for intense follow-up examinations as well as the 30% risk of dying from metastatic bladder cancer in the future have to be weighed against the determination of health-related quality of life following an orthotopic neobladder substitution preferably in young patients. The reported cancer-specific survival associated with immediate cystectomy for these patients is 85–90% (Shariat et al. 2006; Stein et al. 2001; Hautmann et al. 2006). The behavior of stage T1 tumors is directly correlated to their histologic grade. Grade 3 lesions are much more likely to progress to muscle-invasive disease and result in poorer survival rates than grades 1 and 2 cancers. In spite of aggressive intravesical chemotherapy or immunotherapy with BCG, relevant recurrence and progression rates were seen with T1 and for grade 3 lesions (Heney et al. 1983; Althausen et al. 1976). In patients with stage T1 grade 3 tumors, up to 50% develop muscle-invasive disease during the course of the disease. Therefore, the natural history of these more aggressive lesions mandates more aggressive treatment and close monitoring. It is well accepted that once these lesions progress to muscle invasion, radical cystectomy is the treatment of choice. However, muscle invasion is often difficult to predict in these lesions because of clinical staging errors.

15.3.2 BCG-Refractory Bladder Cancer

The standard approach for high-grade, nonmuscle-invasive bladder cancer (Ta, T1, carcinoma in situ) is transurethral resection (TUR) followed by intravesical immunotherapy with BCG. The EAU guidelines recommend intravesical chemotherapy with Mitomycin for nonmuscle-invasive bladder cancer stage pTa, G3 and intravesical immunotherapy with BCG for stages pT1 and CIS. Many intravesical chemotherapeutic agents have been shown to reduce the risk for tumor recurrence and to increase the duration of recurrence-free survival when used in combination with transurethral tumor resection. Unfortunately, however, none of these agents have proved to be of benefit in preventing disease progression. Compared with controls, BCG has a 43% advantage in preventing tumor recurrence, a significantly better rate than the 16–21% advantage of intravesical chemotherapy. In addition, BCG is particularly effective in the treatment of carcinoma in situ, eradicating this lesion in more than 80% of cases. In contrast to intravesical chemotherapy, BCG has also been suggested to decrease the risk of tumor progression. Herr et al. (Herr 2000) monitored patients with high-grade Ta cancers for at least 15 years after transurethral resection that was followed by intravesical BCG therapy. These patients had a 39% rate of progression to muscle-invasive disease, 26% died of bladder cancer.

These rates were similar for patients with stage T1 tumors monitored for the same period of time. Furthermore, Herr et al. (1992) performed a randomized study with BCG versus transurethral resection alone for the treatment of carcinoma in situ associated with papillary bladder tumors. In those patients receiving BCG, the progression-free rate at 10 years was 61.9% versus 37% for patients receiving TURB with or without additional BCG. Currently, six meta-analyses that have tried to determine the preventive effect of BCG toward tumor progression are available. However, due to inhomogeneous patient populations, varying end points, and an overall low frequency of tumor progression, the question whether BCG reduces the risk of tumor progression remains to be answered.

Radical cystectomy is generally recommended for patients with TCC refractory to BCG, because the risk of progression to muscle-invasive cancer is 80–100%. BCG-refractory disease is defined as the occurrence of recurrent high-track nonmuscle-invasive tumor 3–6 months after initiating BCG therapy, muscle-invasive disease during follow-up or progression of the disease. Although other intravesical agents (e.g., gemcitabine, valrubicin, mitomycin) frequently induce responses, long-term responses to salvage intravesical therapy are rare. Thus, salvage intravesical therapy for BCG-refractory disease is generally preserved for patients unwilling or unfit to undergo radical cystectomy. Herr et al. (1988) evaluated 86 patients with high-risk superficial bladder cancer. The time to development of muscle invasion or distant metastasis was significantly prolonged with BCG therapy. Stage progression was noted in 35% of the control patients versus 28% of the BCG-treated patients. Another important finding in this study was that in spite of earlier and more frequent use of cystectomy in the control/untreated population, the mortality rate was reduced from 32% to 14% with the use of BCG treatment. Treatment with intravesical BCG after TURBT results in 70% 5-year survival for T1 tumors (Herr et al. 1990). This is similar to the results achieved with immediate radical cystectomy. However, cystectomy is appropriate for patients who relapse with recurrent T1 or high-track tumor who develop muscle-invasive disease or experience a recurrence of the disease within 6–12 months after combined treatment with TURBT and intravesical BCG.

15.3.3 Miscellaneous Indications

Uncommon indications for radical cystectomy in patients without muscle-invasive bladder cancer include diffuse, low-grade, papillary bladder cancer (papillomatosis) that is not amenable to complete transurethral resection. Radical cystectomy may also be indicated for patients with bladder cancers that cause symptoms (e.g., hemorrhage, urinary frequency) or that are not manageable endoscopically. Cystectomy is discussed as a therapeutic option in T4 prostate cancer. In addition, nonmuscle-invasive cancer of the prostatic urethra is frequently managed by cystectomy, particularly if a complete resection cannot be accomplished (Table 15.3).

Table 15.3 Indications for cystectomy

Muscle-invasive bladder tumors
Superficial bladder tumors characterized by any of the following: • Refractory to cystoscopic resection and intravesical chemotherapy or immunotherapy • Incomplete resection due to diffuse bladder involvement and/or unfavorable location • Invasive prostatic urethral involvement
Stage-pT1, grade-3 tumors unresponsive to intravesical BCG vaccine therapy
CIS refractory to intravesical immunotherapy or chemotherapy
Palliation for pain, bleeding, or urinary frequency
Primary adenocarcinoma, SCC, or sarcoma

15.4 Outcome After Radical Cystectomy for Nonmuscle-Invasive Bladder Cancer

Most reported series demonstrate a good outcome after radical cystectomy for superficial disease (Freeman et al. 1995; Amling et al. 1994; Malkowicz et al. 1990). Freeman et al. (1995) reported the experience from the University of Southern California with radical cystectomy in patients with high-risk superficial bladder cancer. In total, 182 patients underwent radical cystectomy with a median follow-up of 7.2 years. Thirty-four percent of all patients and 40% of patients with stage T1 disease were pathologically upstaged at the time of cystectomy, and these patients had a significantly worse survival and a higher recurrence rate. The median survival for pathologically confirmed superficial tumors was 10.2 years compared with 6.9 years for those with clinically understaged disease. In this series regional lymph node involvement was found in 8% of patients. Amling et al. (1994) reported on 220 high-risk patients subjected to radical cystectomy for clinically nonmuscle-invasive disease. The tumors were either high grade or had not responded to a transurethral resection or intravesical therapy. The surgery-associated mortality rate was 2.3% with an overall complication rate of 20%. The 5-year cancer-specific survival estimates for pathologic Ta, Tis, and T1 tumors were 88%, 100%, and 76%, respectively. Clinical stage T1 tumors that remained pathological stage T1 had a median survival of 12.2 years compared with 5.7 years for those patients with pathological upstaging to muscle-invasive disease ($p = 0.005$). Lymph node positive disease was found in a total of 13 patients (5.9%). Similar survival rates were reported by Malkowicz et al. (1990) in 107 patients with nonmuscle-invasive disease. The 5-year actuarial survival rates for pathologically confirmed nonmuscle-invasive disease were 100% for Ta, 80% for T1, and 85% for Tis lesions. The results of these series that have evaluated the long-term efficiency for nonmuscle-invasive bladder cancer support an aggressive surgical management in patients not responding to conservative therapy. With the development of orthotopic lower urinary tract reconstruction to the native urethra, with or without nerve-sparing techniques, the quality of life has dramatically improved and lessened the socially negative impact formally related to radical cystectomy.

Table 15.4 Summary

Although typically reserved for muscle-invasive disease, radical surgery is more appropriately used to treat some cases of nonmuscle-invasive bladder cancer.
Thirty-five to fifty percent of patients who undergo cystectomy for Ta, T1, or CIS are discovered to have muscle-invasive disease, with 10–15% demonstrating microscopic lymph node metastasis.
CIS in upwards of 80% of affected patients progresses to muscle-invasive disease, with 20% of patients found to have muscle-invasive disease at the time of cystectomy.
High-grade T1 tumors that recur despite BCG have a 50% likelihood of progressing to muscle-invasive disease. Cystectomy performed prior to progression yields a 90% 5-year survival rate. The 5-year survival rate drops to 50–60% in muscle-invasive disease.
Patients with unresectable large superficial tumors, prostatic urethra involvement, and BCG failure should also undergo radical cystectomy.

15.5 Summary and Recommendations

One of the biggest challenges in the management of nonmuscle-invasive bladder cancer is the decision when to abandon conservative therapy and to proceed with radical cystectomy. In general, the initial treatment includes a complete cystoscopic transurethral resection of all visible bladder tumors that can be accomplished by fluorescence light cystoscopy in selected cases. The limited ability to predict the tumor's biological behavior makes individualized therapeutic approaches indispensable. An estimated 40–80% of nonmuscle-invasive bladder cancers will recur within 6–12 months if managed exclusively with a TURBT without additional therapy, and 10–25% of patients will develop muscle-invasive, regional, or metastatic disease, hereby emphasizing the aggressiveness of TCC as well as the need for therapeutic decision adapted to an individual clinical situation (Table 15.4).

Immediate cystectomy should be considered and discussed with patients revealing nonmuscle-invasive tumor for the following indications:

- Multiple recurrent high-grade tumors
- High-grade T1 tumors
- High-grade tumors with concomitant CIS
- BCG failure

Delaying cystectomy in these patients may lead to decreased disease-specific survival.

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Chapter 16
Radical Surgery for Muscle-Invasive Bladder Cancer

Juergen E. Gschwend

Abstract *Background*: Radical cystectomy offers the best opportunity for ultimate cure of high-grade and high-risk invasive bladder cancer.

Objective: To review the available literature on the indication and oncological outcome of radical cystectomy for urothelial carcinoma of the bladder. A database search of the U.S. National Library of Medicine (PubMed) was performed for relevant medical articles using the Medical Subject Headings "invasive bladder cancer" and "radical cystectomy" with restrictions to English language publications.

Evidence: Immediate or early radical cystectomy offers excellent recurrence-free and disease-specific survival rates as well as local tumor control in patients with organ-confined and node-negative disease. Tumor control in nonorgan-confined tumors is satisfactory with long-term recurrence-free survival rates of about 50%. For node-positive disease, surgery may still be curative in approximately one-fourth of patients.

Conclusions: Evidence from the literature supports an early aggressive surgical management for invasive bladder cancer. Risk-stratification of patients with bladder cancer based on pathologic features at initial TUR or at recurrence can select those most appropriate for radical cystectomy early. In patients with organ-confined, lymph node-negative urothelial bladder carcinoma excellent long-term survival rates can be achieved.

16.1 Background

The incidence of urothelial cell carcinoma of the bladder accounts for more than 336,000 cases worldwide (Tyczynski and Parkin 2003). Approximately 75% of bladder cancer cases are primarily nonmuscle-invasive; however, of these tumors, roughly one-fourth are already early invasive with lamina propria involvement.

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Since the early sixties, radical cystectomy has become the treatment of choice for the management of invasive bladder cancer in most western countries (Whitmore 1975). Improvements in surgical technique and modern perioperative care have diminished the perioperative complications rate substantially and even lowered the operative mortality rate from nearly 20% to less than 2% (Wood et al. 1987; Skinner and Lieskovsky 1988; Novotny et al. 2007). Today, pelvic lymph node dissection (PLND) and radical cystectomy are considered to be the optimal therapy for invasive bladder cancer and regarded to be superior to radiation therapy or organ-conserving surgery with local tumor control and ultimate cure of cancer as endpoints (Hautmann et al. 2006). This chapter scopes the indications and the results of contemporary cystectomy series and its impact on overall, recurrence-free and disease-specific survival.

16.2 Radical Surgery for Muscle-Invasive Bladder Cancer

16.2.1 Diagnosis of Invasive Bladder Cancer

The mainstay of correct diagnosis and proper initial treatment of invasive bladder cancer is transurethral resection (TUR) of the bladder. The specimen from different fractions must be sent to the pathologist in separate portions to enable a correct diagnosis. Cauterization should be avoided as much as possible during the resection to prevent tissue damage. The resection material must include the muscular layer and should be as complete as possible without bladder perforation. Tumor visibility and completeness of resection may be aided by fluorescence-guided cystoscopy, although this diagnostic modality is not available everywhere (Witjes and Douglass 2007). Adequate sampling of muscularis propria is important as studies have documented the risk of understaging of invasive lesions at 50–70% in the absence of muscularis propria (Dutta et al. 2001; Herr 1999). Even with muscularis in the specimen, understaging rates are reported between approximately 20–30% after single TUR based on cystectomy pathology (Dutta et al. 2001; Jakse et al. 2004).

In an effort to ease and improve the pathology reproducibility, the World Health Organization (WHO) and the International Society of Urologic Pathologists (ISUP) developed the WHO/ISUP consensus classification of urothelial neoplasms in 1998 (Epstein 2003). This system updated the 1973 WHO numerical grading. Consequently, invasive bladder tumors, virtually all of which are poorly differentiated and classified as grade 3 according to the 1973 WHO criteria, are referred as “high grade” under the WHO/ISUP classification. The impact of this change in grading on oncological outcome of bladder cancer patients is not yet fully understood (Harnden 2007; Burger et al. 2008a), although both grading systems contribute independent information regarding disease progression (Burger et al. 2008b).

For staging purposes, computerized tomography (CT) and magnetic resonance imaging (MRI) are used to assess clinical tumor stage prior to surgery. The purpose of preoperative staging of invasive bladder cancer is to assess the extent of local

tumor invasion, to detect lymph node involvement and tumor spread to distant organs. However, clinical staging of bladder cancer is still related to a significant staging error.

16.2.2 Clinical Relevance of a Secondary TUR for Invasive Bladder Tumors

Correct local tumor staging is extremely important since it will directly affect the treatment modality. A second TUR should always be performed when the initial resection has been incomplete, e.g., when multiple and/or large tumors are present, or when the pathologist has reported the lack of muscle tissue in the specimen. For all cases of newly diagnosed early invasive (T1G3) tumors a secondary TUR 4–6 weeks after the primary TUR is strongly recommended for two reasons. First, a repeat TUR of the previous resection site 4–6 weeks after the initial resection will provide a more accurate staging information. This is particularly important since the probability of understaging of such tumor ranges from 20% to 70%, depending on the presence of muscle fibers in the sample. If muscle is absent from the initial TUR, repeat resection is mandatory because of the well-known high rate of understaging (Herr 1999). Even with muscularis propria sampling at first resection, several reports have documented occult T2 disease in up to 10% of second resections (Jakse et al. 2004; Miladi et al. 2003; Schwaibold et al. 2006). A second TUR often upstages T1 lesions and provides additional pathologic information that can alter the therapeutic management (Herr 1999; Brauers et al. 2001). Furthermore, repeat resection is mandatory in the management of all early invasive tumors penetrating the basal membrane (Soloway et al. 2007).

A second advantage of repeat TUR is the gain of prognostic information. While upstaging of T1G3 lesions to ≥ pT2 disease automatically selects patients for definitive radical therapy, Herr and colleagues demonstrated that evidence of T1 disease on repeat TUR may portend future muscle invasion (Herr et al. 2007). Of 92 T1 patients with residual T1 disease at second resection, 82% progressed to muscle invasion at 5 years. In contrast, of 260 T1 patients without lamina propria invasion on second TUR only 19% progressed at 5 years. Based on these data, residual T1 bladder cancer on repeat TUR is recognized as negative prognostic factors and a potential trigger for immediate cystectomy in these selective patients.

16.2.3 Timing and Delay of Radical Cystectomy for Bladder Cancer

Retrospective analysis of patients with a clear indication for radical surgery of locally advanced bladder cancer revealed that a further delay of treatment beyond 3 months from primary diagnosis caused a significantly increased frequency of

extravesical disease (81 vs 52%) (Chang et al. 2003). A delay of definitive therapy by radical cystectomy may not only alter the oncological outcome but also the type of urinary diversion. In a series of patients with clinically organ-confined urothelial cancer of the bladder, the average time from the primary diagnosis of bladder cancer to cystectomy was 12.2 months for patients who received an ileal neobladder and 19.1 months for patients with an ileal conduit. The average delay from diagnosis of invasive disease to surgery was substantial with 3.1 months in neobladder and even more with 15.1 months in ileal conduit patients (Hautmann and Paiss 1998). Similar results have been observed in another series of 247 patients where superior recurrence-free and overall survival rates were observed in those patients treated within a 3 months period compared to other cystectomy candidates with more delayed surgery (Sanchez-Ortiz et al. 2003). A contemporary cohort of 214 consecutive patients who presented with clinical T2 bladder cancer was analyzed by Lee et al. in regard of time to cystectomy, pathological stage, disease-specific survival, and overall survival. A significant disease-specific and overall survival advantage was observed in patients undergoing cystectomy by 93 days or less compared to greater than 93 days (Lee et al. 2006).

A cystectomy delay of more than 3 months apparently undermines patient survival, probably through the development of micrometastases, since local stage progression is not apparent at this point. Most delays in surgery are avoidable and should be minimized. Despite the need for second opinions and the impact of busy surgical schedules, clinicians must take care to schedule patients efficiently and complete the surgical treatment within this proposed time frame.

16.2.4 General Indication for Radical Cystectomy in Patients with Bladder Cancer

Traditionally, radical cystectomy is recommended for the majority of patients with muscle-invasive bladder cancer (T2-T4a, N0-Nx, M0) with a curative intent [Hautmann 2006]. Other well-accepted indications do include high-risk and recurrent noninvasive bladder tumors, BCG-resistant carcinoma in situ (Cis), high-risk T1G3, as well as extensive papillary disease that cannot be managed with TUR and intravesical therapy alone.

Performance status and age influence the choice of primary therapy, as well as the type of urinary diversion with cystectomy and orthotopic ileal neobladder reconstruction being reserved for patients without severe concomitant disease and better performance status. The value of assessing overall health before recommending and proceeding with surgery was emphasized in a multivariate analysis, which demonstrated an association between comorbid disease and adverse pathological and survival outcome following radical cystectomy (Miller et al. 2003).

There is still controversy about age, radical cystectomy, and the type of urinary diversion. Cystectomy is associated with the greatest risk reduction in disease-related and nondisease-related death even in patients older than 80 years (Miller et al. 2003).

The largest retrospective single-institution study on cystectomy in old patients demonstrated that patients older than 80 years did have an increased postoperative morbidity but mortality was not increased. Some patients can even successfully undergo an orthotopic neobladder procedure in this age-group, but the majority of patients were treated in this series with an ileal conduit diversion (Figueroa et al. 1998).

A so-called salvage cystectomy is indicated for nonresponders to conservative therapy, recurrences after bladder-sparing approaches, nonurothelial carcinomas (some of these tumors respond even less to neoadjuvant chemo- and radiotherapy) and often with a palliative intent for patients with recurrent gross hematuria, local pain, and urgency as well as fistula formation to the bowel due to locally advanced bladder cancer (see paragraph 3.8: palliative cystectomy). There is some evidence that a palliative cystectomy, even in very old patients, may decrease the overall morbidity, lower the frequency of hospital admittance as well as total time of hospital stay.

16.2.5 Extension of Pelvic Lymph Node Dissection (PLND) Along with Radical Cystetcomy

A *standard pelvic lymph node dissection* was recently suggested by Mills et al. and does include removal of all nodal tissue up to and including the common iliac bifurcation (Mills et al. 2007). The anatomic landmarks do further include the following fields: internal iliac (plus presacral), obturator fossa, external iliac, and distal common iliac. Removal of tissue above the common iliac bifurcation up to the crossing of the ureter (as part of the common iliac area) is recommended. The lymphatic tissue medial to the ureters, as well as the common and internal iliac arteries, is spared in order to avoid injury to the hypogastric autonomic nerves which are important in case of potency-sparing cystectomy. However, this type of meticulous lymph node dissection is regarded by others as extended field (Herr et al. 2002).

Stein et al. (2001) defined an *extended pelvic lymph node dissection* as an area including all lymph nodes in the boundaries of the aortic bifurcation and common iliac vessels (proximally), the genitofemoral nerve (laterally), the circumflex iliac vein and lymph node of Cloquet (distally), the hypogastric vessels (posteriorly) including the obturator fossa, presciatic nodes bilaterally, and the presacral lymph nodes.

16.2.6 Localization of Lymph Node Metastases

Lymph node mapping studies are helpful to define common landing sites of lymph node metastases (Abdel-Latif et al. 2004; Vazina et al. 2004; Abol-Enein et al. 2004). Recently, a prospective multicenter study evaluated the pattern of lymphatic

spread in 290 patients who underwent RC and extended PLND (Leissner et al. 2004). Lymph nodes were examined from 12 well-defined anatomical sites. The percentage of metastases at different sites ranged from 14.1% (right obturator nodes) to 2.9% (right paracaval nodes above the aortic bifurcation). Sixteen percent of lymph node metastases were detected above the aortic bifurcation and 8% in the presacral region. The authors concluded that if the dissection had been limited to the obturator sites, 74% of positive lymph nodes would have been left behind and about 7% of patients would have been understaged as lymph node negative.

Vazina et al. evaluated 176 patients (pT1-pT4) who underwent RC and PLND (Vazina et al. 2004). Of the 43 lymph node-positive patients, the percentage of metastases at different sites were as follows: aortic bifurcation 4%, common iliac nodes (right side 5.7%, left side 8%), presacral nodes 5.1%, pelvic nodes (right side 12%, left side 14%), perivesical nodes 2.8%, and pelvic nodes not specified 5.7%. Thirty-three percent of patients with involvement of the common iliac lymph nodes also showed positive nodes in the presacral region.

Abol-Enein et al. (2004) evaluated 200 patients who underwent extended PLND for a cohort with predominant squamous cell carcinoma of the bladder. The removed lymph nodes (median 51) were labeled according to the anatomical location of dissection. In 48 patients (24%), histopathologically positive lymph nodes were detected, 39% of these cases presented with bilateral lymph node involvement. Therefore, the authors advocate mandatory bilateral endopelvic dissection. This is supported by most of the other current series (Stein et al. 2001; Mills et al. 2001; Leissner et al. 2000). As no skipped lesions outside the small pelvis were found in this dataset, the authors concluded that negative lymph nodes in the endopelvic region indicate that a more proximal dissection is not necessary.

Inevitably, there is some overlap between areas of lymph node dissection which complicates the accurate anatomical assignment of a removed lymph node and therefore the comparison between different studies. For instance, a node in the bifurcation of the common iliac artery may be classified as common, internal, external, or obturator, depending mainly on the surgeons´ estimation. This should be taken into account in the interpretation of all mapping studies. Collectively, these studies present debatable data regarding the spread of lymph node metastases in bladder cancer. However, these data indicate that a lymph node dissection encompassing only the nodes along the external iliac vessels and the obturator fossa would not be sufficient in a large number of cases.

16.2.7 Rationale for an Extended Pelvic Lymph Node Dissection

If pelvic or distant recurrence occurs after radical cystetcomy, the prognosis of patients is poor even with subsequent therapy. This emphasizes the need for at least optimal local control at the time of initial treatment. Leissner et al. showed that for patients undergoing extended lymph node dissection, survival for both lymph node-negative and lymph node-positive patients improved, with a reduced local recurrence rate when a greater number of lymph nodes were removed

(Leissner et al. 2000). It has been shown that resection of more than 16 lymph nodes translated into a 5-year recurrence-free survival increase from 63% to 85% in organ-confined tumors, from 40% to 55% in pT3 tumors, and from 25% to 53% in patients with at most 5 positive lymph nodes. Also Poulsen et al. (Poulsen et al. 1998) demonstrate that an extended lymph node dissection is beneficial in patients with organ-confined, lymph node-negative disease. There was a 5-year recurrence-free survival of 90% in patients with organ-confined disease and without lymph node metastasis in the extended PLND group, versus 71% in the standard PLND group ($p<0.02$). Furthermore, an extended PLND reduced the pelvic and distant metastases rate in these patients.

Although very promising at first sight, these results must be interpreted with caution. The so-called Will Rogers phenomenon (Feinstein et al. 1985) has to be remembered when analyzing prognosis outcomes in such series. A patient with only a few negative nodes removed may still have undiscovered positive nodes and thus a poor outcome. If many nodes are analyzed and classified negative, the likelihood of leaving behind undiscovered positive nodes is reduced, which results in a better prognosis for extended lymph node dissection.

Recently, two series were evaluated which included patients with limited pelvic lymph node dissection (336 patients) and extended lymph node dissection (322 patients) (Dhar et al. 2008). All cases were staged preoperatively N0M0 and none received adjunct therapy. The 5-year recurrence-free survival of patients with lymph node-positive disease was 7% for limited dissection and 35% for extended pelvic lymph node dissection. The 5-year recurrence-free survival for pT2 pN0 cases was 67% for limited and 77% for extended pelvic lymph node dissection, and the respective percentages for pT3 pN0 were 23% and 57% ($p<0.0001$). The 5-year recurrence-free survival for pT2 pN0-2 was 63% for limited and 71% for extended pelvic lymph node dissection, and for pT3pN0-2 cases, the respective figures were 19% and 49% ($p<0.0001$).

Collectively, these retrospective data suggest that limited pelvic lymph node dissection along the external iliac vessels and the obturator fossa only is associated with suboptimal staging, and poorer outcome for patients with node-positive and node-negative disease. Extended pelvic lymph node dissection may allow for more accurate staging and removal of undetected micrometastases. This could improve survival of patients with histopathological lymph node-positive and negative disease. Results from the German prospective, randomized multicenter study comparing an extended versus limited lymph node dissection along with cystectomy are awaited.

16.2.8 Principles of Radical Cystectomy

Radical cystectomy for urothelial carcinoma of the bladder does include the removal of the urinary bladder and adjacent organs, that is, prostate and seminal vesicles in men, and the uterus in women (Hautmann et al. 2007; Stenzl et al. 2005). The complete removal of the prostate in male patients and the extent of urethrectomy and vaginal resection in female patients have recently been questioned (Vallancien et al.

2002; Muto et al. 2004). However, urothelial cancer in the prostate was detected in 32 and 33% of patients undergoing radical cystoprostatectomy in recently published studies (Pettus et al. 2008; Shen et al. 2006). In another study, 41% of the cystoprostatectomy specimens removed for urothelial cancer had unsuspected prostate cancer. Twenty-four of 50 tumors (48%) were clinically significant. In the same study, 48% of patients had urothelial carcinoma in the prostate of which 33% had apical involvement (Revelo et al. 2004). Overall, in the above mentioned series, only 26–33% of the patients undergoing cystoprostatectomy for bladder cancer had neither prostate cancer nor prostatic urothelial cancer in the specimen.

In summary, the significant incidence of bladder and prostate cancer involving the prostate at the time of cystectomy, which is difficult to determine preoperatively, may preclude the general application of prostate-sparing techniques in most men requiring cystectomy. Concerns regarding the oncologic outcomes with prostate-sparing techniques, coupled with the excellent results seen with traditional radical cystectomy and orthotopic diversion, suggest that prostate-sparing procedure should be performed only in well-selected individuals (Stein et al. 2009; Hautmann and Stein 2005).

Urethrectomy is recommended if there are positive margins at the level of urethral dissection. If the primary tumor is located at the bladder neck or near to the urethra (in women), or if the tumor is infiltrating the prostate, an intraoperative frozen section is necessary to prove negative margins (Hautmann et al. 2007; Nagele et al. 2006).

Today radical cystectomy should follow the principles of a meticulous nerve-sparing technique whenever technically feasible and oncologically appropriate (Fig. 16.1). Nerve-sparing cystectcomy has shown to translate into improved functional results. Nerve-sparing radical cystoprostatectomy does not compromise cancer control and provides improved postoperative quality of life (Schoenberg et al. 1996). There is urodynamic evidence that the nerve sparing technique improves urethral sphincteric function and, consequently, the continence rate (El-Bahnasawy et al. 2006). Therefore, nerve sparing seems to be associated with improved urinary continence. Attempted nerve sparing has the greatest impact on daytime continence and age has the greatest impact on nighttime continence (Kessler et al. 2004). Nerve sparing is associated with more frequent recovery of erectile function after radical cystectomy for bladder cancer. Moreover, sexual function can be preserved as well in female patients who received neurovascular preservation. In contrast, all domains of sexual function declined in patients who had undergone nonneurovascular preservation (Bhatt et al. 2006).

16.2.9 Oncological Outcome of Radical Cystectomy for Muscle-Invasive Bladder Cancer with Negative Nodes

The outcome of patients undergoing cystectomy for bladder cancer according to a recent multiinstitutional database of 888 consecutive patients undergoing cystectomy and lymphadenectomy for bladder cancer revealed a mean recurrence-free

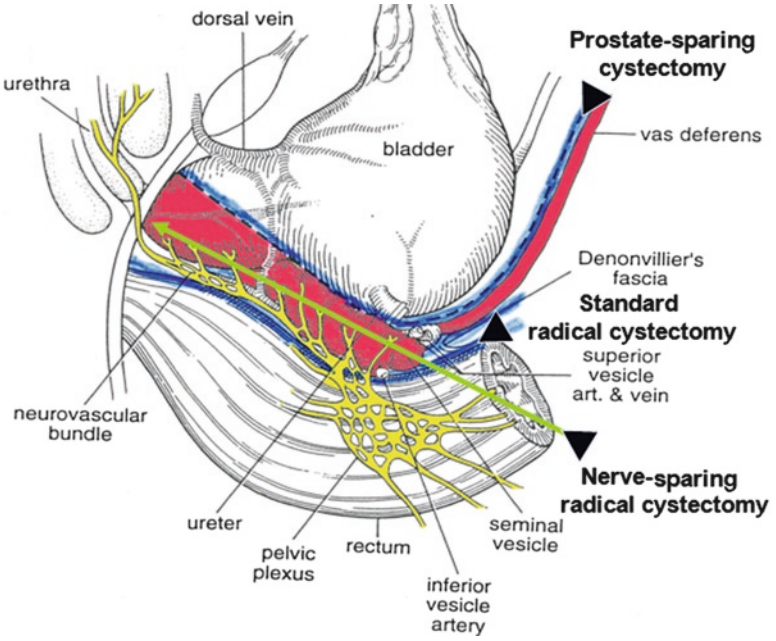

Fig. 16.1 Principles of nerve-sparing radical cystectomy with preservation of autonomic innervation along the anterior rectal wall, seminal vesicles, and the prostate to maintain potency and improve continence rates after orthotopic neobladder reconstruction

and bladder cancer specific survival of 58% and 66%, respectively, at 5 years (Shariat et al. 2006). The recurrence-free and overall survival in the largest single center study of 1,054 male and female patients was 68% and 66% at 5 years and 60% and 43%, at 10 years, respectively. Both, recurrence-free and overall survival, were significantly related to pathologic stage and lymph node status. Increasing pathologic stage and lymph node–positive disease were associated with significantly higher recurrence rates and worse overall survival ($p < 0.001$). The 5- and 10-year recurrence-free survival for the subgroup of organ-confined lymph node-negative tumors was 85% and 82% and overall survival at 5 and 10 years was 78% and 56%, respectively. The 5- and 10-year recurrence-free and overall survival for the subgroup of nonorgan-confined, lymph node-negative tumors was 58% and 55%, and 47% and 27%, respectively (Stein et al. 2001). In another contemporary study analyzing 686 patients, 10-year disease-specific and overall survival rates were 72.9% and 49.1%, respectively for organ-confined disease (defined as < pT3a), versus 33.3% and 22.8% for nonorgan-confined disease (Gschwend et al. 2002). Madersbacher et al. have likewise reported a 5-year recurrence-free survival of 76% in patients with pT1 tumors, 74% for pT2, 52% in pT3, and 36% in pT4 tumors (Madersbacher et al. 2003). Tumor stage and nodal involvement were the only independent predictors of survival (Bassi et al. 1999). Hautmann et al. have reported the results of 788 cystectomy patients who underwent surgery without any

Table 16.1 First site of recurrence in 788 patients who underwent radical cystectomy in a surgery-only series (Hautmann et al. 2006)

	Organ confined (pTa/Cis/1/2 pN0)	Nonorgan confined (pT3/4 pN0)	All pT-stages, node positive	Total # patients
	$n=495$	$n=151$	$n=142$	$n=788$
Local recurrences	4.0%	15.9%	20.4%	9.3%
Distant metastasis	9.5%	19.2%	45.1%	17.9%
Urinary tract recurrences	4.0%	3.3%	0.7%	3.3%
Total	17.5%	37.4%	66.2%	30.5%

neoadjuvant or adjuvant chemotherapy. The 10-year recurrence-free and overall survival rates were 59.1% and 44.9%, respectively. The rate of recurrence-free survival at 5 years was 82.5% for pT2a, 61.9% for pT2b and pT3a, and 53.1% for pT3b node-negative disease (Hautmann et al. 2006).

Overall, radical cystectomy provides excellent local (pelvic) control for the treatment of invasive bladder cancer. Local and distant failure rates (Table 16.1) were 4% and 9.5% for organ-confined tumors, 15.9% and 19.2% for nonorgan-confined tumors, respectively, in the latter series (Hautmann et al. 2006). Accordingly, an overall local pelvic recurrence rate of only 9% was observed in the University of South California (USC) series reported by John Stein in 2001. Patients with organ-confined lymph node-negative tumors demonstrated a 6% local recurrence rate, compared with a 13% local recurrence rate in those with nonorgan-confined, lymph node-negative tumors (Stein et al. 2001).

In patients with lymph node-negative urothelial carcinoma, excellent survival data can be achieved as long as the tumor is confined to the bladder, best to the inner half of the detrusor muscle (Table 16.2). These data, based on a large number of patients from several studies, support an early aggressive surgical management at least for any muscle-invasive bladder cancer.

16.2.10 Oncological Outcome of Radical Cystectomy for Invasive Bladder Cancer and Prostatic Involvement

Involvement of the prostate by urothelial cancer of the bladder was first reported in 1952 by Melicow and Hollowell when they described carcinoma in situ of the prostate coexistent with bladder cancer (Melicow and Hollowell 1952). However, in the past, most authors did not discriminate between carcinoma in situ of the prostatic urethra, invasion of prostatic ducts or the prostatic stroma secondary to urethral or ductal basement membrane invasion or a primary extravesical extension of cancer into the prostate from outside. Pagano and associates first postulated two different pathways of prostatic involvement. Tumor extension from the urethra into the prostate was classified as noncontiguous invasion, whereas direct tumor extension from the bladder wall into the prostate was defined as contiguous extension. The overall 5-year survival rate of patients with pT4a disease in this

series was 21.5%. A subgroup analysis of patients who showed invasion of the prostate through the urethra revealed a significantly higher 5-year overall survival of 46% versus 7% in patients with a direct extension of cancer from the bladder origin into the prostate (Pagano et al. 1996). Esrig et al. analyzed 143 patients who had prostatic involvement by urothelial carcinoma in the cystectomy specimen and reported a 5-year overall survival rate of 74.3% for carcinoma in situ of the prostatic urethra versus 67% overall survival for tumor in prostatic ducts and an overall survival of only 35.8% for patients with stromal invasion of the prostate ($p<0.0001$). Prostatic involvement was further associated with the primary bladder tumor stage. Recurrence-free and overall survival rates for patients with prostatic stromal invasion but otherwise organ-confined disease were significantly higher compared to patients with prostatic stromal invasion and primary nonorgan-confined tumor (Esrig et al. 1996).

These studies have demonstrated that patients with organ-confined tumors and prostatic invasion arising in the urethra exhibit better survival rates than one would expect from real pT4a tumors with contiguous prostate involvement through the bladder wall. The first group of patients can achieve long-term survival rates comparable to those without prostatic involvement. Urothelial carcinoma limited to the mucosa or ducts of the prostate does not add worse prognostic impact to the primary bladder tumor stage and even stromal invasion does not confer itself a poor prognosis as long as the primary bladder tumor is organ confined and surgical margins are negative.

16.2.11 Oncological Outcome of Radical Cystectomy for Patients with Bladder Cancer and Lymph Node Involvement

Regional lymph node status has consistently been found to be one of the strongest predictors of survival. Cystectomy patients found to have positive pelvic lymph nodes at the time of lymph node dissection (PLND) have a poor prognosis, but considerable variation exists among the reported survival rates. Radical surgery in combination with a meticulous PLND (Fig. 16.2) may provide good long-term survival in some cases and patients most likely to benefit from radical surgery are those with favorable primary tumor stage and/or limited or microscopic lymph node involvement (See and Fuller 1992; Vieweg et al. 1994, 1999a, b).

Long-term survival rates of 25% and 21% for patients with positive nodes were reported at 5 and 10 years, respectively. Survival also appeared to be a function of the extent of local disease with a 5-year overall survival of 52% for organ-confined (pT0-pT3a, TNM 1998), and 17% for nonorgan-confined tumors (pT3b-pT4b, TNM 1998). Survival was inversely related to the extent of pelvic node involvement in this series. Among patients with a single positive node (pN1), 33% survived after 5 years, whereas only 22% with pN2 (2–5 lymph nodes involved) disease and no patient in the pN3 category (multiple nodes>3 cm) survived for 5 years (Vieweg et al. 1999b).

Table 16.2 Long-term oncological outcome cystectomy patients according to local tumors stage and lymph node status

Author	# Patients	Survival rate in organ-confined/pN0 tumors	Survival rate in nonorgan-confined/pN0 tumors	Survival rate in pN+tumors
Stein et al. (2001)	1,054	10 year recurrence-free survival	10 year recurrence-free survival	5/10 year recurrence free survival (246/1,054 pN+)
		P0 86%	P3a 76%	35%/34%
		Pis 89%	P3b 61%	
		Pa 74%	P4 45%	
		P1 78%		
		P2 87%		
Gschwend et al. (2002)	686	5/10 year disease-specific survival	5/10 year disease-specific survival	5/10 year disease specific survival (193/686 pN+)
		78.9%/72.9%	36.8%/33.3%	31.2%/27.7%
		5/10 year overall survival	5/10 year overall survival	5/10 year overall survival
		68.0%/49.1%	30.3%/22.8%	25%/20.9%
Madersbacher et al. (2003)	507	5 year recurrence-free survival	5 year recurrence-free survival	5 year recurrence free survival(121/507 pN+)
		73%	56%	33%
		5 year overall survival	5 year overall survival	
		62%	49%	
Lerner et al. (1993)	591	not reported	not reported	5/10 year progression free survival (132/591 pN+)
Hautmann (Hautmann et al. 2006)	788	5 year recurrence-free survival	5 year recurrence-free survival	5/10 year recurrence-free survival (142/788 pN+)
		pT2a 82.5%	pT3a 61.9%	20.9%/14.6%
		pT2b 61.9%	pT3b 53.1%	

According to the USC series, patients with lymph node-positive disease demonstrated significantly worse survival and higher recurrence rates compared to those with no lymph node involvement ($p<0.001$). The recurrence-free and overall survival for 246 patients (23%) with lymph node-positive disease at 5 and 10 years was 35% and 34%, and 31% and 23%, respectively. Survival rates in this group of patients with lymph node-positive disease could be stratified further by the primary bladder tumor (T-stage) and by the total number of lymph nodes involved. Patients with fewer than five positive lymph nodes had significantly higher survival rates than those with five or more lymph nodes involved ($p<0.003$). Similarly, patients with lymph node-positive disease and organ-confined primary bladder tumors had significantly higher survival rates than lymph node-positive patients with nonorgan-confined primary bladder tumors ($p < 0.004$) (Stein et al. 2001). Accordingly, from a Memorial Hospital (MSKCC) series of 688 patients ,10-year disease-specific and overall survival rates of 27.7% and 20.9%, respectively, in node-positive patients were reported (Gschwend et al. 2002).

Herr et al. analyzed the outcome of patients with grossly node-positive bladder cancer after pelvic lymph node dissection and radical cystectomy (Herr and Donat 2001). Included in this study were 83 patients treated with surgery alone (without neo-adjuvant or adjuvant chemotherapy), presenting N2-3 disease. Analyzing the outcome after 10 years, 20 patients (24%) survived and 64 (76%) died of disease. Thus, there is evidence that even a substantial number of patients with grossly node-positive bladder cancer may have a chance of cure with radical cystectomy through pelvic lymph node dissections. However, based on recent results of systemic therapy, neoadjuvant chemotherapy would be preferable in patients with clinically suspected lymph node involvement.

A variety of recent studies hypothesized a chance of long-term survival in patients with lymph node-positive disease. Table 16.2 summarizes results of five large studies of patients treated with radical cystectomy including subgroups of patients with nodal involvement. Data from these analyses highlight that patients with lymph node-positive disease may experience a 5-year recurrence-free survival of approximately 30% (20.9–35%) (Hautmann et al. 2006; Stein et al. 2001; Gschwend et al. 2002; Madersbacher et al. 2003; Lerner et al. 1993). However, in the surgery-only series reported by Hautmann et al. patients with positive lymph nodes had a rather poor prognosis compared to those without lymph node involvement. Of patients with positive lymph nodes as much as 85.4% progressed within 10 years and 68% of all recurrences were attributable to distant metastasis. In face of these poor recurrence-free survival rates in patients with node-positive diseases, the authors emphasized the importance of future randomized trials to test the true efficacy of systemic therapies in combination with radical cystectomy in such patient cohorts (Hautmann et al. 2006).

In summary, PLND and radical cystectomy appears to benefit a small but significant number of patients with node-positive bladder cancer and should be performed especially in cases where in the tumor is still organ confined. Based on the experience reported in the literature an overall cure rate of about 25% can be expected for such patients. Since PLND renders every fourth patient tumor-free, a planned

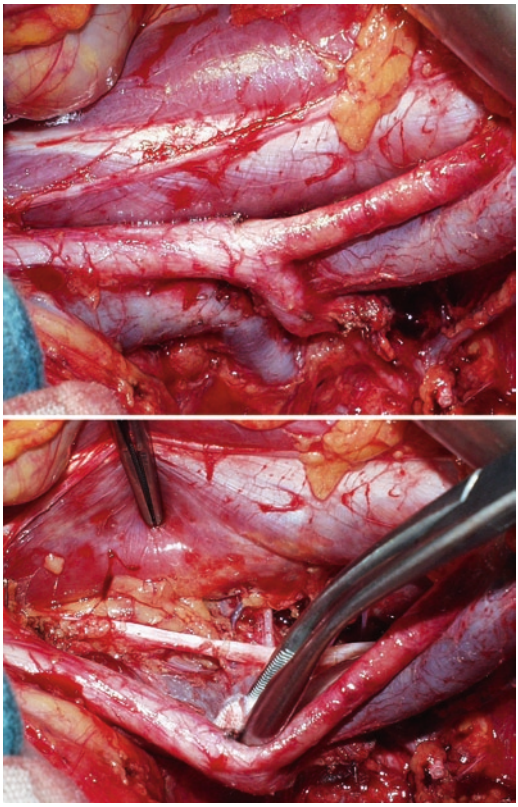

Fig. 16.2 Meticulous pelvic lymph node dissection along the common, external and internal iliac vas, including all lymph node tissue along the obturator nerve

cystectomy should not be abandoned in the face of microscopic lymph node metastases at frozen section.

16.2.12 Overall or Disease-Specific Survival as Endpoint of Outcome for Cystectomy patients?

The majority of older cystectomy series analyzing the outcome of bladder cancer patients have consistently used overall survival rather than disease-specific survival as the endpoint of analysis (Bassi et al. 1999; Lerner et al. 1993; Pagano et al. 1991; Freeman et al. 1995). In patient populations with advanced pathological tumor and nodal stage, the majority of deaths may indeed be attributed to distant metastasis and overall survival may serve as an appropriate surrogate endpoint of disease-specific survival. Moreover, studies using disease-specific survival as the primary endpoint may require higher numbers of patients to reveal statistically significant differences between groups, because patients dying of unrelated causes are censored at their date of death. However, it has been shown that overall survival

Table 16.3 Comparison of overall and disease-specific survival rates stratified according to organ-confined disease, nonorgan-confined disease, node-negative and node-positive disease. Declining statistical differences between overall and disease-specific survival were observed with increasing tumor burden (Gschwend et al. 2002)

Category	Interval (years)	Disease-specific survival	Overall survival
All patients	5	56.8% ± 2.09	47.9% ± 2.02
	10	52.2% ± 2.25	35.1% ± 2.21
Organ-confined tumors (≤T3a)	5	78.9% ± 2.51	68.0% ± 2.77
	10	72.9% ± 3.14	49.1% ± 3.59
Non-organ-confined tumors (>T3a)	5	36.8% ± 2.84	30.3% ± 2.56
	10	33.3% ± 2.86	22.8% ± 2.53
Negative lymph nodes (N0)	5	66.7% ± 2.34	57.0% ± 2.37
	10	61.7% ± 2.65	40.8% ± 2.74
Positive lymph nodes (N+)	5	31.2% ± 3.76	25.0% ± 3.30
	10	27.7% ± 3.73	20.9% ± 3.28

approximates disease-specific survival only if the proportion of cancer-unrelated death is small (Gschwend et al. 2002). As modern surgical technique improves survival, increasing differences between disease-specific and overall survival can be demonstrated proportionally with improved oncological outcome. This is reflected by profound differences when comparing the disease-specific and overall survival of patients with organ-confined and nonorgan-confined disease as well lymph node status. For example, the 10-year disease-specific and overall survival rate in organ-confined disease was quite different with 72.9% and 49.1%, respectively (Table 16.3). In concordance, in node-negative patients, the 10-year disease-specific and overall survival rates were 61.7% and 40.8%, respectively. These differences highlight the advantage of using disease-specific survival as the correct endpoint of outcome analysis for bladder cancer patients.

16.2.13 Palliative Versus Therapeutic Indication of Radical Cystectomy for Bladder Cancer

For patients with advanced bladder tumors (clinical stage T4b, invading the pelvic or abdominal wall), radical cystectomy is usually not a curative option (Ok et al. 2005). However, treatment of these patients does remain a clinical challenge, and surgery should always be considered on the basis of an individual decision. Urinary diversion using intestinal segment with or without cystectomy is a therapeutic but palliative option. Besides surgery, these patients are potential candidates for other treatment modalities, such as palliative chemo- or radiotherapy, repeated TUR, arterial embolization, or best supportive care. Inoperable locally advanced tumors may be accompanied by several debilitating symptoms, including repeated bleeding, chronic local pain, severe dysuria, and obstruction of the lower and upper urinary tract. Surgery in these patients is an invasive treatment and carries a substantial

risk of morbidity. It is usually performed for the relief of symptoms such as pain, recurrent bleeding, urgency, and fistula formation (Ubrig et al. 2004). A retrospective study analyzed older patients aged >75 years, who received radical cystectomy with either curative or palliative intent (Zebic et al. 2005). Analysis revealed that elderly people have a higher risk of perioperative morbidity and mortality, especially those with advanced pelvic malignancies who have undergone palliative cystectomy. The indications for palliative cystectomy were advanced pelvic malignancies with severe irritating voiding symptoms, pain, and recurrent hematuria requiring repeated blood transfusions.

Advanced muscle-invasive bladder cancer is often associated with ureteral obstruction at the level of the intramural section of the ureter or by enlarged pelvic lymph nodes. Bilateral ureteral obstruction, or unilateral obstruction with a solitary functioning kidney, may result in end stage renal insufficiency. El-Tabey et al. retrospectively reviewed the records of bladder cancer patients who presented with bladder cancer, hydronephrosis, and subsequent uremia. Patients with inoperable locally advanced bladder tumors (37.7%) were treated with permanent nephrostomy tubes to relieve obstruction. In 10 patients (26.3%) who underwent surgery, palliative cystectomy without lymphadenectomy was carried out for advanced nodal involvement in four patients and locally advanced disease infiltrating the pelvic wall in six patients. In all patients, local pelvic recurrence was reported within the first year of follow-up (El-Tabey et al. 2005). Another small study reported the postoperative outcome of primary radical cystectomy in T4 bladder cancer patients. The authors concluded that primary cystectomy for the treatment of T4 bladder cancer was technically feasible and had a very tolerable therapy-related morbidity and mortality (Nagele et al. 2007).

In another recent study, a bladder-preservation strategy was investigated in a total of 24 old patients with a mean age of 81 years (68–92) with muscle-invasive bladder cancer who had refused surgery or were not eligible for cystectomy. Patients were followed for a mean time of 680 days and all patients complained of frequency, urgency, and severe nocturia. The second most frequent complication was bleeding which required a salvage cystectomy in seven cases. The mean readmission rate was 8 per patient and the mean time spent at the hospital was 109 (13–253) days. The bladder-preserving strategy failed to be successful in most cases, and complications led to a salvage cystectomy in nearly half of the cases (Lodde et al. 2005).

In summary, palliative cystectomy will rarely extend overall survival, but can relieve severe symptoms such as recurrent bleeding from the tumor, urinary obstruction with renal insufficiency and may avoid repeated hospital admissions.

16.3 Conclusion

Risk-stratification of patients with bladder cancer based on pathological features at initial TUR or at the time of recurrence can select those most appropriate for radical cystectomy early. Immediate or early radical cystectomy should be offered

as treatment of choice to all patients with primary or recurrent tumors at high risk for progression (recurrent or multifocal high-grade T1 tumors, failures of BCG treatment, and muscle- invasive bladder cancer). Radical cystectomy does offer excellent recurrence-free and disease-specific survival rates as well as optimal local tumor control in patients with organ-confined disease. Tumor control in nonorgan-confined tumors is still satisfactory with long-term recurrence-free survival rates of about 50%. For node-positive disease, surgery may still be curative in approximately one-fourth of patients. For patients with inoperable locally advanced tumors, primary radical cystectomy is usually not a curative option. However, the indication for a palliative cystectomy or radical surgery in very old patients is, beside oncological outcome, also based on relief of symptoms in these patients.

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Chapter 17
Urinary Diversion

Richard E. Hautmann and Stefan H. Hautmann

Abstract The goal of urinary reconstruction has become not only to create a means to divert urine and protect the upper urinary tract, but also to provide patients with a continent means to store urine and allow for volitional voiding through the native urethra. These advances in urinary diversion have been made in an effort to give patients a normal lifestyle with a positive self-image following removal of the bladder.

During the past 25 years orthotopic reconstruction has evolved from "experimental surgery" to "standard of care at larger medical centres" and to the "preferred method of urinary diversion" in both sexes. The ileal conduit has remained the standard urinary diversion against which others have to be judged. During the last decade, use of the time-honored conduit has given way to the increasingly frequent use of orthotopic reconstruction.

17.1 Introduction

The goals of urinary diversion following radical cystectomy (RCX) have evolved from simple diversion and protection of the upper tract to functional anatomical restoration as close as possible to the natural preoperative state. This evolution of urinary diversion has developed along three distinct paths of:

- Incontinent, cutaneous diversion (conduit)
- Continent, cutaneous diversion (pouch) and
- Most recently, continent urinary diversion to the intact native urethra (neobladder, orthotopic reconstruction)

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During the last 25 years, orthotopic reconstruction has evolved from "experimental surgery" to "standard of care at larger medical centres" to the "preferred method of urinary diversion" in both sexes. The ileal conduit was described by Bricker (1950) and has remained a standard urinary diversion against which others are judged. During the last decade, the time-honored conduit has given way to the increasingly frequent use of orthotopic reconstruction (Hautmann et al. 2007).

The purpose of this article is not a comprehensive review of urinary diversion but rather a closer look at more recent and controversial developments of urinary diversion. For a standard overview the interested reader is referred to an article that resulted from a consensus conference convened by the World Health Association (WHO) and the Societé Internationale d'Urologie (SIU) that met to critically review reports of urinary diversion. The world literature on urinary diversion was identified through a Medline search. Evidence-based recommendations for urinary diversion were prepared with reference to a four-point scale. Many level 3 and 4 citations, but very few level 2 and no level 1, were noted. This outcome supported the clinical practice pattern. Findings of >300 reviewed citations are summarized. Published reports on urinary diversion rely heavily on expert opinion and single-institution retrospective case series (Hautmann et al. 2007).

17.2 Historical Aspects of Urinary Diversion

The last century undoubtably was the century of urinary diversion. Amazingly urinary diversion began as a tempered continent diversion:

The ureterosigmoidostomy was first published by J. Simon in 1852. Coffey's (1911) modification of the direct ureterocolic anastomosis by constructing a non-refluxing submucosal tunnel tempered the consequences caused by reflux of fecal material. His basic experiments were fundamental for understanding the mode of action of antireflux procedures used today.

At the beginning of this century, the ileocecal segment was used by several surgeons as a continent reservoir. The appendix served as an outlet valve, but continence was compromised by the persisting peristaltic waves in the excluded bowel segment.

Goodwin's cup-patched technique was published in 1959 and turned out to be another landmark in continent diversion (Goodwin et al. 1959). His reservoir was made from a doublefolded ileal segment with a transected antimesenteric border. Because of asynchronous intestinal contractions and the largest possible diameter together with an optimal capacity, a low pressure reservoir was created which, at the same time, had a minimal reabsorbing intestinal surface. This may be of particular importance for good long-term results with continent diversions.

At that time the only alternative was the ileal conduit which was a noncontinent form of supravesical urinary drainage. It was described first by Shoemaker in 1911. The technique was propagated by Seiffert in 1935 and worldwide in the 1950s by Bricker.

Retrospectively, many disappointing results of continent supravesical urinary diversion were not caused by incompetence of the outlet valve, but rather because the reservoir maintained its peristaltic activity resulting in high pressure peaks. The advance in continence, and thus, an improvement in the patient's comfort, was achieved through the combination of the continent stoma and a low pressure reservoir (Studer et al. 1991).

Anastomosing an intestinal reservoir to the urethra instead of using a continent outlet mechanism was proposed by Tizzoni and Foggi (1888). They replaced the native bladder by an ileal segment, which was interposed between ureters and urethra. In 1951, Couvelaire reactivated the idea of an ileal bladder substitute, and actually various forms of intestinal low pressure reservoirs are used as bladder substitutes (Couvelaire 1951).

17.3 Current Situation of Urinary Diversion

17.3.1 Quality of Life

The published literature on quality of life (QOL) after RCX is extensive. However, the scientific quality is low, and flaws in patient selection and evaluation methods are common. No randomized controlled study has been performed. Such a study is desirable but probably difficult to perform. Published evidence does not support an advantage of one type of reconstruction over the others with regard to QOL. An important reason is probably that patients are subjected preoperatively to method-to-patient matching and thus are prepared for disadvantages and advantages associated with different methods as well as the use of totally inappropriate instruments (Hautmann et al. 2007).

17.3.2 Quality of Surgery

RCX quality and PLND extent have a major impact on bladder cancer survival. Who performs the surgery, and where and how well it is done matter. Recent studies show the mortality from RCX is higher at low versus high volume hospitals (3.1% vs 0.7%) (Herr et al. 2007).

Experienced surgeons who frequently perform cystectomy achieve better survival and fewer complications than surgeons who perform an occasional RCX (Elting et al. 2005). Although acceptable standards for PLND are not yet defined, patients who undergo complete bilateral PLND with dissection of the common iliac, external iliac, obturator, and hypogastric nodes have better survival than patients with limited (obturator nodes) or omitted lymp node dissection. An increased number of lymph nodes removed improves survival and decreases pelvic

recurrence in patients with node negative and node positive disease (Herr et al. 2007). Although node counts vary widely due to other uncontrolled factors, multivariate analysis of a prospective study showed that only the surgical extent of node dissection (limited vs standard or extended) was associated with significant differences in node yields (Herr et al. 2007).

Even more compelling evidence of the importance of surgical quality was provided by an analysis of INT-0080 in the USA involving multiple institutions and surgeons (Herr et al. 2007). Negative surgical margins and ten or more lymph nodes removed were associated with better overall survival independent of patient age, pathological stage, nodal status and whether chemotherapy was given. These surgical factors also predicted pelvic relapse, which is a death knell in most patients. Of these patients 15% had local recurrence and all eventually died of disease. Local recurrence developed in 68% of cases with positive surgical margins compared to 6% with negative margins, while high versus low volume urologists had a positive margin rate of 4% versus 14%.

Despite chemotherapy, patients with the best overall 5-year survival received neoadjuvant MVAC, followed by quality RCX and complete PLND, compared to patients who underwent radical cystectomy an inadequate or no PLND (52% vs 34%, $p=0.001$).

The cooperative group trial shows that the quality of RCX and PLND directly impact the chances of survival and it is surgeon dependent (Herr et al. 2007).

17.3.3 Trends in Reconstruction After RCX

Many bladder cancer patients in the USA undergoing RCX are candidates for either neobladder or ileal conduit. Indications for neobladder creation have expanded since 1990, particularly in women. Saigal et al. (2005) sought to identify changes in types of reconstruction among Medicare beneficiaries in response to evolving best practices.

The authors assembled a data set using Medicare claims from 1992, 1995, 1998, and 2001. Claims with a diagnosis code for bladder cancer were searched for procedure codes for neobladder and ileal conduit creation. Data were stratified by age, sex, and region. Data were examined for each year and for all years combined.

During the 4 years examined, 19,620 ileal conduit and 2,980 neobladder procedures were performed. Age-adjusted rates of ileal conduit use did not change significantly over this period, ranging from 1,958 to 2,112 per 100,000 beneficiaries with bladder cancer. Age-adjusted rates of neobladder creation did not significantly increase over this period, ranging from 274 to 407 per 100,000.

Use of neobladder was similar in males and females. The age-adjusted rate of neobladder reconstruction was higher in the West and Midwest than the Northeast.

During this period, rates of neobladder reconstruction were similar in males and females, indicating that evidence regarding effectiveness of neobladders in women has been widely accepted. Regional variation in rates of use of this type of

reconstruction may be related to physician practice patterns or patient references. Although neobladder reconstruction confers superior quality of life for bladder cancer patients, use of neobladder reconstruction has not increased since 1992 in the Medicare population (Saigal et al. 2005).

17.3.4 Circumstances Under Which Urinary Diversion Is Performed

At oncologic centers, the likelihood of receiving an orthotopic reservoir is much higher. The frequency distribution of urinary diversions performed by the authors of the WHO/SIU consensus report in >7,000 patients with cystectomy reflects the current status of urinary diversion after cystectomy for bladder cancer: neobladder, 47%; conduit, 33%; anal diversion, 10%; continent cutaneous diversion, 8%; incontinent cutaneous diversion, 2%, and others, 0,1% (Hautmann et al. 2007) (Table 17.1).

However, we have to acknowledge that geographic, social, ethnic, and religious factors come into play. The health care system, as well as the referral pattern plays a major role. In the USA, the likelihood of receiving a neobladder in the medicare population is <15%. The corresponding figure for Germany is ≥ 30%. This is explained by the referral pattern. The approximately 3,000 urologists in Germany in private praxis do not perform major surgery and refer their RCX to hospitals that exclusively do surgery, that is, this is a system with a high number of high volume hospitals and surgeons.

Table 17.1 Urinary diversions performed by the authors (Hautmann et al. 2007)

	No. of Cystectomies	Period	Neo-bladder	Continent Cutaneous Pouch	Conduit	UC/ TUUC	Anal	Others
Ann Arbor, MI	643	02/1995-09/2004	45.1%	1.4%	53.5%	0.0%	0.0%	0.0%
Bern	327	01/1999-09/2004	54.0%	3.0%	37.0%	0.0%	3.0%	NA
Dallas, TX	228	01/1999-09/2004	30.0%	6.0%	64.0%	0.0%	0.0%	0.0%
Kobe, Japan	87	02/1989-09/2004	46.0%	2.3%	10.3%	41.4%	0.0%	0.0%
Los Angeles	1359	08/1971-12/2001	51.6%	25.8%	22.3%	0.0%	0.0%	0.3%
Lund, Schweden	119	01/2000-09/2004	28.6%	31.1%	40.3%	0.0%	0.0%	0.0%
Mansoura, Egypt	3157	01/1980-01/2004	39.1%	3.5%	34.4%	0.0%	23.1%	0.0%
Ulm, Germany	1209	01/1986-09/2004	66.2%	0.5%	22.6%	8.9%	1.5%	0.4%
Total	7129		46.9%	7.6%	32.7%	2.0%	10.6%	0.1%

NA = not available; UC/TUUC = cutaneous ureterostomy/transureterostomy

17.3.5 Correlation Between Volume of RCX and Outcomes

The correlation between high volume and lower preoperative mortality is well established among cancer patients who undergo cystectomy. The association between volume and preoperative complications has not been studied to date and hospital characteristics contributions to the volume-outcome correlation are unknown. In a recent study, the authors studied these associations, emphasizing hospital factors that contribute to the volume-outcome correlation (Elting et al. 2005).

Treatment at high-volume hospital appears to confer a modest, but statistically significant, survival benefit for patients who undergo cystectomy for bladder carcinoma. Referral to a hospital performing more than ten cystectomies annually is indicated for patients who have access to high-volume centers, which also may provide long-term survival benefits from the multidisciplinary planning and treatment available at such centers. Among patients who do not have access to high-volume hospitals, treatment in a local hospital with a high nurse-to-patient ratio may confer a similar benefit. However, further study is required to confirm these findings.

17.3.6 Indications, Contraindications, and Patient Selection

17.3.6.1 Substantial Change in Paradigm

The goal of patient counselling about urinary diversion should be to determine the method that is the safest for cancer control, has the fewest complications in both the short- and long-term, and provides the easiest adjustment for the patient's lifestyle, supporting the best quality of life. The paradigm for choosing a urinary diversion has changed substantially. Now all cystectomy patients are candidates for a neobladder, and we should identify patients in whom orthotopic reconstruction may be less ideal.

The proportion of cystectomy patients receiving a neobladder has increased at medical centers to 50–90% (Hautmann et al. 2007).

17.3.6.2 Patient Selection Criteria: Absolute and Relative Contraindications

An absolute contraindication to continent diversion of any type is compromised renal function as a result of long-standing obstruction or chronic renal failure, with serum creatinine above 150–200 micro mol/L. Severe hepatic dysfunction is also a contraindication to continent diversion. Patients with compromised intestinal function, particularly inflammatory bowel disease, may be better served by an incontinent bowel conduit. Orthotopic reconstruction is also absolutely contraindicated in all patients in whom simultaneous urethrectomy is indicated based on their primary tumor. The role of relative contraindications and comorbidities is steadily decreasing. However, some of them, such as mental impairment, external sphincter dysfunction, or recurrent urethral strictures, deserve serious consideration.

Paramount to success of anal sphincter-controlled bladder substitutes is an adequate anal sphincter mechanism. Inability of the patient to retain 400–500 mL in the upright position for 1 h is a contraindication (Hautmann et al. 2007). Patients with neurogenic bladder are not suitable candidates either, as there may be associated anal sphincter dysfunction (Hautmann et al. 2007).

Urologists should first consider permanent urinary diversion for patients who undergo total cystectomy due to invasive bladder cancer. In fact, less attention has been directed to palliative urinary diversions in the treatment of bladder cancer. However, mainly in two situations, this type of urinary diversion has significant meaning for patients with bladder cancer. At first, patients who cannot have total cystectomy because of advanced stage or poor general condition sometimes require urinary diversion due to uncontrollable symptoms (such as hematuria or pain) or uremia. Secondly, patients undergoing total cystectomy but not urinary diversion using intestinal segment are candidates for palliative urinary diversion. Patients with severe bowel adhesion or disease, or patients who need short and less invasive surgery due to medical conditions need palliative urinary diversion.

17.3.7 Patient Selection

17.3.7.1 Patient Factors: For (Pros)

The primary patient factor is the "patient's desire for a neobladder." The patient needs a certain motivation to tolerate the initial and sometimes lasting inconvenience of nocturnal incontinence associated with a neobladder. Most patients readily accept some degree of nocturnal incontinence for the benefit of avoiding an external stoma and pouch, but not all patients do, and realistic expectations of the functional outcome are essential for both the surgeon and the patient (Hautmann 2003).

The psychologically damaging stigma to the patient who enters surgery expecting a neobladder but awakens with a stoma plays an increasing role. It should always be remembered that in many parts of the world, a bag may either be socially unacceptable or economically unrealistic as a long-term solution. The pressures drive the urologist toward some form of continent urinary diversion, and, although rectal pouches have been used widely as alternatives to conduits, continent catheterizable reservoirs or orthotopic bladder substitutes in particular represent attractive options (Hautmann 2003).

17.3.7.2 Patient Factors: Against (Cons)

There are still patients who are better served with a conduit. Patient factors against a neobladder are:

1. If the patient's main motivation is to "get out of the hospital as soon as possible" and resume normal, rather sedentary activities. Many frail patients undergoing cystectomy will have less disruption of normal activities with a well-functioning conduit than an orthotopic reservoir associated with less than ideal continence.

2. The “little old lady” living in social isolation.
3. No concern about body image. Most elder patients do not have the same cosmetic concerns that a younger patient might have, and their main goal is returning to their previous life style, which is often quite sedentary (Hautmann et al. 2007).

17.3.7.3 Patient Selection Criteria: Oncologic Factors

Following cystectomy, the rhabdosphincter must remain intact. Nevertheless, the cancer operation must not be compromised. This concern applies to two aspects of selection: urethral tumor recurrence in men and the use of orthotopic replacement in women.

1. One of the initial deterrents to orthotopic diversion is the risk for urethral recurrence of cancer. See below.
2. Orthotopic bladder substitution for women with invasive bladder cancer has been popularized recently (Hautmann et al. 2007). For oncologic justification see below.

Increasing experience with orthotopic reconstruction has fostered fewer restrictions for patient selection based on tumor stage. Should extensive pelvic disease, a palpable mass, or positive but resectable lymph nodes preclude a neobladder because of the high propensity for a pelvic recurrence or distant relapse? There is no convincing evidence that a patient with an orthotopic diversion tolerates adjuvant chemotherapy less well or that a pelvic recurrence is any more difficult to manage with a neobladder than after an ileal conduit. Patients can anticipate normal neobladder function until the time of death (Hautmann et al. 2007).

However, adjuvant chemotherapy may substantially weaken the patient and prolong the time for neobladder maturation. Nevertheless, our philosophy respects the patient’s desire for a neobladder; if the patient is strongly motivated, he or she gets a neobladder. Even though the patient has a poor prognosis and relapse is likely to occur, we still try to construct the diversion they want. Previous radiation therapy, especially with an advanced cancer, usually mitigates against an orthotopic diversion but does not absolutely preclude it. However, all patients should be informed that diversion to the skin either by a continent reservoir or ileal conduit may be necessary due to unexpected tumor extent, and an appropriate stoma site should be marked on the abdominal wall beforehand.

17.3.7.4 Current Practice

Despite the fact that orthotopic bladder replacement provides the ideal method of urinary diversion after cystectomy, many patients treated outside of centers that are dedicated to neobladder reconstruction receive an ileal conduit. Why? These patients often have adverse clinical factors such as increased age, more comorbidities, and more previous cancer therapy, including patients with previously deemed unresectable cancers undergoing desperation cystectomy or after failed combined radiation

therapy and chemotherapy regimens (Hautmann et al. 2007). Thus, despite a strong desire to offer orthotopic diversion whenever possible, some patients do not qualify on the basis of current clinical judgement. An ileal conduit remains an expedient, safe, and appropriate method of diversion in these patients. Many factors go into the decision to perform a urinary diversion and must be kept paramount in discussing the pros and cons of each method with the patient and his or her family.

17.4 General Aspects of Urinary Diversion

17.4.1 Which Gut Segment Should Be Used

(a) Biological consequences of exposing gut mucosa to urine.

Intestinal segments vary in handling of solutes. Length of bowel segment, surface area, duration of urinary exposure, solute concentration, pH, renal function, and urine osmolality all play a role. The reservoir surface is exceedingly difficult to estimate. There is no difference between ileal and colonic mucosa in regard to sodium absorbing capacity. However, in the colon, chloride absorption and bicarbonate excretion are more pronounced, and there is increasing evidence to suggest that inherent chloride absorption is maintained when in contact with urine (Hautmann 2003). Therefore, it may preferable to use ileum rather than colon for bladder reconstruction to reduce the risk of hyperchloremic acidosis, particularly in the presence of renal impairment. There are clear differences between ileum und colon in regard to metabolic consequences, but this is only one consideration when planning orthotopic reconstruction. However, due to the reduced absorption of electrolytes in ileal urinary reservoirs, it seems that ileum is preferable to large bowel for storing urine, at least in patients with decreased kidney function and increased risk for metabolic disorders (Hautmann et al. 2007). An obvious advantage of the sigmoid reservoir is its ease of accessibility. However, there is the substantial disadvantage of high reservoir pressures as compared to cecum or ileum that is confirmed by most urodynamic studies (Hautmann 2003). We recommend using a sigmoid reservoir only in cases in which ileum or right colon is not available (Hautmann et al. 2007). An advantage of the ileocolonic reservoir is its greatest initial volume as a reservoir. However, it requires mobilization of the entire right colon and is potentially the most tedious procedure to perform. The greatest disadvantage of the procedure is the loss of the ileocecal valve. There is also a greater risk of vitamin B 12 deficiency secondary to resection of the terminal ileum.

Most investigators have reported on one single type of diversion. Santucci et al. (1999) performed six different continent urinary reservoir operations and revealed remarkably different continence rates and urodynamic data. Their experience suggests that neobladders composed of stomach or sigmoid should be used only under unusual circumstances because of the high rates of incontinence. Of course, other patient and surgeon issues might supersede these guidelines. Surgeon preference, length of surgery,

ease of construction, potential need for revision, differences in body image, and other patient characteristics are among the many factors that must be considered when choosing which type of orthotopic reconstruction to provide each individual patient.

17.4.2 Difficulty of Operative Technique

Open RCX has been assessed the highest values in terms of difficulty of surgery for any procedure in urology. This is also true for laparoscopic/robotic RCX, which is the most difficult robotic procedure and more so, if the diversion is performed totally intracorporeally.

17.4.3 No Standards for Surgical Complication Reporting

Significant disparity in the quality of surgical complication reporting and the lack of universally accepted reporting guidelines, definitions, and grading systems have made it impossible to compare surgical morbidity and outcomes in urologic oncology patients. Improved surgical outcome reporting will allow more meaningful comparisons between standard open and minimally invasive surgical techniques, when randomized trials for comparison are sparse. Standard guidelines for accruing and reporting surgical morbidity data, defining procedure-specific complications, and severity grading need to be established, perhaps through an American Urological Association panel for guidelines with the participation of members of the Society of Urologic Oncology. In the interim, investigators reporting surgical outcomes should strongly consider incorporating the ten established critical surgical reporting criteria, as outlined by Martin et al. (2002). The quality of surgical morbidity and outcomes reporting with urologic oncology procedures is disparate, and it is now time for a change (Donat 2007).

17.4.4 Early Complications of Radical Cystectomy and Urinary Diversion

Muscle invasive bladder cancer occurs predominantly in an elderly comorbid population with a mean age of 65 years in contemporary RCX series (Hautmann et al. 2006a, b). The incidence of "early complications" defined as occurring either during the hospitalization or within 30 days of surgery, has been reported in the range of 20–57% (Sabsigh et al. 2009). Therefore, accounting for the impact of surgical morbidity on patient outcome is essential for treatment planning, for clinical trial design, for assessing new surgical techniques, and for perioperative patient education.

A recent evaluation of the urologic oncology literature revealed that the majority of series that reported on RC morbidity had not employed a formal complication reporting system, had not utilized grading systems other than categorizing them into "major versus minor," had not accounted for comorbidities, or had not defined complications. This makes it difficult to compare data and most certainly leads to an underestimation of morbidity for the procedure (Sabsigh et al. 2009). In addition, the incidence of perioperative complications have often been utilized as surrogate measures of surgical competency, institutional quality of care, success of new surgical techniques, and have been suggested as benchmarks for financial reimbursement (Sabsigh et al. 2009).

Between 01/86 and 09/08, the Department of Urology at the University of Ulm, Germany performed 1,013 RCXs with orthotopic reconstruction. All complications within 90 days of surgery were defined, categorized, and classified by an established 5-grade modification of the original Clavien system utilized at MSKCC (Table 17.2): grade 1: oral medication/bedside care; grade 2: i.v. therapy, hyperalimentation, enteral feedings, or transfusions; grade 3: intubation, interventional radiology, or reoperative intervention; grade 4: organ resection or chronic disability; grade 5: death. Only 42.4% of the patients had no early complications. The overall rates were grade 1:11.1%; grade 2:25.3%; grade 3:16.7%; grade 4:2.3%; and grade 5:2.3% (Table 17.2).

RCX and ileal neobladder formation presents major surgery with potential relevant early complications even in the most experienced hands. But the rate of severe and lethal complications is acceptably low.

At MSKCC 64% (735/1,142) of patients experienced a complication within 90 days of surgery. Among patients experiencing a complication, 67% experienced a complication during the operative hospital admission and 58% following discharge. Overall, the highest grade of complication was grade 0 in 36% ($n=407$), grade 1–2 in 51% ($n=582$), and grade 3–5 in 13% ($n=153$). Gastrointestinal complications were most common (29%), followed by infectious complications (25%) and wound-related complications (15%). The 30 days mortality rate was 1.5%.

Our own data are presented for comparison in Table 17.3.

The high rate of postoperative morbidity following RCX when a standard reporting methodology is utilized and the value of considering reporting methodology when comparing series becomes obvious. Furthermore, this study confirms prior

Table 17.2 Postoperative complications grading system

Grade	Definition
Grade 0	No event observed
Grade 1	Use of oral medications or bedside intervention
Grade 2	Use of intravenious medications, total parenteral nutrition (TPN), enteral nutrition, or blood infusion
Grade 3	Interventional radiology, therapeutic endoscopy, intubation, angiography, or operation
Grade 4	Residual an lasting disability requiring major rehabilitation or organ resection
Grade 5	Death of patient

Table 17.3 Grading of early complications according the modified Clavien system at the MSKCC (Volkmer et al. 2009)

	0	1	2	3	4	5
Gastrointestinal	85.0%	0.7%	8.1%	3.9%	1.8%	0.5%
Infection	75.4%	3.8%	15.6%	3.6%	0.4%	1.3%
Wound	93.5%	1.9%	1.1%	3.5%	0.1%	0.0%
Cardiac	97.6%	1.2%	0.9%	0.1%	0.0%	0.2%
Genitourinary	83.8%	3.5%	7.1%	5.6%	0.0%	0.0%
Pulmonary	95.3%	0.3%	1.8%	1.7%	0.2%	0.8%
Bleeding	98.1%	0.1%	0.2%	1.5%	0.1%	0.0%
Thromboembolic	97.2%	0.7%	1.4%	0.2%	0.0%	0.5%
Neurologic	97.5%	1.9%	0.5%	0.0%	0.0%	0.1%
Miscellaneous	90.5%	3.0%	1.6%	4.0%	0.0%	0.0%
Surgical	96.7%	2.1%	0.5%	0.6%	0.0%	0.0%
Total	42.4%	11.1%	25.3%	16.7%	2.3%	2.3%

observations that have indicated the significant impact patient comorbidity may have on surgical outcomes such as length of stay, emphasizing the importance of taking this into account when assessing new surgical techniques. Accurate reporting of complications is essential for preoperative counselling, for identifying modifiable risk factors to reduce complication rates, for planning combined modality treatments, for clinical trial design, and for a more accurate assessment of surgical success. It is our hope that our experience will encourage others to begin utilizing the ten reporting criteria and methodology described in this report when publishing their results, so future meaningful comparisons among retrospective series may be possible in lieu of randomized trials.

17.4.5 Neobladder Specific Complications

17.4.5.1 Mucus Production

Bowel mucosa secretes mucus made up of a glycoprotein core of long sequence amino acids with a molecular weight of 2–20 million Daltons with side chains of monosaccharides wrapped around the protein core (Hautmann et al. 2007). The glycoprotein core is made by the rough endoplasmic reticulum of goblet cells. In solution, the glycoprotein becomes hydrated and viscous. In the early postoperative period, the indwelling catheters must be carefully irrigated to prevent initial mucus build-up within the diversion. Patients with good spontaneous voiding and complete emptying usually pass the mucus spontaneously in the urine. In contrast, patients with incomplete emptying or those performing CIC may need to irrigate to remove retained mucus. Mucus accumulation may occur in some neobladder patients with apparently normal voiding. Some investigators report that an increase in mucus production may be an early sign of urinary infection and irritation in the

diversion. Early or late mucus retention has been reported in 0.58–2% and in 3% of patients, respectively (Hautmann et al. 2007). *N*-acetylcysteine and urea are effective mucolytic agents. *N*-acetylcysteine is a water-soluble thiol that reduces the disulfide bonds in the mucus. In contrast, carbocysteine causes mucus precipitation rather than dissolution. Irrigation of urinary diversions with *N*-acetylcysteine at smaller volumes and higher concentrations (30 mL, 20%) also has been found to be effective.

17.4.5.2 Chronic Bacteriuria

The reported prevalence of chronic bacteriuria in patients with neobladders is variable from less than 12–79% (Hautmann et al. 2007).

The prevalence of bacteriuria is fairly steady over time as demonstrated by the findings of Steven and Poulsen that the rate of bacteriuria at 1 year, 3 years, and 5 years in patients with ileal neobladders was 26.0%, 34.3%, and 24.2%, respectively (Steven and Poulsen 2000).

Wood et al. (2003) reported positive urine cultures in only 50% of patients despite bacteriuria in 78%. The same authors reported the incidences of clinical urinary tract infection or urosepsis of only 39% and 12%, respectively. This supports the view that bacteriuria in most neobladder patients is asymptomatic and represents a colonization rather than a clinical infection, provided there is good emptying.

17.4.5.3 Rupture

Multiple cases of spontaneous rupture of continent urinary diversions have been reported (Hautmann 2003). Recurrent episodes of pouch rupture in the same patient have also been reported. The most common cause is acute or chronic overdistension of the diversion. Patients must be instructed to void every 3–4 h even if they can maintain continence for longer intervals and do not feel pressure or a need to void. This is especially important in long-term follow-up as the pouch capacity may enlarge and the efficiency of voiding by Valsalva straining may decrease. Other causes include catheter trauma, mucus retention, and altered sensorium from alcohol intoxication. Neobladder patients should be instructed in the technique of CIC in case an episode of mucus plugging and retention occurs when they do not have access to medical personnel who are familiar with continent urinary diversion. Some authors advocate that continent diversion patients should wear a medical alert bracelet. The reported rates of spontaneous pouch rupture are 1.5–4.3%. Pouch rupture should be considered in all patients with continent urinary diversion who present with acute abdominal pain. Catheterization to check for urinary retention and hematuria suggestive of pouch trauma should be performed initially while hematologic studies are pending. Pouchogram by standard radiography or referably by computed tomography (CT) should be obtained.

Radiographic pouchogram frequently fails to show extravasation in cases of proven rupture. In contrast, free intra-abdominal fluid (urine) usually will be detected by CT even if ongoing extravasation is not shown during the infusion CT cystogram. The overall clinical features of the patient dictate the necessary treatment. Patients who are hemodynamically stable, have uninfected urine, and lack signs of acute peritonitis may be treated with an indwelling catheter, broad spectrum antibiotics, and close observation. Patients with overt sepsis, uncontrollable pain, or a rigid abdomen require surgical exploration and repair. Delayed diagnosis may lead to life-threatening infection with necrosis of the pouch and Fournier's type gangrene of the abdominal wall.

17.4.5.4 Renal Function

While there is rich literature on urinary diversion, few publications give data on renal function. The majority of reports are case series with a low level of evidence. The literature does not support benefits of one type of ureteric implantation over another. Prospective randomized studies are needed to clarify this issue. Renal function decreases at long-term follow-up, but this is at least partly due to normal aging. There is no evidence to suggest that patients with continent reconstruction do less well than conduit patients with regard to renal function.

17.5 How to Obtain Good Results with Orthotopic Bladder Substitution: The Ten Commandments

The ileal neobladder has not only withstood the test of time, but is increasingly becoming a more desirable method of urinary diversion (Hautmann et al. 2009). Complementing orthotopic neobladder features with adequate training allows patients to return to a close to if not normal urinary routine. Ten points must, however, carefully be followed. Three surgeons (Hautmann et al. 2009) from institutions that pioneered orthotopic reconstruction during the last 25 years, with a high surgical volume of radical cystectomy (RCX) and any form of urinary diversion, in particular orthotopic reconstruction present their experience and long-term follow-up of orthotopic reconstruction.

The ten commandments:

I. High volume surgeon
II. Do not over extent the indication
III. You must have experience with nerve sparing RPX and bowel surgery
IV. Use ileum whenever possible
V. Maximum detubularization a must
VI. Use a stented freely refluxive ileo ureterostomy
VII. The low pressure, compliant, freely refluxive reservoir is standard
VIII. Be aware of myriad of potential complications

IX. Full armamentarium of diversion techniques must be available
X. Meticulous follow-up must be guaranteed

For a detailed description of these commandments the interested reader is referred to the original publication (Hautmann et al. 2009). In the context of this chapter, three commandments deserve special mentioning:

17.5.1 Commandment IV: Use Ileum Whenever Possible

A comparison of gastric, ileal, ileocolic, right colic, and sigmoid segments discloses the following:

17.5.2, 17.5.3 Commandment V: Maximum detubularization is a must

Advantage of ileum over any other segment: function/urodynamics, transformation after exposure to urine, change in absorptive capacity, adaption of mucosa absorptive to storage, incidence of metabolic disorders, late volume increase, capacity at first and maximum contraction, involuntary contractions, motor activity, and distensibility, suitable for patients with decreased kidney function (Hautmann 2003).

Advantage of ileocolon, sigmoid: initial volume, accessibility and anastomosis, save ileocecal valve, lack of vitamin B 12 deficit (Hautmann 2003).

Comparison of gastric, ileal, ileocolic, right colic, and sigmoid segments:

- Continence rates and urodynamic data remarkably different: ileal > ileocolic > sigmoid > gastric
- Clear differences in regard to metabolic consequences: ileal > gastric > colonic
- Patient and surgeon issues: surgeon's preference, length and ease of surgery, body image, lowest possible complication rate

17.5.4 Commandment VI: Use a Stented Freely Refluxive Ileo Ureterostomy

Conventional wisdom suggests the need for an antireflux mechanism, as reflux nephropathy can develop with high-pressure neuropathic bladders. However, reflux prevention in a low pressure detubularized orthotopic reservoir might not be as beneficial as anticipated. Some reasons are:

- With detubularized bowel segments and the absence of any coordinated contractions, no appreciable pressure is generated.
- Increase in intra-abdominal pressure results in identical pressure rises in both the neobladder and ureters, allowing no reflux.
- The pressure in the reservoir cannot be higher than the peristaltic force of the ureters and afferent tubular segments.
- The afferent tubular segment itself has certain dynamic antireflux properties due to its coordinated peristalsis (Studer et al. 1996a).
- With a major pressure peak within the reservoir that exceeds the urethral closure pressure, the external sphincter generally acts as a safety valve allowing urinary leakage thus preventing reflux.
- Urine usually remains sterile within the closed system of orthotopic substitutes and therefore nephropathy is less likely.
- All published antireflux techniques have a higher anastomotic stricture and eventual renal damage rate than the Nesbit technique (direct ureterointestinal anastomosis).

17.5.5 Commandment VII: The Low Pressure, Compliant Freely Refluxive Reservoir Is Standard

First of all, it is mandatory to avoid too long ileal or colonic segment because they lead to too large neobladder volume and thus overdistension. The larger the bladder volume, higher the rate of post-voiding residual urine and thus reflux; higher the rate of leakage, higher the risk of metabolic disorders (Studer et al. 1996b). No more than 45 cm of ileum seems safe to avoid metabolic disorders. The rate of bacteriuria is also increased with large bladder and residual post-voiding urine. 450 mL for the bladder has been advocated as an optimal volume (Studer et al. 1996a). Whatever the detubularized technique used, of great importance are the training and the life-long follow-up. Training is necessary to teach and explain the patient how to be continent; how to void the bladder; and how to avoid overdistension. The life-long follow-up, apart from oncologic surveillance, control these data regularly and if needed, corrects it; and remember, confidence does not exclude surveillance.

Uretero-ileal anastomosis: The pressure in the lower part of ureter is 20–30 cm of water (Studer et al. 1996b). The bladder-end filling pressure is 20 cm for an optimal cytometric capacity of 450 cm^3. This pressure difference is a safety margin. During the filling phase of bladder, absence of coordinated contractions guarantees a low pressure reservoir. During voiding the use of Valsalva maneuver increases the pressure in the bladder, abdomen, and renal pelvis at the same time, keeping the safety margin. Thus a direct ureteral neobladder anastomosis can be performed, provided the patient is well trained and controlled (Studer et al. 1996b). Even if there is a reflux during voiding, it is only transient and grade 1 without renal damage. But in case of overdistension of the bladder, whatever the reasons, reflux will occur.

17.5.6 Commandment X: Meticulous Follow-up

Critical components for good long-term results require not only surgical finesse but also patient compliance and meticulous postoperative care (Hautmann et al. 2009).

17.5.6.1 Management Immediately Postoperatively:

- S.c. heparin prophylaxis into the arm instead of thigh to prevent lymphoceles
- Bladder substitute rinsed 6 hourly, aspiration of mucus
- Bowel stimulation: parasympathomimetics from day 2 or 3 on
- Withdrawal of urethral stents days 5–7, after resumed bowel activity
- First removal of the suprapubic tube on day 8–10 (cystogram)
- Withdrawal of urethral catheter on day 10–12

17.5.6.2 Management After Catheter Withdrawal

Patients are carefully instructed on how to void. Initially, they are taught to empty the neobladder every 2 h during daytime in a sitting position by relaxing the pelvic floor and increasing the abdominal pressure. The following points must be observed:

- Voiding without residual urine
- Sterile urine
- Alarm clock at night
- Venous blood gas analysis every second day
- Supplement of bicarbonate (2–6 g) and salt
- Increase fluid intake, check body weight
- Increase reservoir capacity by adhering to regular voiding intervals: first 2 h, thereafter 3, later 4 h. Aim: capacity of 500 mL

17.5.6.3 Meticulous Long-Term Follow-Up Is Essential

- Metabolism (Vitamin B 12, electrolytes, base-excess)
- Continent
- Volume of voided urine 400–500 mL?
- Sterile urine
- Residual urine? If yes: regular voiding intervals?
- “Bladder neck” obstruction? If yes: incision, resection

17.6 Long-Term Results of Orthotopic Reconstruction

Recently the two institutions that pioneered orthotopic reconstruction have lumped their data together for an analysis of long-term outcomes. Not surprisingly, the comparison revealed that these two data sets looked rather identical.

Over the past 20 years orthotopic urinary reconstruction with the techniques developed in Ulm and Bern has become a widely accepted form of urinary diversion. So far, both centers together have performed more than 1,300 orthotopic bladder substitutions with an overall rate of neobladder formation in 58% of all patients undergoing cystectomy. Today, the absolute contraindications for this procedure are urinary stress incontinence, damaged rhabdosphincter, severely impaired renal and liver function, severe intestinal diseases, or an oncologic situation requiring urethrectomy. In patients treated for transitional cell carcinoma of the bladder, the rate of urethral recurrence in both centers was 1.5% and 5%, respectively, and the rate of upper urinary tract recurrence was 2–3%. Local tumor recurrence usually did not affect neobladder function. The rate of outlet obstruction by local recurrence was 2%, that of gross hematuria 1%, and of entero-reservoir fistulas 1–2%. Daytime continence at 12 months was 92%, while night time continence was lower around 80%. Transient or permanent urinary retention was seen in 11–12% of male patients. In both series, long-term upper urinary tract safety was good. The risk of stenoses of the ureterointestinal anastomosis with consecutive loss of renal function decreased with the introduction of non-refluxing implantation techniques. The rate of long-term metabolic complications remains low when adequate substitution by sodium bicarbonate is guaranteed in patients with impaired renal function. Patient selection and meticulous postoperative follow-up contributed to achieve good long-term results after cystectomy and orthotopic ileal neobladder substitution of the two large series of patients from the Universities of Ulm and Bern (Hautmann et al. 2006a, b).

17.7 Operative Technique Ileal Neobladder

Since 1986 over 1,000 ileal neobladders have been performed in Ulm. With two exceptions the operative technique has remained constant. The reservoir construction in both sexes is identical. The operative RCX technique in the two sexes is clearly remarkably different. Most urologists are quite confident with a neobladder in a male patient; however, they are very reluctant to do it in a female patient. This is surprising because a female RCX is much easier to do and the neobladder has never difficulties to get trouble-free down to the urethra. The ileal neobladder replaces the native bladder (i.e., it is located extraperitoneally in the pelvic cavity), and ileo-urethral and ileo-ureteral anastomoses are located retroperitoneally. Depending on tumor stage and location, this goal can be easily achieved by the creation of two large peritoneal flaps obtained from the visceral pelvic peritoneum (Fig. 17.1).

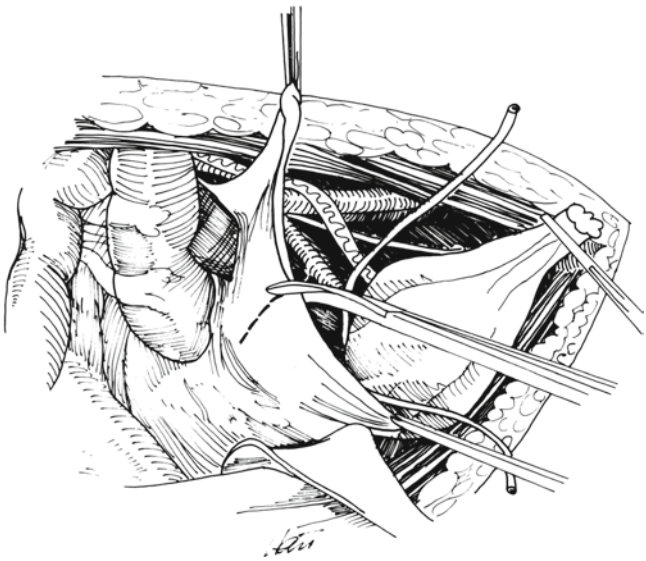

Fig. 17.1 Modified pelvic lymph node dissection has been completed. Ureters are exposed extraperitoneally. Peritoneum over the bladder is bisected to create two large peritoneal flaps for later total extraperitonealization of the ileal neobladder

Better not to use the standard vertical incision lateral to the sigmoid mescolon. Mobilize the peritoneal sac medially on both sides. Continue to locate the ureter extraperitoneally, realizing that it is displaced during exposure, because it adheres to the peritoneum (see Fig. 17.1). Mobilize the ureters with sufficient periureteral adventitia in a cephalad direction on both the right and the left sides.

A plane is established between the ureter and the lateral pedicles of the bladder. As the ureter and the bladder are retracted medially, the lateral pedicle is exposed. Finally the ureter is clamped distally.

Fine traction sutures are inserted in the proximal surface of the ureter, and it is divided against the clamps with scissors. Then the ureter is dissected proximally so that about 6–9 cm is free. Creation of large peritoneal flaps for subsequent extraperitonealization of the ileal neobladder (both sexes). Depending on tumor stage and location, the bladder is completely extraperitonealized, and the peritoneum is bisected over the bladder (see Fig. 17.1). If this cannot be done safely, an incision in the peritoneum is made high on the base of the bladder, leaving a peritoneal patch on the posterior bladder wall.

17.7.1 RCX in a Female Patient with Planned Ileal Neobladder

Preparation of the urethra requires special attention to surgical detail to avoid damage to the proximal urethra, anterior vaginal wall, and urethral support, which could

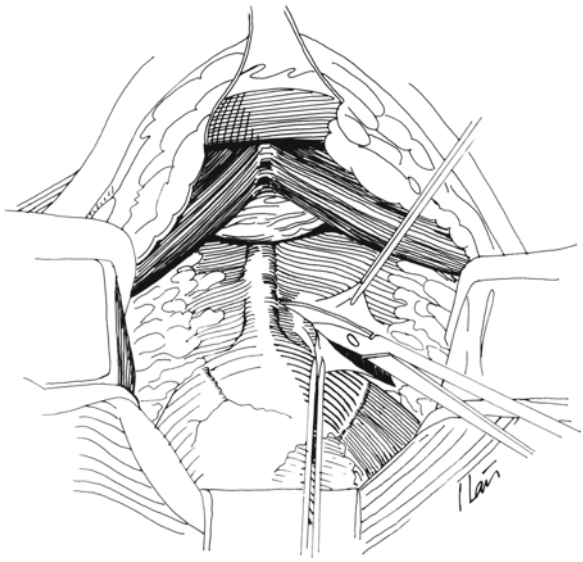

Fig. 17.2 Urethral support-sparing cystectomy in women. Incision of endopelvic fascia is parallel to posterior urethra and urethrovesical junction

jeopardize the continence mechanism and micturition. The endopelvic fascia is incised immediately lateral to the posterior urethra at the urethrovesical junction (Fig. 17.2). As much of the urethropelvic ligament and paraurethral vascular and nerve plexus as possible must be saved. This step is greatly facilitated when a providone-iodine pack is placed into the vagina and the urethra rides on top of the anterior vaginal wall (Fig. 17.3). Without that trick, the urethra falls back into the pelvic cavity, and mobilization of the urethra results in bleeding and jeopardizes the urethral support.

As the proximal urethra or urethrovesical junction is transacted, six interrupted 2–0 polyglycolic acid sutures are placed circumferentially in the urethra (Figs. 17.4a, b).

Frozen sections for pathologic examination are taken from the transacted end of the urethra/bladder neck to identify carcinoma in situ or overt carcinoma, which would result in subsequent urethrostomy.

The urethrovesical junction is dissected off the anterior vaginal wall down to the posterior bladder wall.

The remainder of the cystectomy is done in a descending fashion. The cardinal ligaments are divided anteriorly to avoid the pelvic plexus. The superior vesical artery and uterine vessels are cross-clamped, cut, and tied. Once the uterine artery is transacted, the distal ureter comes into view and can be isolated to avoid damage to the nerve fibers. A sponge on a sponge forceps is inserted into the vagina all the way to the posterior fornix to stretch the vaginal wall. The vaginal cavity is entered by a transverse incision, and the edges of the vaginal incision are held open with Allis clamps (Fig. 17.5). The distal stump of the ureter following its division is pulled medially to facilitate dissection of the lateral bladder wall.

The perivesical vascular plexus and inferior hypogastric plexus, which are located lateral to the vessels, are separated from the lateral wall of the bladder and

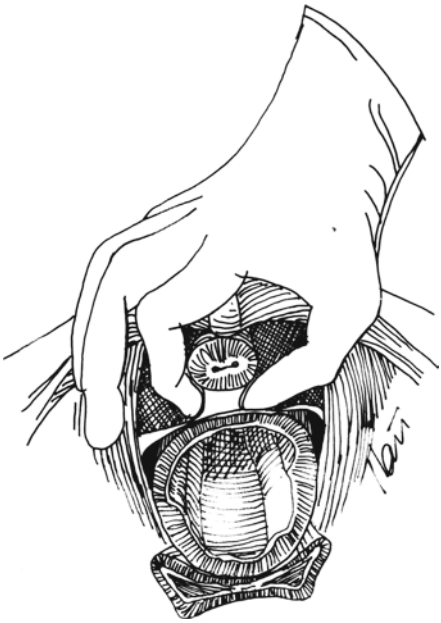

Fig. 17.3 Packed vagina gives ideal exposure to urethra an pelvic support structures

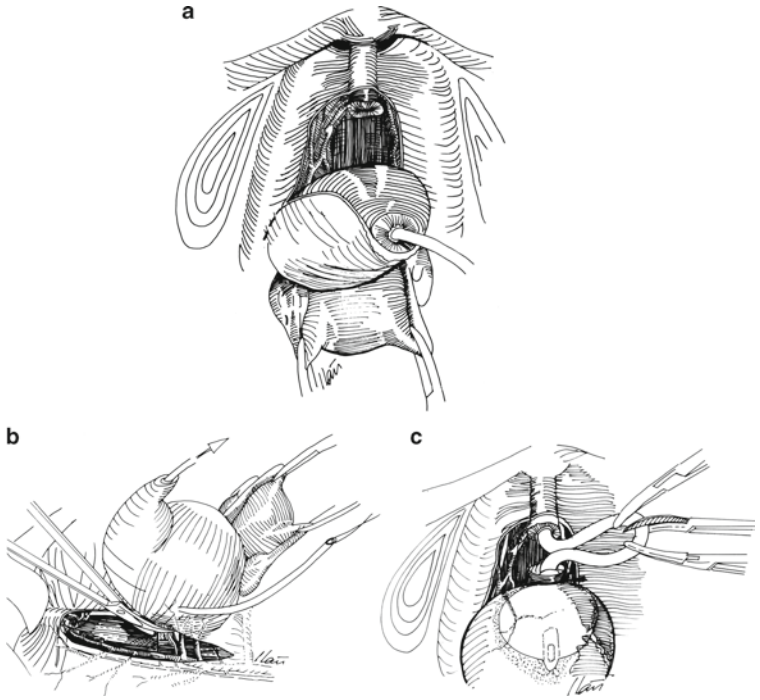

Fig. 17.4 (**a**) Transection of posterior urethra. (**b**) Bladder neck is carefully dissected off anterior vaginal wall. Upward traction of specimen facilitates dissection between lateral bladder wall and supporting structures that must not be jeopardized. (**c**) Balloon is inflated with 50 cc, pulled into bladder neck and proximal urethra including catheter are ligated and transected

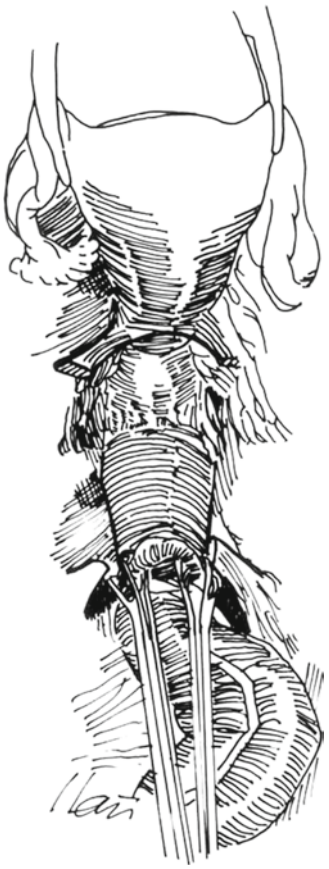

Fig. 17.5 Uterosacral ligaments on both sides are divided. Edges of transverse incision in posterior fornix of vagina are held under gentle traction with Allis clamps. Posterior bladder wall is dissected off anterior vaginal wall down to bladder neck. Autonomic nerve fibers (inferior hypogastric plexus), which run laterally to vessels, are separated from lateral wall of bladder and vagina

the vagina. Ascending and descending cleavage planes are combined, and thus the inferior pedicles of the bladder are developed and transacted close to the bladder (Fig. 17.6) (Hautmann 2001).

The ileal neobladder is anastomosed to the urethra following vaginal closure (Fig. 17.7).

17.7.2 Construction of the Ileal Neobladder in Both Sexes

Four lengths of ileum are arranged in the shape of a W with 3–5 cm long chimneys on each side of the W using five to six Babcock clamps. Other than the two chimneys, the bowel is opened on the antimesenteric border except for a 5–7 cm section centered around the marking suture, which is opened to 3 cm from the tip of that flap; a buttonhole of all layers is excised from the ileal plate. An ileal plate is formed by sewing together the cut edges of the antimesenteric borders of the W using 2–0 SAS on a straight needle (Fig. 17.8).

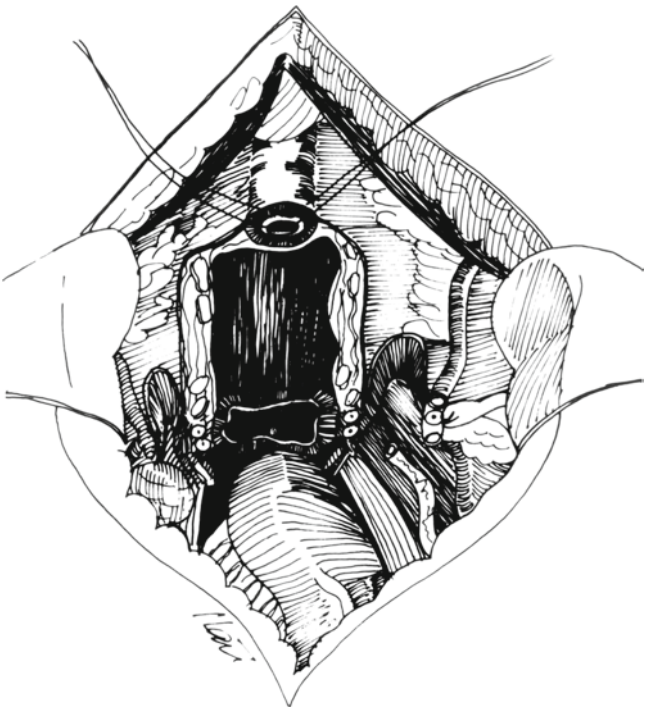

Fig. 17.6 Final results of a precise modified anterior exenteration with maximum preservation of the urethral support structures, the bottom of this defect being the anterior vaginal wall. Note: considerably compromised radicality as compared with standard cystectomy

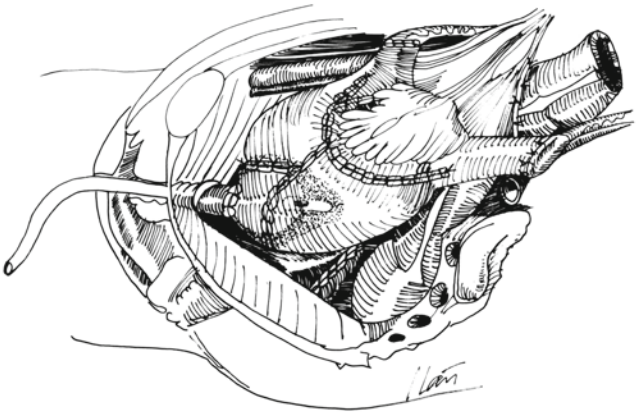

Fig. 17.7 Final situation: Neobladder on top anterior vaginal wall, ileo-ureteral anastomoses above common iliac vessels. Left ureter courses lateral to sigmoid in its physiologic bed. Extraperitonealization of neobladder to follow

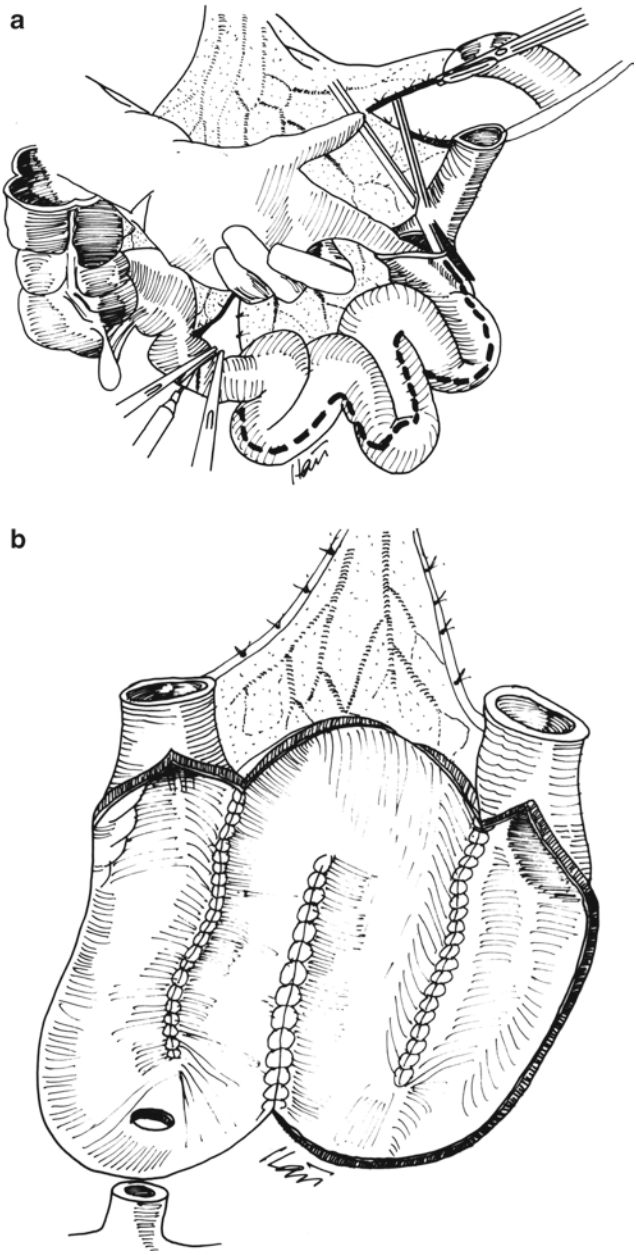

Fig. 17.8 (**a**) Selection of a 60 cm long ileal segment 20–25 cm proximal to the ileocecal valve. (**b**) W-shaped reconfiguration of the intestinal segment after detubularization and asymmetric incision of the ileal wall at the site of the anastomosis to the urethra, forming a U-shaped flap

A 22 F is placed through the buttonhole. For the actual anastomosis, six previously placed double-armed sutures using 3–0 SAS in the urethra are used. The inner sutures are passed through the neobladder outlet in the ileal plate without grasping the ileum, and the corresponding outer sutures grasp the entire ileal wall 5–8 mm lateral to the neobladder outlet (Fig. 17.9).

This guarantees a wide, ideal, funnel-shaped anastomosis so that mucosa is in direct contact with urethral epithelium. Next, under gentle traction on the transurethral catheter, the ileal plate is manipulated down to the urethral remnant, and the knots are tied inside the bowel (Fig. 17.10).

The cut edges of the 5–7 cm U-shaped flap are sewn together over the 22 F. The lower third of the anterior wall of the neobladder is closed, beginning inferiorly with interrupted 3–0 SAS (Fig. 17.11).

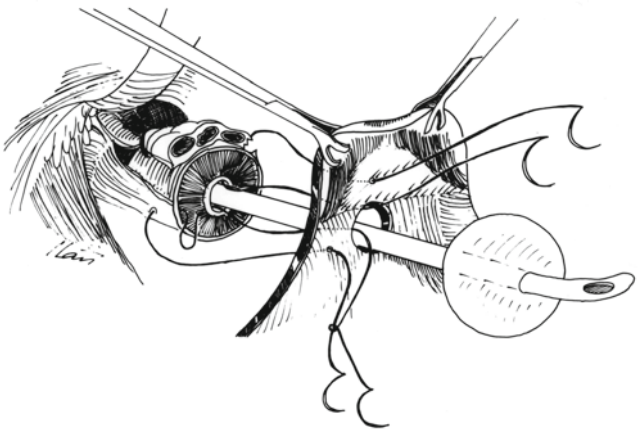

Fig. 17.9 Ileo-urethral anastomosis, anterior view

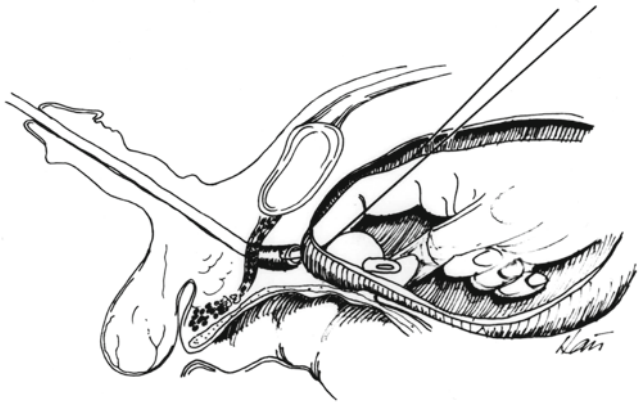

Fig. 17.10 Lateral aspect of the ileo-urethral anastomosis. The sutures are tied from inside the ileal bladder

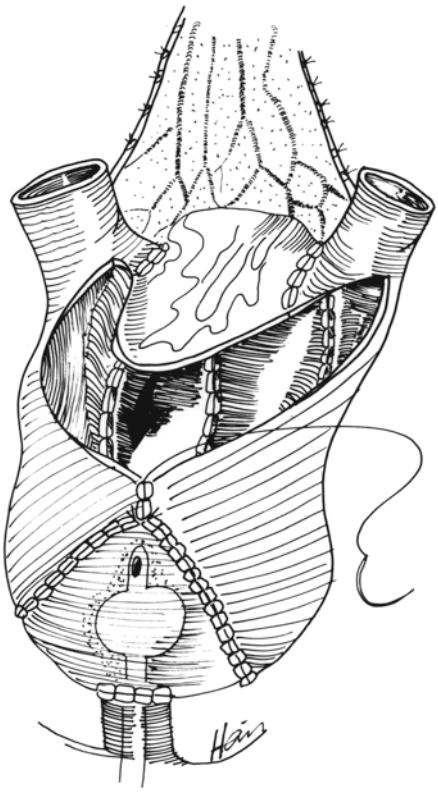

Fig. 17.11 Closure of reservoir

In 10% of male patients, the ileo-urethral anastomosis may cause difficulties. In this case, some or all of the following tricks are helpful to overcome this dilemma (Fig. 17.12) loosening the retractor, straightening the operation table, removing the sacral cushion, neutralizing the extended position of the patient, bringing up the perineum with a sponge stick, freeing the cecum and descending colon as in retroperitoneal lymph node dissection (RPLND), moving up the neobladder outlet to the tip of the U-shaped flap (Fig. 17.13), or performing an end-to-end anastomosis after tubularization of the U-shaped flap. Any incisions into the mesentery of the neobladder should be avoided. The neobladder mesentery should not be pulled roughly to the pelvic floor.

On each side, the ureters are trimmed as appropriate for their chimney (Fig. 17.14). The ureterointestinal anastomosis can be done extraperitoneally above the common iliac vessels using a Bricker or Wallace (our choice) technique without competing with the bowel mesentery for an anastomotic site.

After placing appropriate ureteral stents, they are brought through the anterior neobladder suture line. The remaining anterior neobladder wall is closed in a T-shape with running 3–0 SAS. No cystostomy tube is placed. Two 20 F silicone drains are placed into the small pelvis (Fig. 17.15).

This part of the procedure is facilitated when the distance between the two chimneys is chosen to be 7–10 cm. This chimney modification simplifies and enhances the flexibility of this procedure. This provides three major advantages: (1) extra length for the neobladder to reach the distal ureteral stumps because of an ileal chimney, (2) simplified abdominal or flank access to the postoperative ureterointestinal anastomosis should the patient require reoperation for ureterointestinal obstruction from either stricture or tumor, and (3) technically easier ureterointestinal anastomosis allowing antirefluxing and refluxing anastomotic techniques.

Using the two large peritoneal flaps from visceral pelvic peritoneum, this goal can easily be reached. Both flaps are sewn together, except for the portion where

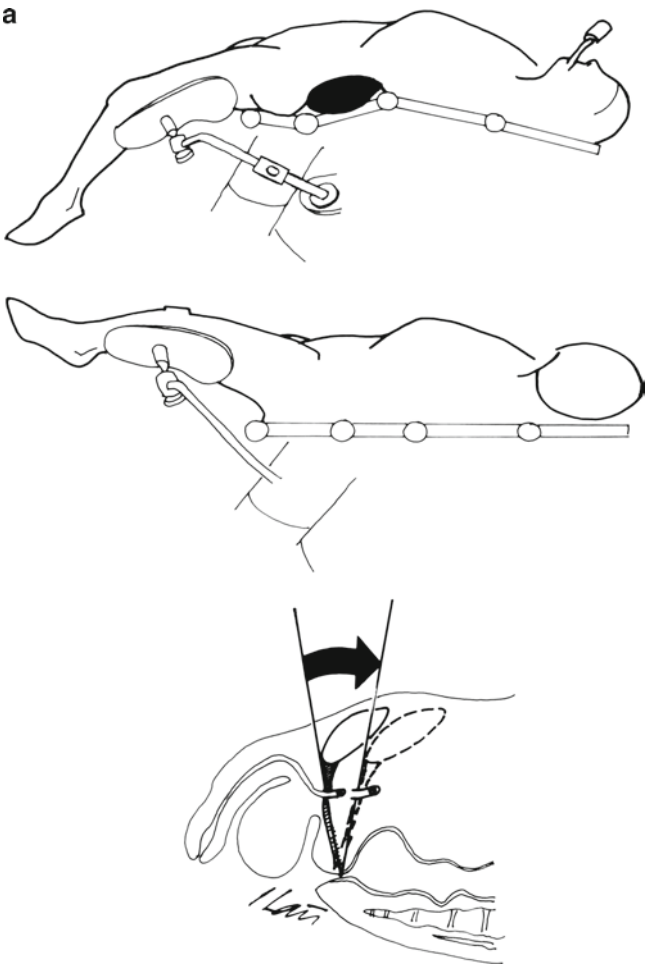

Fig. 17.12 Methods to get the ileal neobladder to the pelvic floor. (**a**) Changing the extended position of the patient to slightly supine and removal of the sacral cushion rotate the floor upward

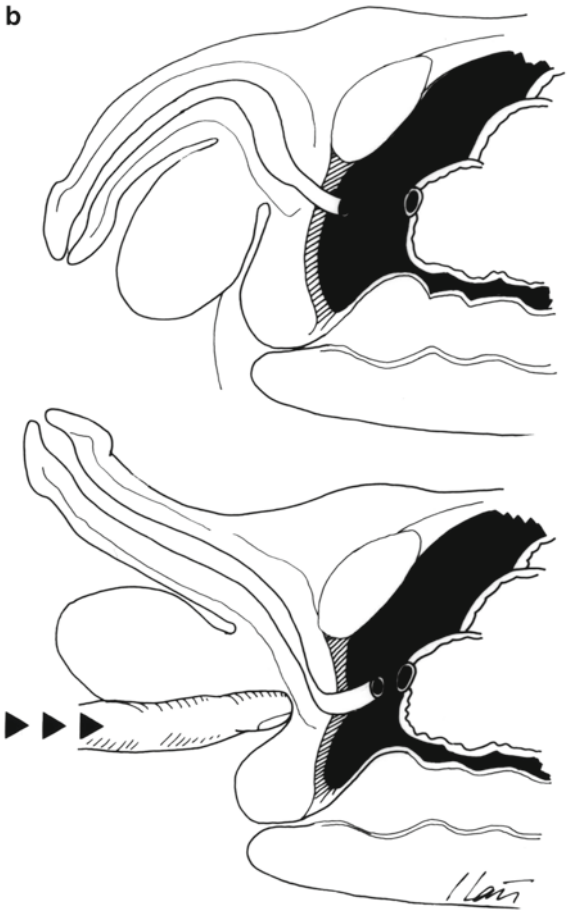

Fig. 17.12 (continued) (**b**)Pushing up the perineum with a sponge stick or finger approximates the urethral remnant and neobladder)

the mesentery of the neobladder runs through them. The peritoneal cavity is closed in a standard fashion. Alternatively, the flaps can be sewn to the posterior wall of the neobladder (see Fig. 17.15) (Hautmann 2001).

17.7.3 Postoperative Management

Excessive mucus production of the ileal bladder may rarely cause a problem in the postoperative course by obstructing the urethral catheter. Therefore, the ileal bladder is rinsed via the cystostomy with 50–100 mL of saline twice a day, starting on the 5th postoperative day. Routinely, the ureteral stents are removed between the 7th and the 14th postoperative day.

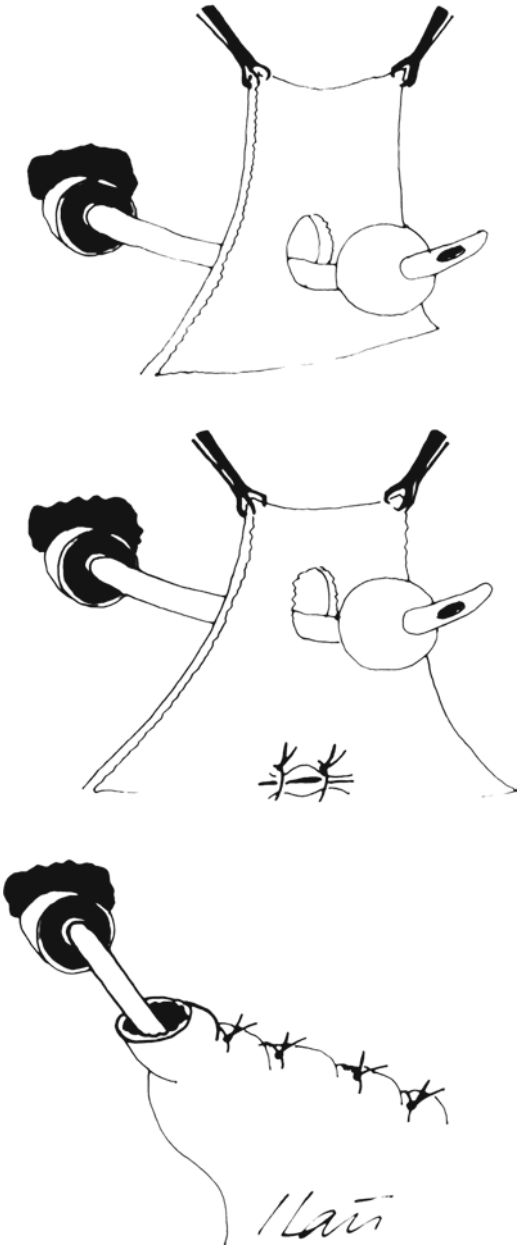

Fig. 17.13 Moving the neobladder outlet closer to the tip of the U-shaped flap of ileal plate. If this still does not allow tension-free anastomosis, one should tubularize the U-shaped flap and perform direct (end-to-end) anastomosis

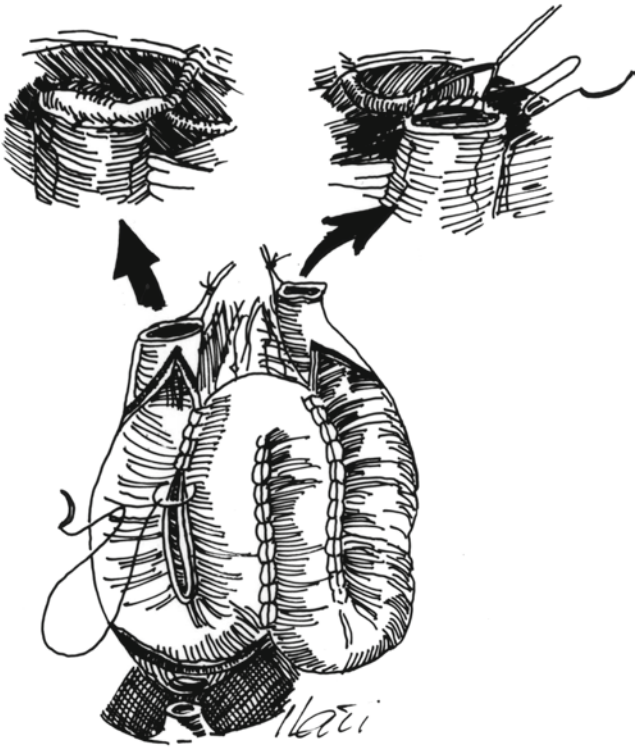

Fig. 17.14 Refluxing ileo-ureteral anastomosis using chimneys of a 3–5 cm afferent limb on each side

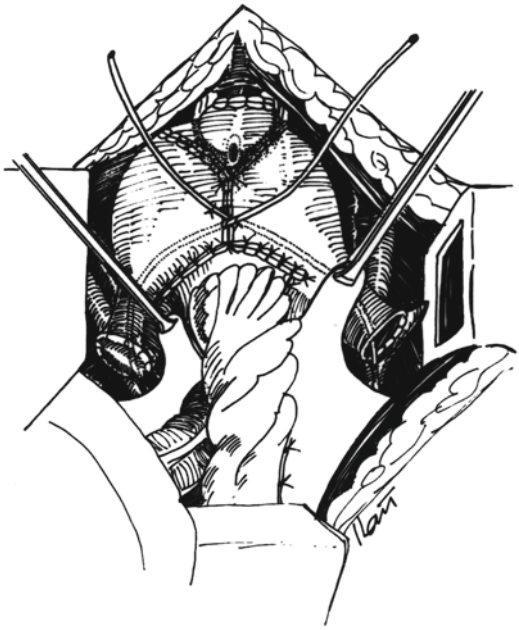

Fig. 17.15 Completely extraperitoneal localization of neobladder as well as ileo-urethral and ileo-ureteral anastomoses

As soon as the urine is in contact with the ileal bladder mucosa, reabsorption of urine electrolytes may occur. Therefore, the base excess is checked at weekly intervals for the first 4 weeks and monthly thereafter. Approximately 50% of all patients need temporary alkalinizing therapy.

The urethral catheter is removed between the 14th and the 21st postoperative days, after a cystogram has demonstrated complete healing of the ileo-urethral anastomosis. Rarely, there is still leakage from the anastomosis. When this occurs, it is treated by prolonged catheter drainage, until the leak has closed spontaneously.

17.8 Prostate Sparing Cystectomy

The standard treatment of high-grade, invasive bladder cancer is radical cystectomy. Prostate-sparing techniques have recently become an alternative surgical approach for the treatment of the disease. The literature regarding the oncologic and functional outcomes for prostate-sparing approaches is controversial. There is a significant incidence of bladder and prostate cancer involving the prostate and prostate apex in men requiring cystectomy for transitional cell carcinoma of the bladder at the time of surgery. This involvement of the prostate with cancer may be difficult to determine preoperatively. Importantly, although prostate-sparing procedures provide good potency results, the functional outcomes following cystectomy and orthotopic diversion to the urethra are not significantly different, particularly regarding daytime continence. Last, several studies suggest that the oncologic outcomes following prostate-sparing cystectomy may be compromised with this surgical approach. The significant incidence of bladder and prostate cancer involving the prostate at the time of cystectomy, which is difficult to determine preoperatively, may preclude the general application of prostate-sparing techniques in most men requiring cystectomy. Concerns regarding the oncologic outcomes with prostate-sparing techniques, coupled with the excellent results seen with traditional radical cystectomy and orthotopic diversion, suggest that prostate-sparing procedure should be performed only in well-selected individuals (Hautmann and Stein 2005; Stein et al. 2008; Kefer et al. 2007).

17.9 Palliative Urinary Diversion by Subcutaneous Nephro-Vesical/Nephro-Cutaneous Bypass

Whenever internal ureteral stenting fails in ureteric obstruction caused by end-stage malignant disease, percutaneous nephrostomy often appears as the only and final solution with deleterious impact on the quality of life.

For a subcutaneous bypass, two F 12 polyurethane tubes are placed as nephrostomy and cystectomy and connected subcutaneously. In patients with impaired

bladder function, the distal end of the system is diverted percutaneously in the lower lateral abdomen to simply drain into an urostoma bag. Between August 1999 and June 2008 52 patients with metastatic malignant disease had this type of palliative diversion (in 12 nephrocutaneous bypass) at the University of Vienna. In all patients, urinalysis, measurement of serum creatinine and quality of life (0 = very poor – 10 = excellent), and renal ultrasonography were evaluated preoperatively, 2 days postoperatively, as well as every 4 weeks for follow-up. With a mean follow-up of 12.9 (range 2–57) months all but four patients died from their progressive metastatic disease.

Preoperative hydronephrosis was completely eliminated in 80.8% of the renal units and dramatically reduced in the remaining units. Serum creatinine dropped significantly from a mean 6.1 (range 2.3–12.8) to 1.55 (range 0.55–6.3) mg%. Mean quality-of-life-score was 3.6 (range 0–6) preoperatively and 7.8 (range 5–9) postoperatively. In eight patients the system had to be replaced due to occlusion after a mean 9.8 months. One bypass was removed and a conduit performed because a vesico-vaginal fistula developed after radiation of cervical cancer. Eleven patients had a single and four patients recurrent urinary tract infection which resolved under antibiotics. In one patient the bypass system had to be replaced twice because of abscess formation. Replacement of the system was easily performed at the side of the connector using a guide wire.

In essence, the subcutaneous bypass system represents a simple minimally invasive treatment for patients with ureteric obstruction due to advanced metastatic disease. Patients gain a better quality of life due to increased mobility and independence during their final period of life (Schmidbauer et al. 2009).

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Chapter 18
Laparoscopic Cystectomy and Robotic-Assisted Cystectomy

Hubert John

Abstract Muscle invasive bladder cancer still has a high mortality rate. Radical cystectomy is the accepted first line therapy for muscle invasive and high-risk urothelial bladder cancer. In order to decrease the procedure-associated morbidity, minimal invasive operative techniques were introduced with increasing interest as well in the ablative and the reconstructive steps of the operation. Laparoscopic and robot-assisted cystectomy in selected cases decreases blood loss respecting the oncological principles including the aim of negative margins and extended pelvic lymphadenectomy. Completely intracorporeal urinary tract reconstruction is feasible; however, it increases the total operative time and perioperative complication rate. So far, it seems that best results can be obtained by laparscopic and robot-assisted extended lymph node dissection and radical cystectomy, while the upper urinary tract reconstruction is performed by limited open access. Pathological and cancer outcome are promising compared to open surgery. Still, the laparoscopic and robotic approaches for radical cystectomy are under clinical investigation. Long-term outcome studies will answer the question, if selected patients profit from a minimal invasive approach.

18.1 Introduction

The incidence of bladder cancer is higher in men than in women. In men, it is the fourth most common malignant tumor after prostate, lung, and colorectal cancer. Cigarette smoking is still the leading etiological factor; bladder cancers due to occupational exposition are rare. In the USA, more than 63,000 new diagnoses appear each year. Death occurs still in more than 50% calculated overall T2–T4 disease (Larsson et al. 2003; Jemal et al. 2006), while overall recurrence-free survival rate

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at 5 and 10 years in largest series is 68% and 66%, respectively (Stein et al. 2001). The overall complication rate in open setting varies between 30% and 60%, whereof 10–12% are considered as major complications. Perioperative mortality in large series is given with 2–3% (Stein et al. 2001; Cookson et al. 2003). These outcomes did not markedly increase in the last few years despite new chemotherapeutic regimens, improved operative technique, and patient selection. There is now increasing efforts with minimal invasive technology to decrease morbidity, replicating the standardized open surgical techniques by laparoscopic approach (Irwin et al. 2009). As laparoscopic and robot-assisted technology has been introduced and widely accepted in prostate and renal surgery, the role of laparoscopic and robot-assisted radical cystectomy with upper urinary tract reconstruction is still in clinical evaluation and waits to be accepted.

18.2 History

Open radical cystoprostatectomy with pelvic lymphadenectomy was first described in 1949 (Marshall and Whitemore 1949). The first laparoscopic cystectomy was performed by Parra in 1992 for pyocystitis (Parra et al. 1992). Badajoz and his group published the first cases of laparoscopic radical cystectomies (LRC) for invasive bladder cancer in 1993 (de Sanchez Badajoz et al. 1993). In females, the anterior pelvic exenteration specimen was brought out through the vagina and upper urinary tract reconstruction performed by a minilaparotomy (Puppo et al. 1995). Intracorporeal urinary tract reconstruction with a sigmoid pouch was described in 2001 by Turk (Turk et al. 2001). Gill performed the first intracorporeal Bricker reconstruction in 2000 and Studer pouch 2002 (Gill et al. 2002). The first robot-assisted radical cystectomy was performed by Menon et al. (Menon et al. 2003) in 2003. Beecken et al. reported in the same year the first intracorporeal robot-assisted neobladder (Beecken et al. 2003) – opening a new field for many surgical teams in the urological community to perform complex ablative and reconstructive laparoscopic urology.

18.3 Patient Selection

Laparoscopic and robot-assisted radical cystectomy is performed in patients with organ-confined, non bulky bladder tumors, ideally without previous pelvic surgery, moderate body mass index and a good cardiopulmonary status. Previous abdominal surgery, radiotherapy, locally advanced disease, uncorrected coagulopathy or morbid obesity may increase the technical challenge and complication rate, thus, compromising oncological and functional outcome. These relative contraindications should, if present, be carefully discussed in an interdisciplinary setting on a case-to-case base. The impact of pneumoperitonism and the Trendelenburg-position on

cardiopulmonary physiology must be kept in mind. Patients with decreased pulmonary complicance or spinal stenosis should, therefore, be excluded. Preoperative patient's informed consent is absolutely mandatory, including catheter and drains, pain, complications, and chance of open conversion. Clear liquid diet at the day before surgery maximizes the intraabdominal working space and minimizes bacterial bowel spillage during the reconstructive operative part. Laparoscopic radical cystectomy for loco-regionally advanced bladder cancer should be considered experimental (Hemal et al. 2008).

18.4 Patient's Positioning and Monitoring

The patient is placed in a lithotomy – 30° Trendelenburg position. The legs are slightly abducted, fixed with towels. The arms are adducted and padded. Shoulder pads should be avoided due to high risk for plexus damages (Fig. 18.1). Monitoring is achieved by oxygen saturation (arrow) and arterial catheter (arrowhead). A central monitoring is mandatory for cystectomies.

18.5 Trocar Placement

Pneumoperitoneum can be achieved using a Veress needle at superior umbilical or upper left abdominal region. Many teams, prefer a supraumbilical open access to prevent any harm of intraabdominal organs. The supraumbilical camera position

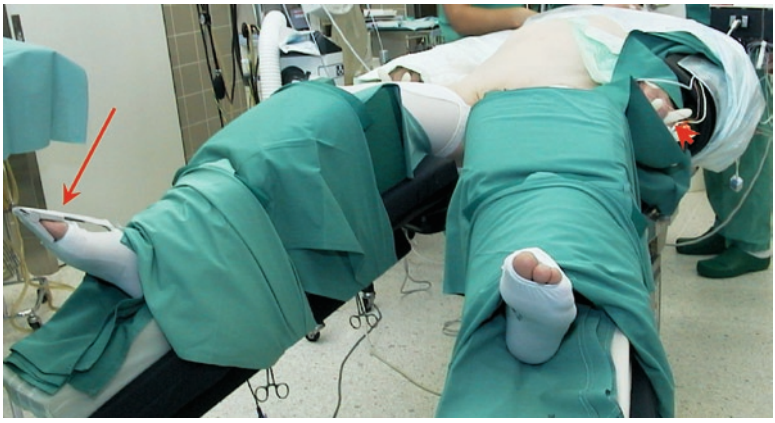

Fig. 18.1 Patient's positioning and monitoring. The patient is placed in supine position, the legs slightly abduced. The legs are fixed with towels, in padded channels. Monitoring is achieved by a 0^2 saturation (*arrow*) at the right big toe, and arterial catheter (*arrowhead*). A central venous monitoring is not routinely necessary

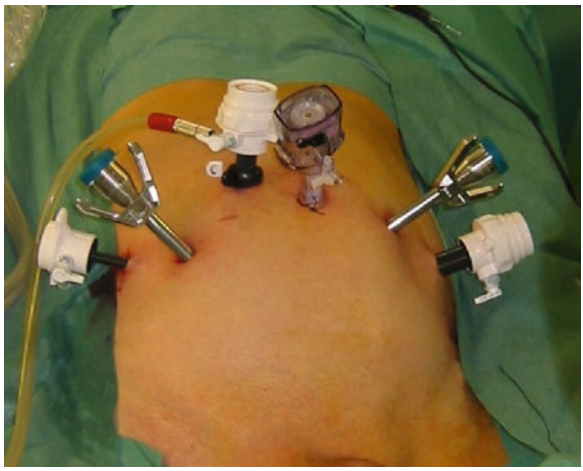

Fig. 18.2 Placement of ports. Port placement for a standard three-arm system

provides an excellent view into the small pelvis and is a prerequisite for the extended pelvic lymphadenectomy template. A total of six trocars are then placed. A 0° lens is used for the whole procedure (Fig. 18.2). The surgeon has a cautery forceps in his left hand (resp. robotic arm) and a monopolar curved scissors in the hand (resp. right robotic arm). The intestinal loops are handled carefully with Cadiere forceps in robotic technique, preventing serosal lesions.

18.6 Extended Lymph Node Dissection

The extended lymphadenectomy is either performed before or after the cystectomy. In contrast to radical prostatectomy, the impact of extended removal of lymph nodes in radical cystectomy has been shown to correlate with cancer-specific survival (Herr et al. 2002). Conventional and robot-assisted technique allow to perform an adequate lymph node removal including the obturator, internal, external, common iliac, and presacral sites up close to the inferior mesentery artery (Fig. 18.3). Laterally, the template extends to the genitofemoral nerve. Proximal landmark is the bifurcation of the common iliac artery. Care is advised doing the skeletonizing of the veins – they appear flat due the 12 mmHg intraabdominal pressure. Steep 45°Trendelenburg-position, mobilization of the sigmoid colon, and the new generation of the DaVinci© robotic system (Intuitive Surgical, Sunnyvale, CA, USA) help to achieve adequate exposure. The correct extended lymph node dissection is time consuming and takes, if carefully performed, over 1 h. Meanwhile, initial criticism regarding oncological correctness of laparoscopic and robot-assisted extended lymphadenectomy has been eliminated.

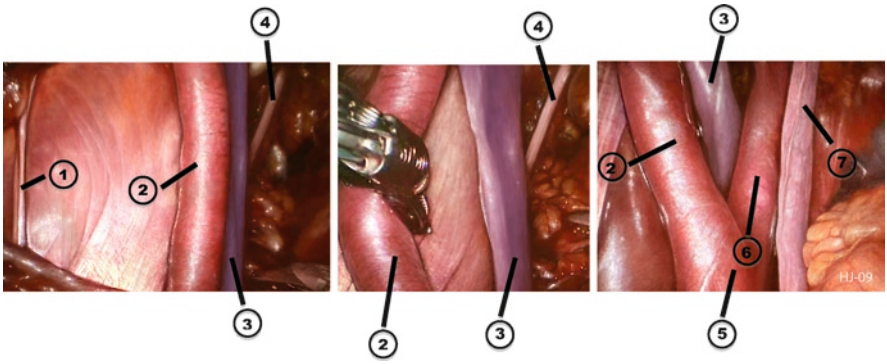

Fig. 18.3 Extended lymphadenectomy template for bladder cancer. The template is dissected laterally from the genitofemoral nerve (*left*), dorsally to the obturator nerve (*middle*) and cranially up to the iliac bifurcation and common iliac artery (*right*). *1* genitofemoral nerve, *2* external iliac artery, *3* external iliac vein, *4* obturator nerve, *5* common iliac artery, *6* internal iliac artery, *7* ureter

18.7 Cystectomy

Open radical cystectomy with pelvic lymph node dissection is the gold standard for muscle invasive bladder cancers. The acceptance of laparoscopy in the urological community and the advances in instrumental design combined with the development of robotic technology. Laparoscopic radical cystectomy was introduced in many laparoscopic centers worldwide. The procedure is feasible with reproducible results. It appears to offer the patient the benefits of minimally invasive surgery with respect to postoperative recovery (Irwin et al. 2009; Cathelineau and Jaffe 2007).

18.7.1 Ureteral Dissection

The procedure starts with inspection of the pelvic cavity and ureteral dissection. Their peristalsis across the common iliac arteries identifies helpful for identification. To maintain vascular supply, abundant periureteral tissue is preserved thereby decreasing later ureteral strictures. Once the ureters are dissected down to the ureterovesical junction, they are clipped with Weck clips (hemo-lock, Weck Pilling, USA). A stay suture at the proximal ureteral border is recommended to facilitate the transfer of the left ureter under the sigmoid colon (Fig. 18.4). The distal ureteral ends are sent for frozen section.

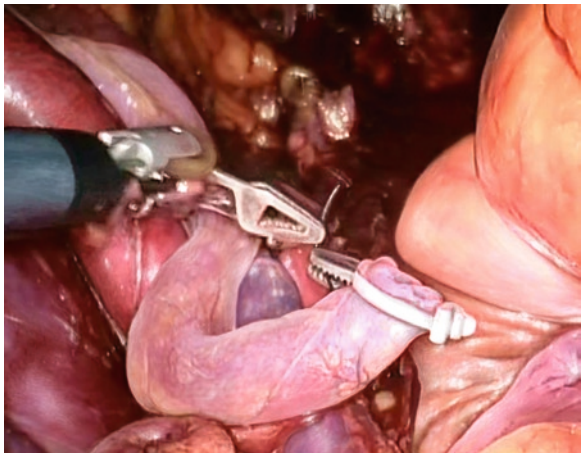

Fig. 18.4 Ureteral transposition. The Cadière forceps is brought from the right to the left transposing the left ureter to the right side through the mesosigma at the level of the promontory

18.7.2 Male Cystectomy

After ureteral mobilization, the posterior dissection is performed first with incision of the peritoneum over about 7 cm at the level of the Douglas pouch. The bladder is lifted and the seminal vesicles localized. Similar to radical prostatectomy, the posterior Devovillier's fascia is incised exposing the yellow prerectal fat as a landmark. The seminal vesicles are not mobilized separately but maintained within the cystectomy specimen. The prostate, seminal vescicles, and bladder are dissected to the anterior, the rectum to the dorsal. The posterior and lateral vascular pedicles of the bladder and the prostate are controlled by Endo-GIA staplers (Ethicon endosurgery, USA), the Ligasure 5 mm or 10 mm device (Valleylab, Boulder, USA).

If planned and discussed before, the nerve-sparing technique with preservation of both neurovascular bundles can be performed as in radical prostatectomy, avoiding thermal energy. The urachus is divided as high as possible and the bladder is extraperitonealized. The endopelvic fascia is incised bilaterally and the dorsal vein is sutured with one to two stitches or secured with a stapler. It is of outmost importance to close the urethra before transection in order to avoid urine and tumor spillage. Once the dissection is completed, the specimen is immediately brought into an Endobag. Frozen section of the urethral stump is added.

18.7.3 Female Cystectomy

The posterior dissection of the Douglas pouch is performed with an inverted U incision. Ovaries and uterus are removed according oncological parameters, patient's age, and need for reproductive function. The uterus is anteverted and the infundibulo-pelvic ligaments, with the ovarian pedicles, are identified close to the uterus and bissected with Weck clips or Endo-GIA stapler. The uterine arteries are then isolated. The round ligaments are divided and the vascular pedicles separated.

The vascular pedicles can also be dissected using the Ligasure technique. The exposure of the uterovaginal junction is facilitated by a sponge stick manipulated by the right-side assistant. The vaginal incision is performed anterior at the vaginal fornix. The sponge is visualized and the vagina incised bilaterally down toward the urethra taking a narrow strip of anterior vaginal wall en bloc with the specimen (Pruthi et al. 2008). Preservation of visceral innervation from the pelvic plexus at the lateral vaginal wall maintains sexual function. If there is no suspected tumor invasion into the uterus and anterior vaginal wall, a vaginal-sparing technique can be chosen and hysterectomy can be performed separately. The specimens are brought into an endobag and removed through the introitus. The vagina is closed with a running interlocking suture, the "clam-shell technique" (Jhaveri et al. 1998).

18.8 Nerve-Sparing Technique

Nerve-sparing technique has been found to improve continence, potency, and sexual function in patients undergoing orthotopic neobladder reconstruction (Lane et al. 2006). These authors found 75% potency in men and 10% day- and nighttime continence in a small series. In males, the neurovascular bundles run at the posterolateral border of the prostate down toward the prostatic apex. After the dissection of the bladder pedicles, the neurovascular tissue is separated from the prostate and the urethra. The risk to find prostate cancer is low, and if found, the tumors are usually intraprostatic pT2 cancers with negative margins. An intrafascial dissection plane therefore facilitates the nerve-sparing approach. In females, the nerve-sparing technique has an impact on urethral competence and continence, and also to preserve vaginal lubrification and sensation (Fergany and Gill 2008).

18.9 Prostate-Sparing Cystectomy

The prostate-sparing cystectomy was proposed with the aim of maximal functional preservation of urinary continence and potency in patients with orthotopic urinary diversions. The bladder is removed over the prostate and seminal vesicles. The prostate adenoma is removed either by transurethral resection or digital enucleation. There should be no infra-neobladder obstruction. The neobladder is sutured onto the prostate capsule. Best potency rates are reported with 84% and full urinary continence (Arroyo et al. 2005).

18.10 Female Reproduction Tract-Sparing Cystectomy

Fertile women or female patients with the strong wish to remain sexually active can undergo cystectomy with preservation of ovaries, the uterus, and the vagina. Uni- or bilateral preservation of the ovaries can be performed by dissecting the

ovarian ligament but sparing the vascular pedicle (Fergany and Gill 2008). However, this technique is reserved to tumors with clinically Ta or T1 stages that are situated away from the posterior bladder wall.

18.11 Urinary Tract Reconstruction

Minimally invasive techniques today offer endoscopic reconstruction of conduits, continent pouches, as well as orthotopic neobladders in extra- or intracorporeal approach.

18.11.1 Ileal Conduit (Bricker)

A 15–20 cm terminal ileal segment is isolated and brought out of the surgical wound in extracorporeal technique. The distal 20 cm of the terminal ileum should be preserved to maintain abundant vitamin B-12 absorption. Ileo-ileostomy is achieved by continuous seromucular 4–0 polydioxanone (PDS) suture. Ureteroileal anastomosis is then performed. The author prefers an end-to-side technique. In dilated distal ureters and intracorporeal laparoscopic reconstruction, an ureteral plate according Wallace II can be performed. In intracorporeal technique, the ureteral stenting through additional suprapubic 3 mm ports with 90 cm single-J stents can be very challenging, as well the suture of the ureteral plate with thin, spatulated distal ureters (John et al. 2008). The aboral end of the conduit is finally brought through the abdominal wall using Alice clamps and an everted skin stoma is performed. The ureteral stents are removed after 7–10 days.

18.11.2 Orthotopic Neobladder

A total of 55 cm terminal ileum is isolated. The proximal 10 cm of the ileal segment is used as Studer afferent segment (Matin and Gill 2002). The uretero-ileal anastomosis is performed end-to-side. The remaining 45 cm are detubularized along its antimesenteric border. The posterior plate of the neobladder is sutured. After the ileo-urethral anastomosis with PDS 2-0 or Biosyn 3-0 and a UR-six needle, the neobladder is completed by completion of the anterior wall. In intracorporeal robot-assisted technique, Wiklund et al. proposed to perform the ileo-urethral anastomoses in van Velthoven technique (Van Velthoven et al. 2003) before ileal detubularization and dorsal reconstruction with Biosyn 4–0, thus, avoiding tension on the urethral anastomosis (Woods et al. 2010). Again, dorsal ureteral plate reconstruction, ureteral stenting with 90 cm Mono J (Fig. 18.5), and ureteral plate anastomosis is technically highly demanding with an intracorporeal approach (John et al. 2009).

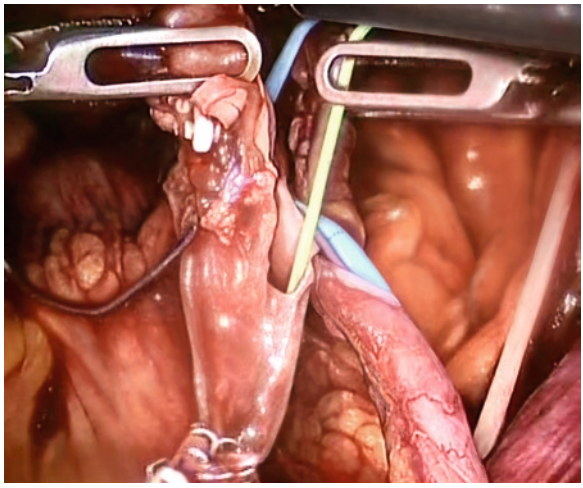

Fig. 18.5 Ureteral stenting. The ureters were stented with two Cadière forceps

18.12 Outcome and Comparison to Open Radical Cystectomy

Robotic technology has proven the feasibility to accomplish even the technically most demanding laparoscopic procedures in urology. There are only few studies comparing outcome of robotic versus open radical prostatectomy. The benefits of minimally invasive surgery in radical cystectomy are well documented, including decreased blood loss, less postoperative pain, faster recovery, and return of bowel function, and a shorter hospital stay (Porpiglia et al. 2007; Haber et al. 2008). On the other hand, critical analyses mention increased cost, longer operative duration, and absence of median- and long-term studies. A 3-year follow up of DeGer et al. (DeGer et al. 2004) showed a progression-free survival rate of 85%, while others report only 65% (Simonato et al. 2005). In a prospective comparison of 21 open and 33 robot-assisted radical cystectomies, Wang et al. (2008) found in the robotic group significantly lower blood loss and transfusion rate, and shorter hospital stay. The complication rates were similar with 24% in open versus 21% in the robotic group. A comparative analysis of ten patients in each group of open, laparoscopic, and robotic radical cystectoctomy for invasive bladder cancer by Elhage et al. (Elhage et al. 2008) showed a shorter operative time in the open series (325 vs 345 vs 365 min). Blood loss (1,300 vs 350 vs 150 mL), complication rates (60% vs 50% vs 20%), and hospital stay (16 vs 16 vs 11 days) were lower in the robotic group. Plus, recovery after the procedure was quicker in the conventional laparoscopic (3 weeks) and robotic (4 weeks) procedure compared to patients in the open surgical group (8 weeks). Similar data found Galich et al. comparing 24 cases with open approach and 13 with robot-assisted technique (Galich et al. 2006). Pruthi et al. (Pruthi and Wallen 2008) report in 50 robotic cystectomies a 0% margin rate. The lymph node count was 19, compared to an open series of the same authors with 16 nodes (Pruthi and Wallen 2007).There were no port site metastases or peritoneal seeding. Haber and Gill reported medium-term follow-up in 37 patients that underwent conventional

laparoscopic radical cystectomy (Haber et al. 2008) with extracorporeal reconstructon. After 5 years 11/37 (30%) were dead whereof 2/37 (5%) from metastases. Thus, so far, the minimal invasive approach does not compromise oncological outcome compared to open radical cystectomy (Irwin et al. 2009; Wang et al. 2008), while reducing morbidity associated with the procedure. The available studies suggest some benefits of the robot-assisted radical cystectomy technique including intraoperative complications (blood loss and transfusion rate) and perioperative outcome (hospital stay, decreased convalescence, reduced short-term disability, quicker return to normal activities) (Novara and Ficarra 2009). The goal of robotic radical cystectomy must be at least to replicate the oncologic and functional outcome of the open surgery, including the ability of extended pelvic lymphadenectomy and maintenance of oncologic surgical principles.

18.13 Final Considerations

Laparoscopic and robot-assisted radical cystectomy are technically feasible procedures. In general, laparoscopic surgery has to maintain the oncologic principles and transfer established open surgical techniques not compromising oncological or functional outcome. Tumor stage, location, and size, as well patient's characteristics must be factored to plan an optimal intervention. Oncological risks must be minimized by clipping the ureters before dissection, stapling, clipping or suturing of the urethral stump to avoid tumor spillage and by performing an extended pelvic lymph node dissection with a supraumbilical camera access.

In well-selected patients, conventional laparoscopic and robot-assisted radical cystectomy with extracorporeal urinary tract reconstruction may combine the benefits of minimally invasive techniques with the safety of open surgery, that is, the potential for reduced blood loss and faster reconvalescence with similar complication rate and comparable oncological outcomes. Total intracorporeal reconstruction of the urinary tract is technically feasible, but so far, these initial series suffer from significant increased operative time and perioperative complications. Nevertheless, the proof of non-inferiority of conventional laparoscopy and robot-assisted techniques compared to open technique has to be studied in multicenter prospective trials. In 2009, the open radical cystectomy is still considered to be the gold standard. Even that laparoscopic and robot-assisted radical cystectomy were accepted in the 2008 EAU guidelines as grade C recommendation, the exact role of laparoscopic cystectomy is not yet defined.

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Chapter 19
Neoadjuvant Chemotherapy

Amir Sherif

Abstract In 1985, MVAC (methotrexate, vinblastine, doxorubicin, and cisplatin) was introduced, and it became the first successful chemotherapy regimen used for metastatic bladder cancer. Overall response proportions were achieved in 72% of patients, including complete responses in 36%. Because MVAC also produced significant responses in the primary tumor, the regimen was introduced in a neoadjuvant setting for muscle invasive bladder cancer. Presently, there are two standard chemotherapeutic regimens: MVAC or GC (gemcitabine and cisplatin). It is considered that the gemcitabine and cisplatin combination has a significantly better toxicity profile, still both regimens carry risk for significant toxicity.

19.1 Rationale of Neoadjuvant Chemotherapy

The excellent overall response rates achieved by the MVAC-regime and reported of in 1985, forms the starting point for trying to use neoadjuvant approaches (Sternberg et al. 1985). There are mainly two reasons for offering patients with invasive urothelial urinarybladder cancer neoadjuvant chemotherapy. The first one is efficacy related to micrometastatic dissemination and the second one is downstaging of the primary tumor. Relating to both aspects of efficacy, the two regimes MVAC or GC are considered being eqvivalent (Van der Maase et al. 2000).

19.2 Pathoanatomical Background of Disseminated Invasive Urinary Bladder Cancer

In 1999, Wallmeroth et al. published an autopsy study on invasive urinary bladder carcinoma. The presence of metastatic deposits was higher than expected (Fig. 19.1). Totally 367 patients with muscle invasive urinary bladder carcinoma underwent

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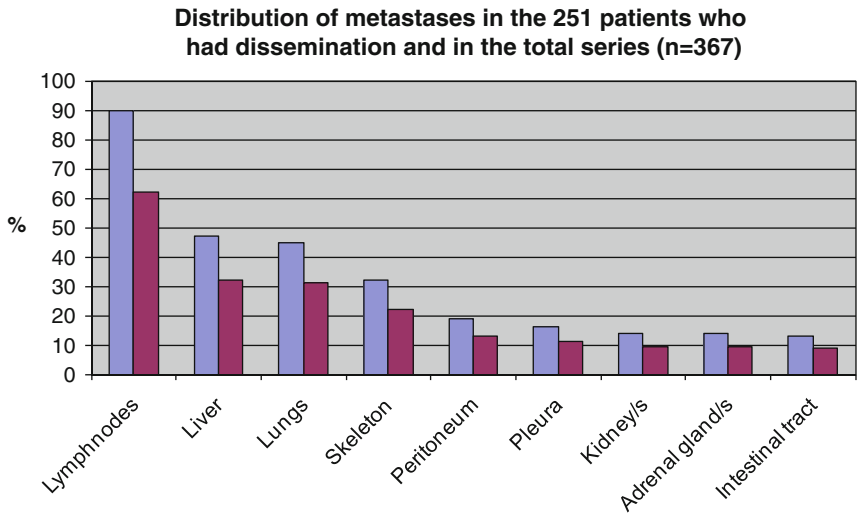

Fig. 19.1 In this autopsy series including totally 367 patients with muscle invasive urinary bladder carcinoma, 251 patients had metastatic spread in one or more localizations. It is noteworthy that in the group with dissemination, nodal spread was found in 90% of the patients. Furthermore in the metastatic group, the investigators, detected 47% with liver metastases and 45% with metastases to the lung/s (Adapted from Wallmeroth et al. 1999)

autopsy and 68% of the patients had dissemination. There was a significant correlation between nodal metastases and concomitant distant metastases ($p<0.0001$). Approximately 47% of the patients had both nodal metastases and distant dissemination. Only 12% of the patients had nodal metastases as sole metastatic manifestation. Of great interest, it was detected that in 215 patients with nodal dissemination the frequency of metastasis was 92% in regional (perivesical or pelvic), 72% in retroperitoneal, and 35% in abdominal lymph nodes. Distribution over pT-stages showed that patients with pT2 had metastatic spread in 36%, pT3a in 45%, pT3b in 69%, and pT4 in 79%. The study did not differentiate between micrometastatic deposits and macrometastatic dissemination (Wallmeroth et al. 1999).

19.3 Definitions of Nodal Dissemination

Isolated tumor cells (ITC) and tumor cell clusters measuring less than 0.2 mm are regarded as metastatic isolated tumor cells (Singletary et al. 2003).

Micrometastases are defined as tumor deposits between 0.2 and 2 mm.

Macrometastases are defined as tumor deposits > 2 mm. Macrometases can further be defined as being (1) intracapsular (relating to the capsule of the node) or having (2) extranodal capsular extension (ECE).

19.4 Dissemination and Neoadjuvant Chemotherapy

All major randomized trials on neoadjuvant chemotherapy have had as one of the inclusion criteria that patients should be preoperatively staged as non-disseminated, that is, N0M0. Thus neoadjuvant chemotherapy is mainly focused on treatment related to micrometastatic deposits. Included patients in which CT-scans have not been performed are, of course, not adequately staged, and this may be considered as a major caveat in these studies.

Both macrometastatic and micrometastatic dissemination represent a generalization of the disease. Thus local treatment, definitive surgery, or definitive local radiotherapy without, for instance, neoadjuvant generalized treatment does not address the problem of a cancer that has spread to both regional and distant sites.

19.5 Present Status of Neoadjuvant Chemotherapy

The MRC presented their first meta-analysis of all known randomized trials in 2003, and the results were updated with an additional trial in 2005. Noteworthy is that the MRC did not just collect published data, but instead established a solid contact with all the investigators for data collection. Thus, independent patient data (IPD) could be collected and reassessed for recalculation of the meta-analysis. Totally 11 trials were included, comprising a total of 3,005 patients. It was clearly noted that cisplatinum-combination therapy was the most beneficial in terms of overall survival and disease-free survival. This is in comparison with single platinum regimens. Thus the 2,433 patients who had received combination therapy were also separately assessed in the analysis. There was a significant survival benefit associated with platinum-based combination chemotherapy (HR = 0.86, 95% CI 0.77–0.95, $p = 0.003$), equivalent to a 5% absolute improvement in survival at 5 years. This is equivalent to a number needed to treat 20 patients. There was also a significant disease-free survival benefit associated with platinum-based combination chemotherapy (HR = 0.78 95% CI 0.71–0.86, $p < 0.0001$), equivalent to a 9% absolute improvement at 5 years (Vale 2005). Furthermore, the Nordic cystectomy study group performed a combined analysis of two prospectively randomized trials (NCS1 and 2) (which also were included in the ABC meta-analysis) and an increased overall survival was clearly detected. The absolute difference at 5 years follow-up was 8% with a number of 12.5 patients needed to treat. Subgroup analysis revealed that the highest overall survival was found in the clinical T3-group with an absolute difference at 5 years follow-up of 11%, translating into 9 patients needed to treat. (Sherif et al. 2004). Noteworthy is that the MRC/ABC-trial did not find any survival-differences in any of the patient subgroups defined by age, sex, clinical T or N category, or performance status. The Nordic results have on the other hand led to cooperative efforts between urologists and oncologists in three out of six University Hospitals in Sweden, to single out patients with T2b-T3b urothelial

urinary bladder carcinoma – receiving neoadjuvant chemotherapy. This in an effort to define the subgroup having the highest benefit of this treatment. The main caveat of the mentioned nordic subgroup analysis was that neither of the two nordic trials were statistically designed for specific subgroup analysis.

19.6 Neoadjuvant Chemotherapy and Downstaging

The downstaging effect on the primary tumor of neoadjuvant chemotherapy is well known, and has also been utilized as indicator of treatment-response (Schultz et al. 1994). In a follow-up investigation of selected patients from the prospective first and second Nordic Cystectomy studies, 424 eligible patients (T2-T4aNXM0) were analyzed in terms of T-stage and postoperative pT and pN-stage. Downstaging as relative occurrence of pT0N0 was calculated, furthermore actual downstaging defined as pT < original T-stage was analyzed. The outcome of pT0 stratified for T-stage and treatment regime, showed small differences. The actual downstaging analysis showed a significantly higher rate of downstaging in the experimental T3-group ($p < 0.001$). In the T3-group we saw a significant difference ($p = 0.0056$) between the occurrence of organ confined cancer in patients of the experimental regime compared to patients of the control regime, with 62.3% in the former and 43% in the latter. There was also a tendency for lower occurrence of nodal metastases in the experimental clinical T3-group ($p = 0.0635$) (Fig. 19.2) (Sherif et al. 2006a). Some investigators have interpreted downstaging after neoadjuvant chemotherapy as a surrogate marker for efficacy and improved survival (Takata et al. 2005, Herr et al. 2007). Theoretically,

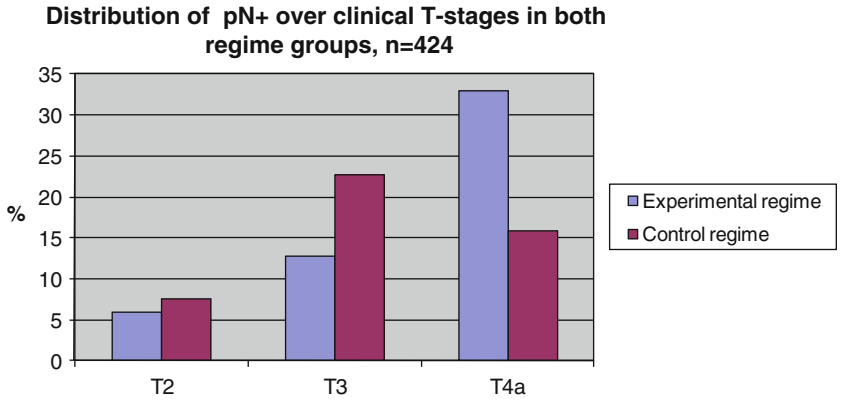

Fig. 19.2 The distribution of final positive pN-stages over clinical T-stages. In the clinical T3-group we encountered a marked difference between the patients who had received neoadjuvant chemotherapy versus the control group. In the former subgroup 12.8% of the patients were classified as pN + and in the latter, 22.7%. Still this difference did *not* reach statistical significance ($p = 0.0635$). The T2-group displayed almost no difference. The T4a-patients were too few ($n = 28$) to be taken in consideration for evaluation (Adapted from Sherif et al. 2006a)

downstaging of the primary tumor might indicate efficacy relating to nodal status in a favorable manner, thus improving survival chances. Still it should be noted that downstaging has, in the aspect of denoting improved survival, *not yet been tested* in a proper randomized prospective manner. Although it might be tempting to translate successful downstaging with improved survival, selection bias can lure the investigator, especially with the background of the TUR-B in itself being a strong instrument in terms of tumor reduction. Any future prospective evaluation of survival benefit from the downstaging per se would need to carefully delineate the exact preoperative T-stage prior to TUR-B and also most probably with only a limited diagnostic resection as starting point. Another likely, but still not investigated advantage of the downstaging, might be the improved resectability of the primary tumor at the time of cystectomy. Although this is an accepted notion among surgeons performing the procedure, the hypothesis has still not been subject to scientific evaluation.

19.7 Advantages and Disadvantages of Neoadjuvant Chemotherapy

19.7.1 Advantages

- Chemotherapy is delivered early in the process, when the burden of micrometastatic disease is expected to be low.
- The tolerability of chemotherapy is expected to be better before than after cystectomy.
- The patient's general status is expected to be better before than after cystectomy

19.7.2 Disadvantages

- Theoretically delayed cystectomy might compromise the outcome in patients not sensitive to chemotherapy.
- Although present randomized prospective trials, and meta-analyses thereof, have shown survival benefits in favor of the neoadjuvant regimens, the burden of postponing the definitive treatment lays heavily on the minds of the patients. Thus the "delay" can be a psychological problem for the patients.

19.8 Future Perspectives

The methods we have today for deciding which patients are suitable for neoadjuvant treatment and which are not, are still very blunt. The essence of our quest is to identify patients likely to be responders of the neoadjuvant treatment and likewise to identify the non-responders, in order to tailor our treatment. The uncertainty in

defining preoperatively what patients are to be staged as T2b-T3b versus other T-stages (especially T2a-patients) is one major problem in clinical praxis. Still utilizing the T-stage system in itself for decision making might also be inadequate. Some investigators have instead decided to use neoadjuvant chemotherapy for all muscle invasive T-stages in light of the results of the ABC-metanalyses and also because of the lack of prospective investigations among different T-stage subgroups associated with muscle invasive disease (Herr 2009).

The most optimal and cost-effective decisions could be made if we had tools and methods for discerning two aspects of tumors to be treated. First of all, if we had reliable tests on chemosensitivity, it would have enabled us to detect early in the process what patients would benefit the most. By testing tumor samples from the original TUR-B we would be able to determine what patients would have tumors that might respond best to treatment. Second, there is a need for having a reliable method of preoperative imaging for detection, not only of macrometastatic deposits, but mainly of micrometastatic dissemination. Thus, patients who theoretically would be more likely to benefit from the treatment could be chosen. This would not only add to the proper choice of responders, but would also optimize the situation for non-responders. Thus allowing the latter group to proceed to immediate cystectomy and spare them chemotherapy with low or none efficacy.

19.9 Chemosensitivity

Chemoresistance and chemosensitivity for different malignant tumors have started to be explored; the aim has been to identify molecular markers predictive of response versus non-response to chemotherapy. In an attempt to correlate mdm-2, p53, and bcl-2 with survival and chemosensitivity in a group of patients with muscle invasive urinary bladder carcinoma, T2-T3N0MO, who had undergone neoadjuvant chemotherapy and radically intended surgery, the authors could not pinpoint any prognostic factor or combination of factors (Maluf et al. 2006).

In an investigation from 2005, genome-wide gene expression profiling was performed on patients undergoing neoadjuvant MVAC-regiments for invasive urinary bladder carcinoma (Takata et al. 2005). Fourteen predictive genes were identified, whereof 12 finally were validated by quantitative RT-PCR. The end-point in this study was downstaging and not cancer-specific or overall survival. Furthermore, a multivariate analysis including T-stage and pN and pM-stages was not performed. In a validation trial from 2007, the same group investigated the use of the identified 14 markers in a small set of patients and found that the scoring system correctly predicted clinical response for 19 of the investigated 22 patients. The group of patients with positive predictive scores had significantly longer survival than that with negative scores. Thus, the results implied that patients with positive scores were likely to benefit from M-VAC (Takata et al. 2007). In a Danish trial from 2007, global gene expression profiling was used to identify possible prognostic molecular markers in a cohort of 30 patients. Two of the markers (Emmprin and

Survivin) were validated using immunohistochemistry on tumor samples from an independent cohort of 124 patients. All the patients in the experimental group had histology locally advanced (T4b and N2-3) or metastatic (M1) transitional urinary bladder cell cancer. Multivariate analysis identified emmprin expression (hazard ratio, 2.23; $p<0.0001$) and surviving expression (hazard ratio, 2.46; $p<0.0001$) as independent prognostic markers for poor outcome, together with the presence of visceral metastases (hazard ratio, 2.62; $p<0.0001$). If the tumor was negative for both emmprin and survivin, the response rate to the treatment was 82%, as opposed to 27%, if both immunohistochemistry results were positive (Als et al. 2007). Although this was not a trial on neoadjuvant chemotherapy, the implications are obvious. A rather non-complicated and cost-effective method could be utilized for further prospective investigations on patients planned for neodjuvant chemotherapy.

19.10 Problems with Accuracy in Tested Chemosensitivity

Apart from technical and methodological problems related to reliability of, for instance, immunohistochemistry or other more complicated and less cost-effective methods, there might even be other inherent obstacles in the whole concept of predicting chemosensitivity/chemoresistance.

1. Cellcycle-dependant resistance to chemotherapy
2. The phenotypical status of micrometastatic and small macrometastatic deposits in terms of transformational status at the time of received neoadjuvant treatment
3. The genotypical status of micrometastatic and small macrometastatic deposits in terms of survival selection and genetic evolution in contrast to corresponding status in primary tumor as evaluated from the TUR-B specimens

The rationale of delivering multiple cycles of neoadjuvant chemotherapy is to affect micrometastatic deposits and preoperatively undetected smaller areas of macrometastatic dissemination, which might contain cells in different phases of cell division. Cells considered to be dormant in G0-phase at one time of treatment, might be in a more susceptible phase at the time of cycle two or three. Still there might be substantial amounts of cells, which escape treatment just due to timing logistics, and thus remain intact after finalized treatment. Another aspect of the problem, are the cancer cells being in transition through the body at time of administered chemotherapy. The process of epithelial to mesenchymal cell transition (EMT) and back through mesenchymal to epithelial transition (MET) is a verified process in propulsive dissemination of cancer cells, in which the phenotypes in the specific cancer cells are altered. (Baumgart et al. 2007) This is in order to enhance transportation in the body and in known routes of dissemination and then returning to their original phenotype once they have reached their target organ. We have no conclusive data on how this altered phenotype either is an advantage or disadvantage

in terms of susceptibility to chemotherapy. The third aspect relates to evolutionary differences between primary tumors and disseminated deposits. There are data in favor of an evolutionary drive, in which established tumor colonies have undergone survival selection, thus rendering them a different genotype compared to the genotype of the primary tumor (Malmstrom et al. 2002). Any preoperative tests on tumor biopsies and/or TUR-B specimens might then not reflect the true nature of the target population of disseminated cells.

19.11 Methods of Preoperative Imaging of Nodal Dissemination

CT-scan, MDCT, and MRI have each shown to be not sufficiently accurate in detection of nodal dissemination. In an open prospective trial published in 2009 the investigators tried to utilize 2-deoxy-2 [F] fluoro-D-glucose (FDG) positron emission tomography (PET) in combination with CT (FDG-PET/CT) for improving preoperative nodal staging. However, mainly relating to accuracy and sensitivity, the results did not show any substantial benefit (Swinnen et al. 2010). Preoperative sentinel node detection, especially with enhancement in form of SPECT/CT has in small series showed interesting results in detection of nodal drainage (Sherif et al. 2006b). Still, in a neoadjuvant protocol, sentinel node detection would request different logistics than presented with an additional surgical nodal dissection. This is prior to both treatment decision, that is, neoadjuvant chemotherapy or not, and to definitive treatment.

19.12 Conclusive Remarks

Neoadjuvant chemotherapy as part of the therapeutic arsenal in radical treatment of advanced urinary bladder cancer is an option being utilized more and more. The ABC-meta-analysis paved the way for the routine introduction of this regimen. Still there remain a number of unanswered questions. What patients are to be considered for this treatment?

Some investigators have proposed that neoadjuvant chemotherapy should be the standard care in *all* muscle invasive urothelial urinary bladder cancers, others would like to focus on the T2b-T3b subgroups. The question relates directly to the problem of identifying responders versus non-responders. Some interesting and primarily successful attempts have been made to identify molecular markers denoting susceptibility to the treatment and it is now up to the scientific community to investigate these kind of matters accordingly. Large prospective series need to be investigated with reference to suggested molecular markers. Defining which patients will benefit from the treatment will enhance our chances to improve long-

term survival results, increase quality of life, and to be optimal on matters of cost-effectiveness.

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Chapter 20
Diagnosis and Treatment of Upper Tract Urothelial Carcinoma

Mesut Remzi and Matthias Waldert

Abstract Urothelial carcinoma of the upper tract (UTUC) is a not common and only level 2b evidence is found in the literature. Typical symptoms are gross hematuria and/or flank pain, but it can be also asymptomatic. Further evaluation is done best by multislice CT scans or retrograde pyelography (RP). The value of cytology is only minor. Diagnostic ureterorenoscopy (URS) has only a place if diagnostic workup is unclear or if minimal invasive therapies will be performed. Standard treatment is still radical nephroureterectomy (RNU) with a bladder cuff, which can be performed open or laparoscopically. However, in distal single ureter tumors, ureterectomy with bladder reconstruction has a place in treatment options, and thus nephron-sparing surgery is also a matter of debate, especially in low-grade low-stage tumors.

20.1 Diagnosis of UTUC

Typical symptoms of upper tract urothelial carcinoma (UTUC) are gross hematuria and/or flank pain. In a recent European study, 40% of patients were detected with gross hematuria, 8% had flank pain, 12% had gross hematuria and flank pain, and 40% had no symptoms (Waldert et al. 2009). Patients with no symptoms are usually detected because of dilatation or hydronephrosis of a kidney, which was found by chance during evaluation of other diseases. In a large multicentre trial from the UTUC collaborations, 32% ($n=200$) had no symptoms, 63% ($n=395$) had local symptoms, and 5% ($n=34$) had no symptoms (Fernández et al. 2009).

About ¼–1/3 (Margulis et al. 2009; Waldert et al. 2009) of patients with UTUC have a previous diagnosis of transitional cell cancer (TCC) of the bladder. In two

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thirds of patients, the index UTUC is found in the renal pelvis and one third in the ureter (Margulis et al. 2009; Remzi et al. 2009; Waldert et al. 2009).

Diagnostic workup in case of gross hematuria includes physical examination, ultrasound of the kidney and bladder, cytology, and cystoscopy. In suspicion of UTUC, an excretory urography (EU), a computer tomography (CT), or magnetic resonance imaging (MRI) can be performed to further stage the tumor (Table 20.1). Additionally, a retrograde pyelography (RP) can be performed. Assessment of the entire urothelium is essential because of the multifocal nature of UTUC (Hall et al. 1998; Hisataki et al. 2000; Hafner et al. 2001; Novara et al. 2007; Gupta et al. 2008; Fernández et al. 2009; Kikuchi et al. 2009; Margulis et al. 2009; Remzi et al. 2009; Waldert et al. 2009) (Figs. 20.1 and 20.2).

20.1.1 Urinary Cytology

Most data on cytology are related to TCC of the bladder. The impact of cytology in UTUC is not well defined. Cytology can be performed from spontaneous urine, washing urine of bladder or as barbotage, washing or brush cytology from the upper urinary tract (Hughes et al. 2000; Lodde et al. 2001; Xu et al. 2002; Siemens et al. 2003; Painter et al. 2007).

The sensitivity of urinary cytology correlates closely with pathologic tumor grade and presence of CIS as in bladder cancer.

Table 20.1 Advantages and disadvantages of imaging utilities for diagnosis and staging of upper urinary tract urothelial carcinoma (UTUC)

Imaging technique	Advantages	Disadvantages
Ultrasound (US)	– Easy available – Not time consuming – No radiation – No contrast media – Detection and grading of hydronephrosis	– Only indirect signs of UTUC – No tumor visualization in the ureter – Only large tumors visible – No adequate staging possible
Excretory Urography (EU)	– Easy available – Evaluation of the contralateral kidney function	– No staging – High false-positive rate: blood clots, stones, fungi – High false-negative rate (40%) – Low sensitivity – Radiation/contrast media
Multidetector computed tomography urography (MDCTU)	– High accuracy in diagnosis and staging – Evaluation of the contralateral kidney – Widespread availability	– Radiation/contrast media

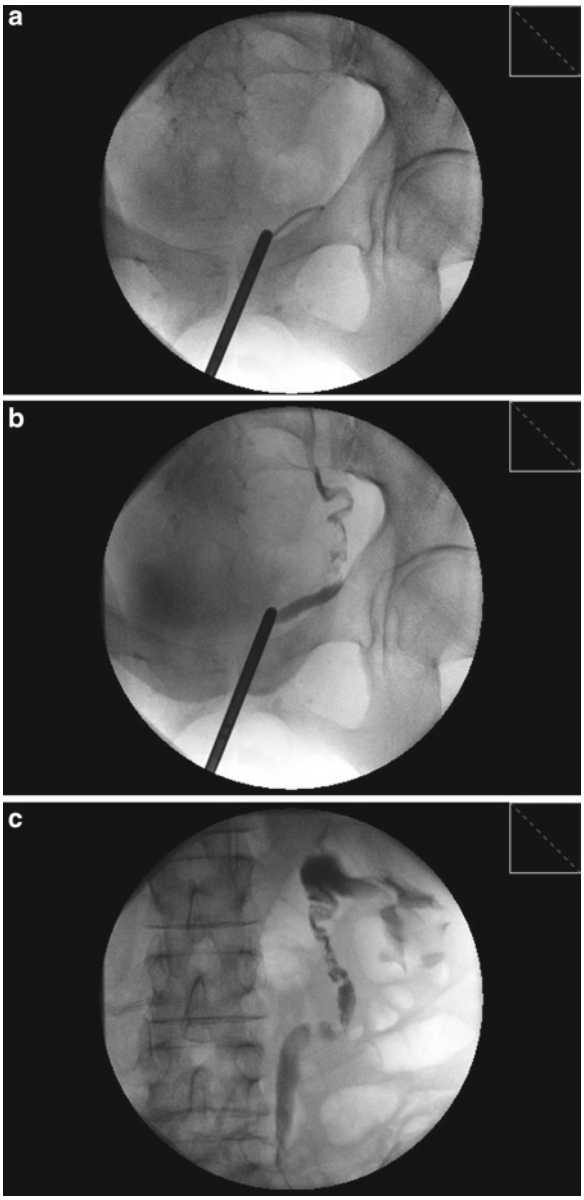

Fig. 20.1 Retrograde pyelography (*RP*) showing multiple filling defects. Final histology pT2, pN0, transitional cell cancer of the ureter. (**a**) Plain film showing the insertion of the uretercatheter for RP. (**b**) After insertion of a few milliliters, contrast media "typical" filling defects are shown. (**c**) Showing the multifocality of this UTUC

Cytology for UTUC: On the whole, it has been shown to be poor, with detection rates as low as 29% (Siemens et al. 2003). Selective upper tract cytology of high-grade

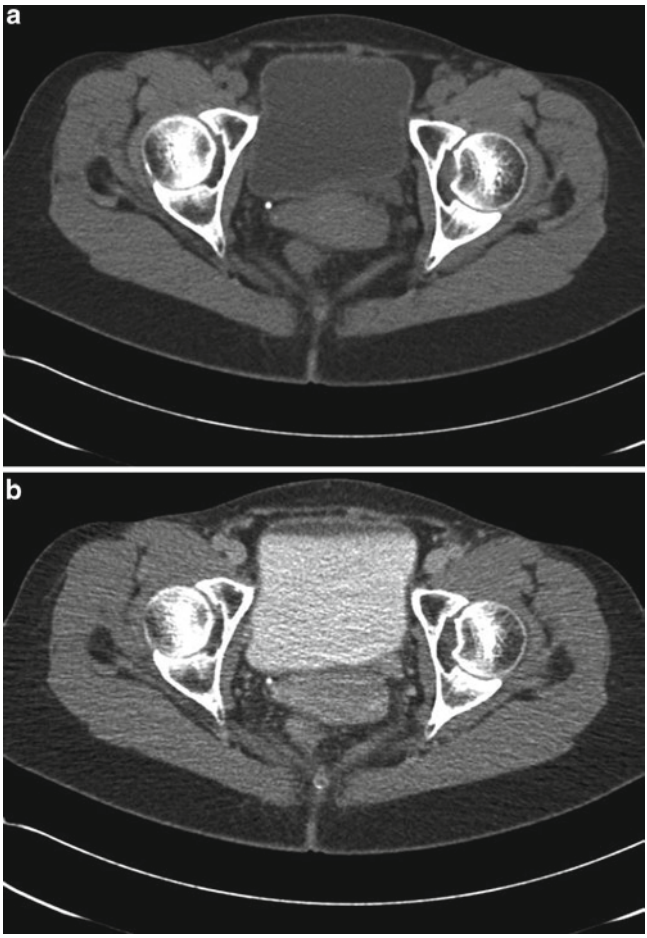

Fig. 20.2 Showing the same tumor from Fig. 20.1 in contrast-enhanced CT. Solid tumor of the distal ureter. (**a**) Plain CT 15 HU, (**b**) venous phase 95 HU

lesions (including carcinoma in situ) has a sensitivity approaching 80–100% (Hughes et al. 2000), and is sometimes the only pathology found at initial assessment. For well-differentiated tumors, the accuracy rates are even poorer with only 10–40% (Xu et al. 2002; El-Hakim et al. 2004) UTUC of the ureter is often combined with hydronephrosis and partial or complete obstruction; therefore, no urine from the kidney passes the ureter to the bladder and thus no cells can be found in the bladder.

Other urine tests are also available as for TCC of the bladder; however, the value of these is limited and they are not widely used (Lodde et al. 2001).

Urine cytology directly from the ureter by ureteral catheterizations provides more accurate results, but false-negative findings are reported in up 22–30% and instrumentation can lead to false-positive results. (Highman 1986; Akkad et al. 2007).

Thus, urine cytology has a limited role in diagnosis and monitoring of UTUC.

20.1.2 Ultrasonography

The value of ultrasonography (US) depends on availability and skill of the investigators. In many countries, ultrasound is 24-h available and the urologist performs it in every patient. With US, the degree of hydronephrosis can be measured, which can be an indirect sign of UTUC of the ureter. Renal pelvic UTUC typically appears as a central soft-tissue mass in the echogenic renal sinus, with or without hydronephrosis (Kirkali and Tuzel 2003; Browne et al. 2005). However, US has a limited role in the evaluation of ureter tumors and for staging in both renal pelvic and ureter UTUC. The ureter can rarely be visualized in its entirety, even if dilated. Moreover, ultrasound only allows limited information of periureteric tissues. UTUC grows infiltrative and does not distort the renal contour (Wong-You-Cheong et al. 1998). In selected cases, focal contour distortion can be observed. US is unable to stage UTUC on routine base.

20.1.3 Excretory Urography

In many departments, the imaging method in evaluating hematuria was the EU. Khadra et al. studied the efficacy of renal US and EU in detecting UTUC in 1930 patients presenting with hematuria (Warshauer et al. 1988). When used in isolation, both tests had significant false-negative rates of 42% for US and 27% for EU. Consequently, the authors advocated the use of a combination of both methods in all patients. More recent studies suggested that the use of EU can be reserved for specific groups of patients. In a retrospective study in over 4,000 patients, Edwards et al. (2006) used EU in those with abnormal US or in patients with persistent hematuria after normal first-line investigations. Thirteen cases (0.32%) of UTUC were found in patients older than 50, of which three were missed by US. One of the drawbacks of this study was that it did not report the long-term outcome of the apparently negative cohort.

The appearances of UTUC at EU are well described. In the pelvis, it usually manifests itself as a filling defect within the contrast-enhanced collecting system, which may be single or multiple and stippled, irregular, or smooth. The stipple sign is caused by diffusion of contrast media into the interstices of a papillary lesion (Kirkali and Tuzel 2003; Wong-You-Cheong et al. 1998) Possible other lesions causing that phenomenon may be blood clot and fungus balls. UTUC also can mimic stricture-like lesions of the pelvicaliceal system. If multiple, this sign may also be seen with renal tuberculosis (Lee et al. 1997). If the tumor causes obstruction of the caliceal infundibulum, filling defects within dilated calices may occur and lead to caliceal “amputation.”

Ureteric TCC is typically seen as a ureteric filling defect with or without surface stippling and ureteric dilation proximal to it. Long-standing obstruction of the ureter may lead to generalized hydronephrosis and poor or no excretion. Moreover, upper tract filling defects may be nonspecific at EU and obstruction and consecutively poor

excretion may obscure distal synchronous ureteric tumors. Overall EU is relatively poor at detecting upper tract malignancies, missing up to 40% of tumors (Mariani et al. 1989; Murakami et al. 1990; Khadra et al. 2000) Moreover, EU suffers by comparison to CT, not only for detection of UTUC, but also for staging.

20.1.4 Multidetector Computed Tomography Urography (MDCTU)

MDCTU is the gold standard for detection and staging of UTUC. MDCTU allows characterization of the UTUC lesion and also provides an evaluation of the contralateral renal unit as well as information of the adjacent structures and metastases.

UTUC has three appearances on CT (Caoili et al. 2005; Baron et al. 1982).

20.1.4.1 A Focal Intraluminal Enhancing Soft-tissue Density Mass

UTUC of the renal pelvis can often be seen as soft-tissue attenuation mass. The attenuation is typically lower than that of urinary calculi with the exception of indinavir stones. Since UTUC enhances after administration of contrast media, differentiation can be made from stones or clot. In rare cases, calcifications may be seen (<3%). Tumors appear as sessile or papillary lesions. (Remzi et al. 2009).

20.1.4.2 Urothelial Wall Thickening with Lumen Narrowing

In case of wall thickening of the renal pelvis or ureter, the appearance is normally symmetric. In some cases, it can be eccentric and is associated with circumferential narrowing of the lumen. The affected wall may also enhance after i.v. contrast administration, which may help in determining the local extension of the tumor. An important differential diagnosis is a thickening caused by inflammation related to infection, nephrolithiasis, hemorrhage, and after surgical intervention such as ureterrenoscopy or stent placement.

20.1.4.3 An Infiltrating Mass

The third observed form is a locally aggressive infiltrative renal mass (Baron et al. 1982; Hartman et al. 1988) Typically, infiltration of the renal parenchyma can be suspected if a hypoenhancing mass involving the renal parenchyma or a distortion of the regular renal architecture with heterogenous attenuation (Urban et al. 1997a, b) is observed. Renal sinus fat may be obliterated, and the tumor can show necrotic components (Urban et al. 1997a, b, Hartmann et al. 1988). Normally, the mass originates from the central region of the kidney and may or may not compromise

the renal contour. Differential diagnoses are: renal cell carcinoma, lymphoma, or metastasis (Hartmann et al. 1988). Typically, a more uniform enhancement is seen in comparison with renal cell cancers. Acute bacterial pyelonephritis can also pose as an infiltrative renal lesion but usually shows typical clinical symptoms.

In case of multifocality, multiple areas of wall thickening or masses are seen on CT.

In cases of hydronephrosis and hydroureter, a decreased perfusion of the involved kidney and delayed excretion of contrast media are observed (Urban et al. 1997a, b; Hartman et al. 1988). UTUC may be seen as an enhancing soft-tissue mass partially or entirely surrounded by a fluid-filled collecting system.

The reported sensitivity of MDCTU for UTUC detection with a dedicated protocol ranges from 89% to 100%. (Caoili et al. 2005; Fritz et al. 2006; Mueller-Lisse et al. 2007) Older studies report nonvisualization in 20% of renal pelvis and 40% of ureteral tumors (McCarron et al. 1983; Auld et al. 1984). Problems in detection may arise where the ureter is not filled with contrast medium or is not dilated Caoili et al. (2005) reported in a retrospective series that MDCTU detected 89% of UTUC (24 out of 27).Ten lesions were seen as a mass and 14 as circumferential urothelial wall thickening. Five masses were smaller than 5 mm in diameter. Of the three undetected lesions, two were carcinoma in situ. MDCTU has also been compared to EU, with intrarenal and midureteric results favoring CT. (Heneghan et al. 2001) In a study in 86 patients presenting with microscopic hematuria and negative EU, CT detected eight renal parenchymal neoplasms (Lang et al. 2003) A recently published meta-analysis, which evaluated the performance of CT in UTUC diagnosis in patient with hematuria confirmed that MDCTU is a highly specific and sensitive imaging method for the evaluation of patients with hematuria (Chlapoutakis et al. 2009). MDCTU alone identified the cause of hematuria in 25.5% (Albani et al. 2007) to 76% (Tsili et al. 2007).

Other studies led to similar results so that in our institution MDCTU (Gray Sears et al. 2002) is used routinely as the initial assessment of hematuria.

CT is the standard in the assessment of the local and distant spread of UTUC. Unfortunately, CT or any other imaging technique cannot accurately differentiate between Ta, T1, and T2 tumors (Baron et al. 1982). But clinically the more important question is the differentiation between early-stage (T1 and T2) and advanced-stage (T3 and T4) tumors. (Huben et al. 1988) Stage T3 disease is diagnosed when the UTUC infiltrates the renal parenchyma or the peripelvic fat. Thus, the peripelvic fat is obliterated by soft-tissue density (Nyman et al. 1992). Problems in staging can arise when the tumor shows no signs of infiltration on CT, thus leading to understaging or infiltration may be simulated on CT by superimposed infection, hemorrhage or secondary inflammation. (Nyman et al. 1992; Urban et al. 1997a, b; Fritz et al. 2006).

Metastasis to lymph nodes and other organs can also be detected on CT, but microscopical nodal involvement remains a weak point of CT staging.

Studies that predate the MDCT era reported a variable accuracy with a range of 36–83% (Anderson et al. 2007). Recently Fritz et al. (2006) reported a MDCT detection rate of 100% and an accuracy of 88% in predicting T0a-T2 versus T3-T4 stage in 36 of 41 UTUC.

20.1.5 Magnetic Resonance Imaging

MRI is rarely used in the primary assessment of UTUC, and tumor characteristics are not well described. MR imaging evaluation of UTUC should always include MR urography. TCC has lower signal intensity than the urine on T2-weighted images, permitting a good visualization of tumors in a dilated collecting system. However, since TCC is isointense to renal parenchyma gadolinium, contrast media is necessary for accurate staging of tumor extent. (Wong-You-Cheong et al. 1998; Walter et al. 2003) Although TCC is a hypovascular tumor, moderate enhancement is seen after gadolinium administration, although not to the same degree as normal renal parenchyma. Gadolinium-enhanced MR urography with or without diuretics is a helpful alternative in patients in whom urography with iodinated contrast material is not possible. Ureteric TCC typically appears as an irregular mass, whereas stones appear as sharply delineated filling defects, although differentiation may be difficult. (Yousem et al. 1988) Tumor enhancement after gadolinium administration can improve the differentiation. Soft-tissue stranding in the periureteric fat can be a sign of periureteric extension.

Despite its advantages, MRI remains second line to CT. The main disadvantage is the inability to detect urinary stones and air, which limits its use as a first-line test. Moreover, spatial resolution is poor compared to CT urography, making detection of small UTUC less likely (Kawashima et al. 2003). Several studies reported a significant incidence of false-positive filling defects and one a missed UTUC. (Verswijvel et al. 2000; Girish et al. 2004).

However, no recent data are available for new high-resolution (3–10 T MRI) or special MRI methods (spectroscopy, etc.) are available.

20.1.6 Image-Guided Biopsy

Whereas image-guided biopsy, especially CT-guided biopsy gained popularity in preoperative diagnostic setup of small renal cell carcinomas (Schmidbauer et al. 2008; Remzi and Marberger 2009), it is not recommended for UTUC evaluation, because of the high probability of tumor seeding. (Remzi and Marberger 2009).

20.1.7 Retrograde Pyelography

RP is an invasive examination and can be performed during cystoscopy or preoperatively under local or general anesthesia to further evaluate abnormalities detected, in nonexcreting kidneys or in case of contrast media allergy. It allows confirmation of the radiologic diagnosis and selective localized urine collections

of the upper urinary tract with subsequent cytologic examination. UTUC appears as an intraluminal smooth, irregular or stippled filling defect. If the TCC is located at caliceal infundibulum, an amputated calix may be seen with or without focal hydronephrosis. Ureteric TCC appears as a polypoid filling defect with dilation of the ureter and collecting system proximal to it. In case of infiltration of the paraureteral tissue, the ureter may be fixed. Localized dilation around and distal to the tumor may give rise to the "goblet" signs, which is caused by slow tumor growth with consecutive lumen expansion. It does not occur in acute obstruction. Malignant strictures may be circumferential or eccentric accompanied by ureteric fixation and nontapering margins. In our department, an RP is performed in all cases preoperatively. In the last 14 years, no patient had a benign tumor or pT0 after radical nephroureterectomy (RNU). (Waldert et al. 2009)

20.1.8 Diagnostic Ureterorenoscopy Plus Biopsy

Diagnostic ureterorenoscopy (URS) allows the direct visualization of the lesion and biopsies can be performed. Inspections of the lesions might accurately order the lesion in high grade and low grade. El-Hakim et al. (2004) have been shown that 20/28 (71%) and 8/10 (80%) of the lesions believed to be low or high grade were actually low and high grade, respectively. Tumor architecture (papillary vs sessile) correlated well with tumor aggressiveness (Remzi et al. 2009). Thus, it could be a useful marker in selected patients to guide conservative versus extirpative further therapy. The correlation of ureterorenoscopy biopsy and pathological stage and grade has been assessed by several studies (Table 20.2). In the current literature (Keeley et al. 1997; Guarnizo et al. 2000; Shiraishi et al. 2003; Skolarikos et al. 2003; El-Hakim et al. 2004, Williams et al. 2008) the diagnostic accuracy of URS biopsies correlated to final pathology was only 75%. Thus, diagnostic URS and biopsy have only limited roles and should be followed when performed by an organ-preserving endoscopic approach in low-risk UTUC.

Table 20.2 Role of biopsy during diagnostic ureterrenoscopy. Correlation between biopsy grading and final histology grading

Author	No. cases (*n*)	No. of evaluated (%)	No. grading correct (%)
Guarnizo et al. (2000)	40	40 (100%)	31(78%)
Shiraishi et al. (2003)	40	31 (88%)	18 (58%)
Williams et al. (2008)	30	30 (100%)	23 (75%)
Keeley et al. (1997)	51	42 (82%)	38 (90%)
Skolarikos et al. (2003)	62	51 (82%)	35 (69%)
Overall	223	194 (87%)	145 (75%)

20.2 Surgical Treatment

Standard treatment for UTUC is the radical nephroureterectomy (RNU) with resection of a bladder cuff. This can be performed open or laparoscopically. The role of laparoscopy for the treatment of renal malignancies has been examined especially in renal cell cancer, and for T1 and T2 diseases it has been considered to be the gold standard (EAU guidelines update 2010) (Ljungberg et al. 2010). This is due to the fact that laparoscopic radical nephrectomy has the same long-term oncological outcomes with significant less morbidity. In a recent review of laparoscopic radical nephrectomy, it was shown that this approach has less blood loss, lower postoperative pain, quicker reconvalescence, less postoperative complications like hernia and wound infections, and a better cosmetic result compared to open RNU (Nguyen et al. 2008). For the same reasons laparoscopic RNU (LRNU) has attracted considerable interest, but the treatment of the distal ureter remains under debate, and urothelial carcinoma has a much higher potential risk for spreading at the surgical management. Therefore, LRNU must copy the oncological results of open RNU (ORNU), with no surgical compromises, i.e. "no-touch" technique, excision of the entire ipsilateral tract with a bladder cuff as a whole specimen, and no opening of the collecting system.

20.2.1 Oncological Results of ORNU and LRNU

RNU is the standard treatment for UTUC because tumor recurrence rates are reported in up to 58% in the remaining distal ureter if left in place after simple nephrectomy, multifocality on the same side in up to 44%, and a low incidence of consecutive bilateral tumors (2–5%) (Oosterlinck et al. 2004; Zigeuner and Pummer 2008). Additionally, follow-up after complete resection of the entire ureter and bladder cuff is easier to perform.

Surgical procedures for RNU include a perifascial nephrectomy including the removal of the perirenal fat capsule and Gerota's fascia (Johansson and Wahlqvist 1979; Arancibia et al. 2007) and without transecting the ureter (Oosterlinck et al. 2004) and removal of a distal bladder cuff (no touch technique).

Oncological outcomes after ORNU and LRNU are shown in Tables 20.3–20.5. In a large study from Graz, Austria also, tumor grade was important with a 5-year metastasis-free survival of 85% in low-grade disease compared to 32% in high-grade disease (Langner et al. 2006).

Most series only reported short-term follow-up. Nevertheless, there were no statistically significant differences concerning all relevant oncological results such as bladder recurrence, local recurrence, and occurrence of distant metastasis (Tables 20.3–20.5).

Until now, only about ten cases of port site metastasis after LNU have been reported (Ahmed et al. 1998; Schaeff et al. 1998; Otani et al. 1999; Ong et al. 2003;

Table 20.3 Single-center series comparing cancer-specific survival after open and laparoscopic RNU

	Waldert et al. (2009)	Roupret et al. (2007)	Hsueh et al. (2007)	Capitanio et al. (2009)
Years	1999–2006	1994–2004	1998–2005	1987–2007
Number of patients	102	46	143	1,249
ONU	59	26	77	979
LNU	43	20	66	270
Mean follow-up (month)				
ONU	41	78	54	62
LNU	41	69	38	
5-year CSS survival				
ONU	80%	62%	88% [a]	73.1%
LNU	85%	90%	92% [a]	85.8%

[a] Only pT1 tumors

ONU Open nephroureterectomy, *LNU* Laparoscopic nephroureterectomy

Table 20.4 Series of laparoscopic nehproureterectomies

Author	*N*	2 years CSS%	5 years CSS%	Bladder recurrence%
Barrett et al. (1998)	22	91	na	22.7
Shalhav et al. (2000)	13	77	na	23
Cicco et al. (2000)	7	69	na	na
McNeill et al. (2000)	25	74	56	25
Jarrett et al. (2001)	25	86	na	48
El Fettouh et al. (2002)	116	87	na	24
Klingler et al. (2003)	19	94.7	na	10.5
Rassweiler et al. (2004)	23	89	81	34.8
Bariol et al. (2004)	26		72	28
Chung et al. (1996)	39		90	44
Hsueh et al. (2007)	66		92 (pT1)–80 (pT3)	20
Muntener et al. (2007)	115		68	55
Roupret et al. (2007)	20		89 (low grade) 63 (high grade)	10

Muntener et al. 2007; Yasuda et al. 2009). In the previous reported cases, removal of the resected organ was done without an organ bag or with a defective organ bag. The remaining cases were explained due to unsuspected TCC, where the strict rules of en bloc removal of the kidney and ureter without opening of the collecting system were not adhered to (Ahmed et al. 1998; Barrett et al. 1998; Schaeff et al. 1998; Otani et al. 1999; Ong et al. 2003; Matsui et al. 2004; Naderi et al. 2004; Muntener et al. 2007; Yasuda et al. 2009). Most series of LNU reported no port site metastases, all of them used an endobag for specimen retrieval. In one recent report from Muntener et al. (2007), out of 115 patients treated with LNU, only 1 had port site metastases (0.9%) Most recently, a Japanese group (Yasuda et al. 2009) reported one port site metastases after LNU in a pT3, pV1, pN2 patient (Fig. 20.3).

Table 20.5 Series of open radical nephroureterctomies

Author	*N*	2 years CSS%	5 years CSS%
Corrado et al. (1991)	124	81	67
Charbit et al. (1991)	92	76	67
Komatsu et al. (1997)	36	–	74
Jurincic-Winkler et al. (1993)	54	71	62
Hall et al. (1998)	194	–	49
Angulo et al. (1998)	15	80	–
Miyake et al. (1998)	72	78	58
Shalhav et al. (2000)	13	69	–
McNeill et al. (2000)	42	68	64
Rassweiler et al. (2004)	21	83	63
Lehmann et al. (2007)	145	–	96-29
Kikuchi et al. (2005)	173	–	85-40
Miyata et al. (2007)	125	–	80-50
Kondo et al. (2007)	181	–	85-16
Secin et al. (2007)	252	–	61
Munoz and Ellison (2000)	9,072	–	95-17
Racioppi et al. (1997)	100	–	70
Chung et al. (1996)	36	–	86
Park et al. (2004)	86	–	83
Novara et al. (2007)	269	–	76

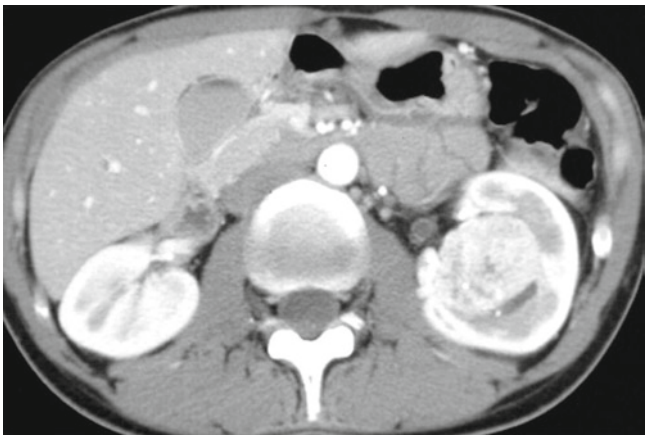

Fig. 20.3 Contrast enhancing central lesion. Pelvic TCC pT3 pN0

20.2.2 Morbidity of ORNU and LRNU

Rassweiler reported a range of 0 and 40% (mean 12.9%) of minor complication for LNU versus 0–45% (mean 11.2%) for ONU. The rates for major complications were similar ranging from 0% to 19% (mean 5.6%) in LNU patients and 0–29% (mean 8.3%) in ONU patients (Rassweiler et al. 2004). The major complication rate

in modern series was 3.4% for ONU and 2.3% for LNU and thus comparable (Waldert et al. 2009) Postoperative morphine usage was lower and hospital stay shorter after LNU. In most studies that reported complications after LNU, some variability is caused due to learning curve time, surgeon skill, and patient selection. Further, in LNU the nephrectomy part is only a component of a larger procedure, and LNU patients are generally older and may suffer from other comorbidities.

In a meta-analysis, Pareek et al. (2006) noted a total of 2.3% minor and 18.8% major complications for nine different laparoscopic renal interventions. LNU and partial nephrectomy had the highest complication rates.

20.2.3 Management of the Bladder Cuff During LNU

The distal ureter and bladder cuff can be managed in four ways:

1. Open excision
2. Laparoscopic stapling
3. Transvesical laparoscopic detachment and
4. Ligation and the "pluck" technique

20.2.3.1 Open Excision

In the open technique, the kidney is removed laparoscopically. After completion, the ureter is clipped to avoid downstream seeding and spillage in the retroperitoneum of tumor cells. Afterward either midline, Gibson or Pfannenstiel incision is performed, through which the distal ureter is removed en bloc with a bladder cuff. The open technique has been evaluated by Klingler et al. (2003) and more recently by Waldert et al. (2009) who found no statistical significant difference in tumor free survival rate compared to ONU. Matsui et al. (2002) reached the same conclusion. The risk for 2 year tumor recurrence and cancer-specific survival rates were similar between ONU and the laparoscopic approach with an open technique of handling the distal ureter and bladder cuff.

20.2.3.2 Laparoscopic Stapling

Laparoscopic stapling of the bladder cuff and distal ureter is combined with ureteral unroofing via cystoscopy and placement of balloon catheter in the intramural ureter. Stapling is subsequently performed laparoscopically during the distal dissection. Shalhav et al. (2000) and Yoshino et al. (2003) compared this technique with ONU. Both techniques had identical oncological outcomes. In contrast, Matin and Gill (2005) reported a higher rate of positive margins and a poorer recurrence-free survival with a laparoscopic stapling approach compared to ONU or the transvesical laparoscopic approach (25% positive margins vs 2.8%). Brown et al. (2005)

reached a similar conclusion. They compared open dissection, transvesical approach, laparoscopic stapling, and a hand-assisted laparoscopic approach. The positive margins rate was 29% for the stapling approach, followed by the hand-assisted approach with a 10% rate.

Further, the risk of stone formation due to migration of staples into the bladder mucosa should be kept in mind. Not described in the initial series in 1993, there has been a recent case study, which reported the presence of intravesical titanium staple line 6 months after LNU (Baughman et al. 2003).

20.2.3.3 Transvesical Laparoscopic Detachment Technique

Gill et al. (1999) first reported the transvesical laparoscopic technique. Needlescopic ports are placed suprapubically into the bladder. Then uretheral catheter is placed in the ipsilateral orifice through an endoloop, and the bladder cuff and ureter are dissected with a Collins knife. Compared to ONU patients, the transvesical laparoscopically treated patients had a comparable cancer-specific and recurrence-free survival (Gill et al. 2000). A potential risk of this technique is the risk of fluid extravasation and subsequent tumor seeding although a meta-analysis of the literature revealed no reports of tumor seeding in over 50 patients (Matin and Gill 2005).

20.2.3.4 Ligation and the "Pluck" Technique

The "pluck" technique combines two techniques. Initially an aggressive transurethral resection of the ipsilateral ureteral orifice is performed. The renal unit and ureter are removed laparoscopically (McNeill et al. 2000) The major drawbacks of this technique are the tumor seeding and the possibility to leave behind a segment of an incompletely resected ureter (Hetherington et al. 1986; Jones and Moisey 1993). The exact incidence of tumor seeding is unknown but the theoretical potential and one reported fatal recurrence led some groups to abandon this technique.

New aspects include the use of a flexible cystoscope combined with a hand-assisted laparoscopic approach. Vardi et al. (2006) and Kurzer et al. (2006) did not close the bladder after ureteral resection. The first group had no retroperitoneal/abdominal recurrence after a mean follow-up of 31 months, whereas the second reported one retroperitoneal metastasis after 10 months. Caution is advised in drawing conclusions from these findings since patient numbers are small and follow-up is short in both studies. First reports reporting the usage of a robot to perform distal ureterectomy are also available (Nanigian et al. 2006; Rose et al. 2006).

The message in almost all reports discussing distal ureter management is that the best approach is the one the surgeon has the most experience with as long as the above-stated oncological principles are adhered to.

20.2.4 Endourologic Treatment

The goal of endoscopic management is cancer control while preserving renal function and integrity of the ipsilateral renal unit. Recommendations for endoscopic management include patient with anatomic or functional solitary kidneys, renal insufficiency, bilateral UTUC or significant comorbidities. The 5 year survival rate of dialysis patients is around 40%, thus lower than the anticipated 5-year survival of conservatively treated patients (Soderdahl et al. 2005; Argyropoulos and Tolley 2007) Treatment can be performed via two pathways: retrograde endoscopic or percutaneous antegrade approach.

Today the ureteroscopic resection is performed using semirigid or flexible ureteroscopes and holmium or Nd:Yag lasers (Arancibia et al. 2007). Limited by the size of the instruments only tumors <1.5 cm in diameter can be treated (Soderdahl et al. 2005; Argyropoulos and Tolley 2007). There is also a significant risk of understaging or grading because the small size of the ureteroscopes permits only limited tissue sampling. Due to this size limitation, a second look procedure is required in larger tumors to ensure complete removal. Furthermore, some areas like the lower pole calyces are not easily accessible and retrograde manipulation can be difficult in patients who have a urinary diversion.

The main complications are ureteral perforation and strictures. The incidence of perforation is low (<10% in most studies) and treatable with ureteral stenting or percutaneous nephrostomy drainage. The stricture rate ranges from 4.9% to 13.6% (Raman and Scherr 2007).

The recurrence rate for ureteroscopicaly treated tumors ranges from 31% to 65%, the disease free rate from 35% to 86%. Disease-specific survival has been reported from 81% to 100%, reflecting selection of patients with good prognosis. Disease recurrences were related to the location, size, grade, and multifocality of tumors (Raman and Scherr 2007).

Percutaneous treatment of UTUC can be offered to patients with large tumors of the pelvis (>1.5 cm), large tumors of the proximal ureter, and those inaccessible by ureteroscopy. Advantages include better visualization of the tumor and the accommodation of larger instrument to handle larger tumors. The main disadvantage of this approach is the violation of urothelial integrity with reported tumor seeding around the kidney or in the nephrostomy tract. It has however only been reported twice (Huang et al. 1995; Yamada et al. 2002). Published local recurrence rates range from 23% to 88%, disease-specific survival from 69.2% to 94.1% (Argyropoulos and Tolley 2007). Generally, stage, grade, and location of the UTUC are more important for recurrence and survival than extensive surgical resection (Soderdahl et al. 2005).

Complication rates are generally low and related to the number of sessions. Transfusion rates are >20% in most series. Stricture of the ureteropelvic junction with consecutive hydronephrosis is less common (Argyropoulos and Tolley 2007).

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Chapter 21
Chemotherapy for Metastatic Bladder Cancer

Maria De Santis and Mark Bachner

21.1 Introduction

Approximately 30% of patients with urothelial carcinoma (UC) present with muscle invasive disease. Depending on the pathological stage of the primary tumor, the nodal status, and the quality and extent of surgery (Ghoneim et al. 1997; Bassi et al. 1999; Dalbagni et al. 2001; Stein et al. 2001; Stein 2006; Stein and Skinner 2006), about half of them will relapse after radical cystectomy. Local recurrence accounts for about 30% of relapses. Distant metastases are more common (Rosenberg et al. 2005; Calabro and Sternberg 2009). Furthermore, a considerable number of patients are already metastatic at the time of diagnosis (David et al. 2009). All of these patients are in need of systemic treatment options.

Before the development of effective chemotherapy, the median survival of metastatic UC rarely exceeded 3–6 months (Sternberg et al. 1999). UC is considered to be chemosensitive.

However, results with chemotherapy in UC have reached a plateau with no recent evidence of survival improvement using new combinations or targeted agents (Garcia and Dreicer 2006; Bellmunt and Albiol 2007; Gallagher et al. 2008a).

This chapter will focus on the development and potentialities of chemotherapy and its optimal use, as well as on its limits in metastatic bladder cancer.

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21.2 Prognostic Factors and Treatment Decisions

Although UC is a chemosensitive tumor, chemotherapy will not be beneficial for all patients and not every patient will tolerate standard treatment. Response rates (RR), progression-free survival (PFS), and overall survival (OS) differ as a function of patient-related factors and the extent of pretreatment disease. Prognostic factors for response and survival were identified. In several phase II and randomized phase III trials, potential markers were evaluated. On multivariate analyses, a poor performance status (PS), the presence of visceral metastases, and elevated alkaline phosphatase levels were statistically significant predictors of poorer survival (Bajorin et al. 1999; Sengelov et al. 2001; Stadler et al. 2002; von der Maase et al. 2005; Lin et al. 2007b) (see Table 21.1).

In a publication by Bajorin et al., a Karnofsky performance score of 80% or less and the presence of visceral metastases were independently predictive of poor survival after treatment with MVAC (methotrexate, vinblastine, adriamycine, and cisplatin) (see also Sect. 20.4). Depending on the number of poor risk factors (0, 1 or 2), patients lived 33 months, 13.5 months, or only 9.3 months, respectively (Bajorin et al. 1999).

These clinicopathologic markers are strong predictors of treatment outcome and especially of survival. Until today, they have been shown to be independent of the treatment used. They are also valid for newer combination chemotherapy regimens, such as taxane-containing triplet therapy (Bellmunt et al. 2002b). Of note, these markers predict survival in patients treated with cisplatin-containing combination chemotherapy, but they are inadequate for predicting the optimal therapeutic regimen for an individual patient.

Awareness of the presence and distribution of prognostic factors is crucial for the assessment of phase II study results and for stratification of phase III trials (Bajorin 2004).

In elderly patients, a retrospective analysis after cisplatin-based chemotherapy showed an ECOG PS of 2–3 and a hemoglobin level of <10 mg/dL to be independent predictors of poor survival (Bamias et al. 2005). Age itself had no impact on response or adverse events.

Apart from these prognostic factors, treatment decisions should also be based on patient-related factors like renal function or other comorbid conditions, in order to decide whether the patient is "fit" or "unfit" for receiving a cisplatin-containing combination regimen. A creatinine clearance of ≥60 mL/min, a PS of 0–1 or 2, and no severe

Table 21.1 Independent prognostic factors of overall survival

Source	Bajorin et al. (1999)	Stadler et al. (2002)	von der Maase et al. (2005)	Bellmunt et al. (2002b)	Lin et al. (2007a)
PS	+		+	+	+
Visceral metastases	+	+	+	+	+
Alkaline phosphatase			+		+

PS performance status

cardiovascular comorbidity are pertinent factors (Yagoda 1980; Bellmunt et al. 1992; Carles et al. 2000; Bellmunt et al. 2001; Raj et al. 2006). So far, there are no generally accepted definitions of "fit" or "unfit" patients (see also Sect. 20.7) (Sternberg et al. 2007; de Wit 2003; De Santis and Bachner 2007; De Santis et al. 2009).

In summary, poor PS, visceral metastases, and elevated alkaline phosphatase are factors that are related to the natural history of the tumor ("prognostic factors"), but not predictive of the efficacy of a given chemotherapeutic agent in an individual patient ("predictive factors") (Bellmunt et al. 2009b).

21.3 Single-Agent Chemotherapy

Chemotherapy for UC was first and systematically explored in the 1980s by Yagoda et al. (1987), who showed that this tumor entity responded to different chemotherapeutics used as single agents with varying response rates (RRs) (see Table 21.2). Trials with other monochemotherapies like 5-fluorouracil (5-FU), vincristine, mitomycin C, cyclophosphamide, bleomycin, and etoposide showed RRs ranging from 0% to 16% (Yagoda 1987).

With newer drugs like gemcitabine, paclitaxel, carboplatin, docetaxel, and ifosfamide, RRs of up to 42% were reported (see Table 21.3). Of note, patient selection might be the reason for variable RRs in these studies, e.g., the percentage of

Table 21.2 Single-agent first-line chemotherapy (Yagoda 1987)

Agent	N	RR (95% CI)
Cisplatin	320	30 (25–35)
Methotrexate (all doses)	236	29 (23–35)
Adriamycin (doxorubicin)	248	17 (12–23)
Vinblastine	38	16 (4–28)

CI confidence interval, *RR* response rate

Table 21.3 Single-agent first-line chemotherapy – newer agents

Agent	RR (%)	Source
Gemcitabine	27	Pollera 1994
	24	Moore 1997
	28	Stadler 1997
Paclitaxel	42	Roth 1994
Carboplatin	0	de Wit 1991
	14	Trump 1990
	8	Raabe 1989
Docetaxel	31	de Wit 1998
	13	McCaffrey 1997
Ifosfamide	40	Gad-el-Mawla 1989
	29	Otaguro 1981 (Roth and Bajorin 1995)

RR response rate

patients with a good PS and no visceral metastases ("Bajorin prognostic factors"). The most robust data are on gemcitabine with an RR of about 25% in the first- and second-line settings in several larger phase II trials (von der Maase 2003). Generally, complete responses (CR) are rare with monochemotherapy, and no long-term disease-free survival (DFS) has been reported (Sternberg 1995; Stenzl et al. 2009). The median survival in such patients usually does not exceed 8 months.

21.4 Standard First-Line Chemotherapy and Its Development

Cisplatin-containing combination chemotherapy has been the standard of care for more than 20 years (Stenzl et al. 2009). In large-scale randomized phase III trials, MVAC has been superior to the following regimens: cisplatin monotherapy (Loehrer et al. 1992), CISCA (cisplatin, cyclophosphamide, and doxorubicin) (Logothetis et al. 1990), cisplatin/docetaxel (Bamias et al. 2004) and the triplet 5-fluorouracil/interferone/cisplatin (Siefker-Radtke et al. 2002). With MVAC, an overall response rate (ORR) of 72%, half of it CR (25% clinical and 11% surgical), was reported for the first time (Sternberg et al. 1989). Also for the first time, the median survival of MVAC-treated patients rose to about 13 months. This is the survival in bladder cancer that has to be challenged by all the other and upcoming combinations. The main disadvantage of MVAC was its toxicity profile with febrile neutropenia (in up to 25% when given without GCSF-support), nausea, emesis, fatigue, mucositis, and toxic deaths.

21.4.1 Newer Platinum-Containing Combination Chemotherapies

There was hope to increase survival with new cisplatin-containing doublets. Gemcitabine/cisplatin (GC) (Moore et al. 1999; von der Maase et al. 1999; von der Maase et al. 2000; Kaufman et al. 2000) (see Table 21.4) and different taxane and platinum-containing combinations (Burch et al. 2000; Dreicer et al. 2000; Sengelov et al. 1998; Dimopoulos et al. 1999; Garcia del Muro et al. 2002) (see Table 21.5) were explored and showed promising results in phase II trials.

Table 21.4 First-line combination chemotherapy with gemcitabine and cisplatin

Source	*N*	RR (%)	CR (%)	OS (months)
Moore (1999)	28	57	21	13.2
von der Maase (1999)	38	42	18	12.5
von der Maase (2000)	203	49	12	13.8
Kaufman (2000)	46	41	22	14.3

CR complete responses, *OS* overall survival, *RR* response rate

Table 21.5 First-line combination chemotherapies with taxanes and cisplatin

Regimen	Source	*N*	RR (%)	CR (%)	OS (months)
Paclitaxel/cisplatin	Burch (2000)	34	70	32	12.7
	Dreicer (2000)	52	50	8	10.6
Docetaxel/cisplatin	Sengelov (1998)	25	60	26	13.6
	Dimopoulos (1999)	66	52	12	8
	Garcia del Muro (2002)	38	58	18	10.4

CR complete responses, *OS* overall survival, *RR* response rate

Following these developments, MVAC and GC were compared in a more recent international multicentric randomized phase III trial. The primary endpoint was to show a 33% improvement in median OS with GC. Four hundred and five patients were included. RR were reported to be 46% versus 49% and survival 14.8 versus 13.8 months for MVAC and GC (von der Maase et al. 2000). Because equivalence was not tested in this trial and none of the two combinations proved to be superior, the question of OS with GC versus MVAC remained controversial and unanswered. In a later update, long-term survival results confirmed the anticipated equivalence of the two regimens (von der Maase et al. 2005). The major difference was toxicity, which gave GC a lead over MVAC (von der Maase et al. 2000). For this very reason and the most probably equivalent efficacy, GC has become widely used in the international community and has become a new standard of care together with MVAC (Stenzl et al. 2009). Of note, MVAC is better tolerated with GCSF (granulocyte colony-stimulating factor) support (Gabrilove et al. 1988; Bamias et al. 2004).

High-dose-intensity MVAC (HD MVAC) with GCSF was compared to standard MVAC in a randomized phase III trial (Sternberg et al. 2001b). The investigational arm of this study proved to be less toxic, but more efficacious in terms of dose density, complete responses, and the 2-year survival rate than standard MVAC, yet with no significant difference in median survival on first and on long-term analyzes (Sternberg et al. 2006).

In summary, cisplatin-containing combination chemotherapy with gemcitabine/cisplatin, MVAC – preferably with GCSF - and, better still, HD-MVAC with GCSF are standard chemotherapy regimens for first-line treatment in patients eligible ("fit") for cisplatin.

21.4.2 Non-Platinum-Containing First-Line Combination Chemotherapy

The platinum-free doublet paclitaxel/gemcitabine was evaluated in the first-line setting in a phase II trial in mainly "fit" patients. Most patients had a PS 0–1 and the median creatinine clearance was 62 mL/min. Results were below what would be expected with a platinum combination. At a follow-up of 30 months, the overall

RR was no better than 36.9%, the PFS 5.8 months, and median survival 13.2 months.

This regimen should be studied in clearly defined patients who are unfit for cisplatin combination chemotherpay (Calabro et al. 2009). Furthermore, there are multiple studies with this combination showing promising data in second-line treatment.

21.5 Long-Term Overall Survival after Chemotherapy for Metastatic Disease

Although RR of up to 50% have been reported in phase III trials with standard cisplatin combination chemotherapies, they did not translate into a median OS of more than 13–15 months. Yet a small albeit nonnegligible percentage of patients will achieve long-term PFS (De Santis and Bachner 2007) (see Table 21.6). This was confirmed by long-term follow-up data of two randomized phase III trials in patients after cisplatin-containing combination chemotherapy for metastatic disease (von der Maase et al. 2005; Sternberg et al. 2006).

In a 7-year update of the above-mentioned randomized phase III study of HD MVAC versus standard-dose MVAC, more overall responses and, even more importantly, more complete responses with HD MVAC translated into a significantly longer PFS, but an only borderline overall survival (OS) benefit at a follow-up of 7.3 years. Interestingly, the 5-year survival rate was 21.8% in the HD MVAC arm versus 13.5% in the standard-dose arm (Sternberg et al. 2006).

The 5-year update of a large randomized phase III trial comparing GC and MVAC showed similar results in terms of the proportion of long-term survivors in both treatment arms. In addition, the Bajorin prognostic factors (Bajorin et al. 1999) were confirmed as independent prognostic variables for survival, irrespective of the type of treatment (MVAC or GC): On multivariate analysis, patients with a KPS of 80–100% had a median OS of 16.0 months and a 5-year survival probability of 16.5%, whereas patients with a KPS of 70 had a median OS of 8.3 months and a 5-year survival rate defying calculation due to the small number

Table 21.6 Long-term survival after cisplatin combination chemotherapy

Source	Regimen	N	RR (CR)	OS (months)	5-year survival rate (%)
Sternberg (2006)	HD MVAC vs	134	72% (25%)	15.1	21.8
	MVAC	129	58% (11%)	14.9	13.5
von der Maase (2005)	Gemcitabine/ cisplatin	203	49.4% (12.2%)	14.0	13.0
	vs MVAC	202	45.7% (11.9%)	15.2	15.3

HD MVAC high dose MVAC, *MVAC* methotrexate/vinblastine/adriamycine/cisplatin, *OS* overall survival, *RR* response rate

of patients meeting this criterion. Patients without visceral metastases had a median OS of 18.4 months and a 5-year survival probability of 20.9% versus 10.3 months and 6.8% for patients with visceral metastases (von der Maase et al. 2005).

21.6 Refining Standard Chemotherapy

In an attempt to improve clinical outcomes, dose intensification protocols, modern triplets, and combinations with targeted agents were studied.

Responses and CR were generally high (ORR up to 78%, CR 4–32%) even in patients with visceral metastases (up to 58% of the patients included had visceral metastases) (see Table 21.7).

Only one of these new triplets was further developed and advanced to a randomized phase III comparison. In an EORTC/Intergroup trial with 627 randomized patients, GC, for the first time used as the standard arm, was compared with the taxane-containing triplet paclitaxel/cisplatin/gemcitabine (PCG) (Bellmunt et al. 2007b). In this trial, a 14% relative risk reduction of death with the triplet was not statistically significant for the intent-to-treat (ITT) population. Only in the post-hoc analysis of the bladder primary patients of this study did the difference reach significance. With PCG, more neutropenic fever was observed. With GC, there was more thrombocytopenia and bleeding.

In summary, despite substantial efforts to develop new treatment options in UC, only one randomized phase III trial was conducted, which failed to show a clear benefit of adding a taxane to standard cisplatin combination chemotherapy with GC.

Table 21.7 Modern triplets

Source	Regimen	N	RR (CR)	OS (months)
Bellmunt et al. (2000, 2002b)	Gemcitabine/paclitaxel/cisplatin	58	78 (28)	15.6
Bajorin (2000)	Ifosfamide/paclitaxel/cisplatin	44	68 2(3)	20.0
Hussain (2001)	Paclitaxel/carboplatin/gemcitabine	49	68 (32)	14.7
Pagliaro (2002)	Gemcitabine/cisplatin/ifosfamide	51	41 (4)	9.5
Pectasides (2002)	Docetaxel/cisplatin/gemcitabine	35	65 (29)	15.5
Lara (2004)	Paclitaxel/methotrexat/gemcitabine	25	57 (29)	18.0
Hainsworth (2005)	Paclitaxel/carboplatin/gemcitabine	60	43 (12)	11.0
Tsukamoto (2006)	Gemcitabine/cisplatin/etoposide	31	68 (19)	13.1
Hussain (2007)	Paclitaxel/carboplatin/gemcitabine/ trastuzumab	44	57 (9)	14.1
Lin (2007a)	Paclitaxel/cisplatin/5-FU	44	68 (25)	17.0

5-FU 5-fluorouracil, *OS* overall survival, *RR* response rate

21.7 Fit and Unfit for Cisplatin

The European Organization for Research and Treatment of Cancer Genitourinary Tract Cancer (EORTC GU) Group was the first to categorize patients with UC as eligible or not for cisplatin-containing chemotherapy in order to develop separate investigational strategies (de Wit 2003). For the first time, the expressions "fit" or "unfit" for cisplatin were used. Patients unfit for cisplatin were defined by either a PS ≥ 2 and/or impaired renal function (GFR < 60 mL/min) versus those with a PS 0 or 1 and a GFR of ≥60 mL/min, who were considered fit enough to tolerate cisplatin combination chemotherapy. Following this discrimination, different studies were developed for these distinct groups of patients. Patients with a PS > 2 are generally not thought to qualify for combination chemotherapy.

21.8 Carboplatin Versus Cisplatin Combination Chemotherapy in "Fit" Patients

Although cisplatin combination chemotherapy is a well-established standard in UC, several attempts to replace cisplatin have been undertaken even in patients eligible for the drug. The main reason was its toxicity and inconvenience.

Carboplatin-based regimens are widely used as an alternative to cisplatin combination chemotherapy and have been shown to be equally effective and less toxic in other tumor entities (Schiller et al. 2002; du Bois et al. 2003). Carboplatin is a less nephrotoxic platinum analog than cisplatin. It can be used safely even in patients with impaired renal function and does not need hyperhydration, which would limit its use in patients with cardiac comorbidity (de Wit 2003). In bladder cancer, carboplatin-containing chemotherapy has, however, not been proven to be equivalent to cisplatin combinations. It is most likely inferior and should therefore not be considered interchangeable or standard. The only randomized phase III study that addressed this question had a disappointing response rate of only 14% in the investigational arm (paclitaxel/carboplatin) compared to MVAC and had to be closed early because of low patient accrual (Dreicer et al. 2004). Therefore, there is no level I evidence that this doublet might have adequate efficacy for first-line use. Furthermore, three randomized phase II studies comparing carboplatin with cisplatin combination chemotherapies showed lower CR and a shorter overall survival (OS) for the carboplatin arms (Petrioli et al. 1996; Bellmunt et al. 1997; Dogliotti et al. 2007) (see Table 21.8).

Smaller phase II studies with carboplatin/paclitaxel were disappointing with only about 9 months median survival and RR below 50% (Redman et al. 1998; Vaughn et al. 1998; Small et al. 2000).

In summary, cisplatin combination chemotherapy is standard and carboplatin instead of cisplatin is most likely inferior. Selecting those patients for cisplatin combination chemotherapy who will profit from its benefits in terms of response,

Table 21.8 Randomized trials comparing carboplatin and cisplatin regimens

Source	Regimen	N	RR (CR)	OS(months)
Petrioli (1996)	MVEC vs	29	71% (25%)	13
	MVECa	28	41% (11%)	9.5
Bellmunt (1997)	M-CAVI vs	23	39% (0%)	9 (DRS)
	MVAC	24	52% (13%)	16 (DRS)
Dogliotti (2007)	Gemcitabine/ cisplatin vs	55	49.1% (14.5%)	12.8
	gemcitabine/ carboplatin	55	40.0% (1.8%)	9.8

DRS disease-related survival, *MVAC* methotrexate/vinblastine/adriamycine/cisplatin, *M-CAVI* methotrexate/carboplatin/vinblastine, *MVEC* methotrexate/vinblastine/epirubicin/cisplatin, *MVECa* methotrexate/vinblastine/epirubicin/carboplatin, *OS* overall survival, *RR* response rate

complete response, and potential long-term progression-free survival, and who tolerate treatment well, is of critical clinical importance.

21.9 Treatment of Elderly and Frail Patients and Those with Impaired Renal Function

Only recently, cancer treatment in the elderly and frail and in those with specific comorbid conditions has become an increasingly important issue worldwide (Launay-Vacher et al. 2004; Lichtman et al. 2007). The definition of "elderly" is chosen arbitrarily and usually used for patients ≥65 years. The incidence of UC peaks at >60 years (Jemal et al. 2009). Patients aged ≥65 years, however, have been significantly underrepresented in clinical trials, although they account for more than half of our UC population (Yee et al. 2003; Hutchins et al. 1999; Lichtman 2006).

For patients above the age of 65 years treated within clinical trials there is no evidence of poorer survival or increased treatment-related mortality than for younger patients (Kumar et al. 2007; Bamias et al. 2005). Chronologic age does not necessarily correlate with functional impairment or frailty (Extermann et al. 1998).

Because elderly patients and those with functional impairment have been poorly studied, one of the prime questions in the treatment of this distinct group is whether dosages and results derived from general trials with younger patient populations are applicable to elderly patients in daily clinical practice. In general, chemotherapy dosages are derived from studies with "fit" patients. This might be one of the reasons why chemotherapy might be more toxic in the elderly and "unfit" outside clinical trials (Repetto 2003; Wedding et al. 2007).

There are, indeed, age-related changes in the pharmacokinetic profile of chemotherapy. The most important reason is the physiologic decline in renal function.

Renal function assessment in elderly UC patients as a necessary basis of treatment and dosing decisions is a challenge. Calculated creatinine cleareance (crcl) using current formulas tends to underestimate crcl in patients >65 years compared to measured crcl (Dash et al. 2006) with resultant inadequate noncisplatin treatment

selection. A cross-check with measured crcl in these patients is recommended. Furthermore, pretreatment hydration and urinary drainage for renal dysfunction caused by hydronephrosis (percutaneous nephrostomy/ureteric stents) should lead to an improvement of the glomerular filtration rate (GFR) and make patients eligible for standard cisplatin combination chemotherapy.

A further point to be considered in the treatment of elderly UC patients is that therapeutic goals may differ from those for their younger counterparts and depend on their functional status and the individual preconsultation treatment preference (Koedoot et al. 2003).

For patient counseling and adequate treatment selection in the elderly and more unfit, we need to identify the seemingly frail older individual who is likely to benefit from and tolerate standard therapy, as well as the seemingly fit older individual who is apt to experience undue side effects and needs a modified treatment plan (Repetto et al. 2001; Balducci and Extermann 2000).

Consensus guidelines already recommend the routine use of geriatric assessments in the older patient with cancer. However, additional studies are needed to confirm that they are useful and valid in this setting.

21.10 Treatment of the "Unfit" Advanced or Metastatic Bladder Cancer Patient

It is a well-known fact in urologic oncology that up to 50% of patients with UC are not eligible for cisplatin-based standard chemotherapy due to impaired renal function, PS, or comorbidity. So far no standard chemotherapy has been established for this patient group. Renal functional impairment increases with age (Brenner et al. 1982; Lichtman et al. 2007) and is a well-known comorbid condition in UC patients (Dash et al. 2006; Nogue-Aliguer et al. 2003).

The first randomized phase II/III trial evaluating two chemotherapy regimens in purely "unfit" UC patients was conducted by the European Organization for Research and Treatment of Cancer Genitourinary Tract Cancer (EORTC GU) Group. The phase II data were published in 2009 (De Santis et al. 2009). The concept of this study as well as the entry criteria reflected a clinical need that had been poorly addressed in the past. This study shed light on the difficulties of treating UC patients with either a PS 2 or impaired renal function or both, and mirrored problems from daily clinical practice (Extermann et al. 2003).

Furthermore, the median age per treatment arm of this study was 71 and 72 years, respectively. This is about 8–10 years more than in trials studying patients with cisplatin-based standard chemotherapy (von der Maase et al. 2000; Bellmunt et al. 2007a). One hundred and seventy five patients in the phase II part of the study were randomized to receive either carboplatin/methotrexate/vinblastine (M-CAVI) or gemcitabine/carboplatin (GC). Both regimens were active in these

unfit patients (RR 42% for GC and 30% for M-CAVI) with different toxicity profiles. The severe acute toxicity rates were 14% and 23% for GC and M-CAVI, respectively.

The main upshot of the phase II part of this study was a change in strategies for future clinical trials in unfit patients. This was prompted by the fact that patients with both stratification factors (impaired renal function and PS 2) or 2 Bajorin poor prognostic factors experienced low RR (26%), a high probability of severe acute toxicity (26%) and of receiving only one chemotherapy course (20%) as seen in both treatment arms. In these subgroups of unfit patients, treatment strategies other than combination chemotherapy should, therefore, be considered.

In these subgroups of unfit patients, monochemotherapy, special trial settings using agents with novel mechanisms of action (e.g., targeted therapies), or best supportive care (especially if the PS declines rapidly) should be considered. Unfortunately, neither monochemotherapies nor other more targeted agents have so far been adequately studied in unfit patients.

21.11 Second-Line Chemotherapy

There is no clear standard chemotherapy for second-line treatment in UC patients. Whenever standards are lacking, the best choice is to treat patients within clinical trials. If no trials are available, one option is to retreat patients with a prior chemotherapy regimen, which had provided a response for at least several months (Kattan et al. 1993; Cara and Tannock 2001). Furthermore, treatment decisions may be based on prognostic and predictive factors, on the individual patient characteristics, and on the results of second-line phase II trials.

21.11.1 Second-Line Chemotherapy in Patients Who Are Still Eligible for Cisplatin

Re-exposure to the primary first-line chemotherapy is feasible, provided the progression-free interval was ≥12 months.

For patients progressing on GC or relapsing within 6–12 months who are still cisplatin-eligible, MVAC could be a reasonable choice (Bachner and De Santis 2009). In a small-scale phase II study ($n=30$), patients who had previously been treated with GC received MVAC (Han et al. 2008). Seven out of 16 patients who had responded to GC also responded to second-line MVAC versus only 2/14 of those refractory to GC. 6.7% of patients achieved CR. However, the overall RR was only 30%. The quality of pretreatment response seems to be closely associated with second-line treatment results.

21.11.2 Second-Line Monochemotherapy

Single agents were mainly studied in small phase II trials. Low RR (0–29%) and short progression-free and median survival times were rather disappointing in this setting (see Table 21.9).

21.11.3 Vinflunine for Second-Line Use (see Table 21.10)

Vinflunine (Javlor®; VFL) is a 3rd generation semisynthetic vinca alkaloid obtained with superacidic chemistry by selectively introducing two fluorine atoms

Table 21.9 Single-agent second-line chemotherapy

Regimen	*N* (evaluable)	RR (%)	TTP (months)	OS (months)	Source
Docetaxel	31 (30)	13	NR	9.0	McCaffrey (1997)
Piritrexim	17 (13)	23	NR	NR	Khorsand (1997)
Paclitaxel	14 (14)	7	NR	NR	Papamichael (1997)
Ifosfamide	20 (20)	5	6	8.0	Pronzato (1997)
Ifosfamide	60 (56)	20	2.2	5.1	Witte (1997)
Gemcitabine	35 (31)	23	3.8	5.0	Lorusso (1998)
Topotecan	46 (44)	9	1.4	6.2	Witte (1998)
Gemcitabine	24 (24)	29	NR	13.0	Gebbia (1999)
Pyrazoloacridine	14 (14)	0	NR	9.0	Dodd (2000)
Gemcitabine	30 (28)	11	4.9	8.7 (DSS)	Albers (2002)
Piritrexim	35 (27)	7	2.1	7.0	Roth (2002)
Paclitaxel	31 (31)	10	2.2	7.2	Vaughn (2002)
Oxaliplatin	20 (18)	6	1.5	7.0	Moore (2003)Winquist (2005)
Paclitaxel	45 (37)	9	3	7.0	Joly et al. (2004, 2009)
Pemetrexed	47 (47)	28	2.9	9.6	Sweeney 2006
Gemcitabine	46 (44)	25	3.1 (PFS)	12.6	Akaza 2007
Epothilone B	45 (42)	12	2.7 (PFS)	8.0	Dreicer 2007
Pemetrexed	13 (12)	8	NR	NR	Galsky 2007

DSS disease-specific survival, *NR* not reported, *OS* overall survival, *PFS* progression-free survival, *RR* response rate, *TTP* time to progression

Table 21.10 Vinflunine, second-line chemotherapy

Source	*N* (evaluable)	RR (%)	PFS (months)	OS (months)
Culine (2006)	58 (51)	17.6	3.0	6.6
Vaughn (2009)	175 (151)	14.6	2.8	8.2
Bellmunt (2009a)	253 (185)	8.6	3.0	6.9

RR response rate, *PFS* progression-free survival, *OS* overall survival

at the 20´-position of vinorelbine, a part of the molecule previously inaccessible to classical chemistry (Fahy et al. 1997, 2008). Of note, VFL is far less neurotoxic than other vinca alkaloids and other microtubule inhibitors. Therefore, VFL appears to be a reasonable option for UC progressing after first-line platinum-containing chemotherapy (Bachner and De Santis 2008). In UC, VFL showed moderate activity and an excellent safety profile at a dose of 320 mg/m^2 every 3 weeks in two phase II trials (Culine et al. 2006; Vaughn et al. 2009). The most common side effects were myelosuppression and constipation.

The first second-line phase III trial for UC in "modern times" was published in 2009 (Bellmunt et al. 2009a). Three hundred and seventy platinum-pretreated patients were randomly assigned to VFL and best supportive care (arm A) versus best supportive care alone (arm B). Only patients with a PS 0 or 1 were eligible. Forty percent of them had bulky disease, 74% suffered from visceral involvement, and over 80% had relapsed or progressed within 6 months after first-line platinum-containing chemotherapy. In the VFL arm, grade 3/4 neutropenia was observed in 50% of patients, but febrile neutropenia occurred in only 6%. There was one toxic death. As for grade 3/4 nonhematological toxicities, fatigue and constipation were most common, but still below 20%.

The results showed a modest overall response rate (8.6%), a statistically significant improvement in PFS (3.0 months) and in duration of disease control (5.7 months), compared to BSC alone.

The statistical hypothesis in this study was an OS benefit of 2 months in the VFL-group (6 vs 4 months). In the intention-to-treat analysis, a 2.3 months' improvement in OS by adding VFL to BSC did not reach statistical significance (6.9 vs 4.6 months, $p=0.2868$). However, a secondary analysis of eligible patients did show a statistically significant 2.6 months improvement in OS for the VFL arm. Of note, PS (PS 0 vs PS 1) was the most important prognostic factor with an HR of 0.48.

For second-line treatment in advanced or metastatic urothelial cancer, this trial reached the highest level of evidence ever reported by 2010. Vinflunine was the first approved second-line treatment in Europe. It will permit a comparison of further potential second-line regimens with a standard of care rather than with placebo or best supportive care (Bachner and De Santis 2009).

As it is true for patients eligible for cisplatin in the first-line setting (Bajorin et al. 1999; Bellmunt et al. 2002b; von der Maase et al. 2005), PS and metastatic disease location have also been shown to be the most important clinical pretreatment prognostic factors for survival in patients at relapse after a platinum-containing regimen (Bellmunt et al. 2009a). In addition, in this patient cohort, a low hemoglobin level was a statistically significant predictor for a shorter OS at multivariate analysis, independent of the treatment arm (Bellmunt et al. 2010). In an external validation set, renal function was first noted as an independent pretreatment prognostic factor among patients who relapse after platinum-containing chemotherapy. However, this was the case in the primary phase II trial cohort; the reason might be that different inclusion criteria that were used in the retrospective studies (De Santis et al. 2010).

21.11.4 Combination Chemotherapy for Second-Line Use

Response rates as well as toxicity of combination chemotherapy are usually higher than those of single agents. This has been a common experience in several tumor entities and seems to be also true for second-line treatment of UC. It is still unclear, whether there is a survival benefit in favor of combination chemotherapy (see Table 21.11).

So far, paclitaxel/gemcitabine second-line combination chemotherapy has been studied extensively in phase II (Meluch et al. 2001; Sternberg et al. 2001a; Kaufman et al. 2004; Fechner et al. 2006; Li et al. 2005; Takahashi et al. 2006; Kanai et al. 2008; Suyama et al. 2009) and one phase III trials (Albers et al. 2008) (see Table 21.12) showing RR of about 50%. Several schedules of this combination chemotherapy regimen have been used. So far it is unclear which is the best and which is least toxic (Gallagher et al. 2008a). A 2- versus 3-week schedule (Fechner et al. 2006) were compared by the German group. In their randomized phase III trial (Albers et al. 2008), the 3-week schedule was used. Six courses of gemcitabine/paclitaxel were compared with treatment until progression. Preliminary data

Table 21.11 Second-line combination chemotherapy

Regimen	*N* (evaluable)	RR (%)	OS (months)	Source
5-FU/α-interferon/cisplatin	28 (NR)	61	NR	Logothetis (1992)
Paclitaxel/methotrexate/cisplatin	25 (25)	40	NR	Tu (1995)
Paclitaxel/ifosfamide	13 (13)	15	8	Sweeney (1999)
5-FU/α-interferon/cisplatin	43 (40)	13	4.9	de Mulder (2000)
Docetaxel/ifosfamide	22 (20)	25	4	Krege (2001)
Methotrexate/paclitaxel	20 (19)	32	5	Bellmunt (2002a)
Cisplatin/gemcitabine/ifosfamide	51 (49)	41	9.5	Pagliaro (2002)
Docetaxel/gemcitabine/carboplatin	NR (9)	56	NR	Chen (2004)
Carboplatin/paclitaxel	44 (44)	16[a]	6	Vaishampayan (2005)
Gemcitabine/ifosfamide	23 (23)	22	4.8	Lin (2007b)

RR response rate, *TTP* time to progression, *NR* not reported, *PFS* progression-free survival
[a]Including four unconfirmed PR

Table 21.12 Second-line gemcitabine/paclitaxel in bladder cancer

Source	*N* (evaluable)	RR (%)	OS (months)
Meluch (2001)	15 (15)	47	NR
Sternberg (2001a)	41 (40)	60	14.4
Kaufman (2002, 2004)	6 (6)	33	NR
Fechner (2006)	30 (27)	44	NR
Takahashi (2006)	23 (23)	30	12.1
Kanai (2008)	20 (20)	30	11.5
Suyama (2009)	33 (30)	33	11.3
Albers (2008)	102 (57)	35/50	pending

RR response rate, *NR* not reported, *OS* overall survival

of this study by Albers et al. confirmed RR of up to 50%, but a PFS of only about 3 months. Prolonged therapy (more than six cycles) was rarely achievable and if so, it did not show any benefit. OS data were still pending (as of 2010).

21.11.5 Novel Approaches for Second-Line Treatment

Second-line treatment in UC is an unmet need, frequently used, although there is only one approved drug (which was tested against best supportive care) in Europe. As already stated above, chemotherapy at second line in the setting of a usually very palliative oncologic situation provides only low RR, adds toxicity, and potentially lowers the quality of life. Therefore, novel approaches and new agents with a better efficacy/toxicity ratio would be highly appreciated. The second-line setting in UC is a suitable situation for testing new agents in clinical trials. So far, these results have been rather disappointing, maybe also caused by flawed patient selection and inappropriate endpoint selection (see Table 21.13).

21.11.6 Conclusion

There is still no globally accepted standard strategy for second-line UC management. With this respect, apart from the European approval of vinflunine, little progress has been made in the past few years.

Therefore, the first choice should still be to treat patients within clinical trials whenever possible. Outside clinical trials, retreatment might be an option for selected patients. Vinflunine and BSC, for the first time in this clinical setting, significantly improved OS, PFS, and disease control compared to BSC alone. Second-line single-agent chemotherapy generally provides low response rates and

Table 21.13 Single-agent second-line treatment: targeted therapy

Regimen	*N* (evaluable)	RR (%)	TTP (months)	OS (months)	Source
Bortezomib	18 (11)	0	NR	NR	Sridhar (2005)
Lapatinib	59 (59)	2	2.0	4.1	Wülfing et al. (2005, 2009)
Vorinostad (SAHA)	14 (12)	0	1.1 (DFS)	2.1	Cheung et al. (2008)
Sorafenib	27 (22)	0	2.2 (PFS)	6.8	Dreicer et al. (2008, 2009)
Sunitinib 50 mg	45 (41)	7.3	2.4	7.1	Gallagher et al. (2008b, 2010)
37.5 mg	32	3.0	2.3	6.0	
Bortezomib	25 (24)	0	1.4	5.7	Rosenberg et al. (2008)

DFS disease-free survival, *NR* not reported, *PFS* progression-free survival, *RR* response rate, *TTP* time to progression

a short duration of PFS and OS. Patient selection and prognostic factors, although not sufficiently validated, contribute to these results. Combination chemotherapy for second-line use is beneficial in terms of response rates, adds to toxicity, and its OS benefits are still unclear.

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Chapter 22
Nontransitional Carcinoma of the Bladder

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Abstract In this article, we review available evidence on the treatment of patients with nonurothelial cancer of the bladder. More than 150 published works were reviewed in preparation for this summary. Squamous cell carcinoma and adenocarcinoma are ideally treated with radical cystectomy. High-risk groups for these diseases are defined. Small cell carcinoma should be treated with multimodality therapy, including chemotherapy. Other rarer tumors of the bladder are also discussed.

Worldwide, urothelial carcinoma (formerly known as "transitional cell carcinoma") is the most prevalent histologic type of bladder tumor. Superficial and invasive diseases have been extensively studied. At the other end of the bladder tumor spectrum lie squamous cell carcinoma (SCC), adenocarcinoma, and other uncommon tumors. This latter group consists of small cell carcinoma, sarcoma, carcinosarcoma and sarcomatoid tumors, paraganglioma, lymphoma, melanoma, and pseudotumors. Other epithelial abnormalities can mimic tumors, and biopsy is frequently indicated for proper diagnosis.

22.1 Squamous Cell Carcinoma

Squamous Cell Carcinoma (SCC) occurs in bladders infected with and those free of bilharziasis. The incidence, epidemiology, and natural history of the two subpopulations are different.

22.1.1 SCC Not Associated with Bilharziasis

22.1.1.1 Epidemiology

Although bilharziasis is the leading cause of SCC worldwide, schistosomal infections are rare in Western countries. Primary SCC in the nonbilharzial bladder is uncommon.

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SCC represents the second most common bladder malignancy in Western countries, accounting for 2–5% of cases in most contemporary cystectomy series (Miller et al. 1969; Johnson et al. 1976; Rundle et al. 1982; Lopez 1994; Serretta et al. 2000). The tumors are most often diagnosed during the seventh decade of life.

Using data obtained from the Surveillance, Epidemiology, and End Results (SEER) program conducted between 1973 and 1997, Porter et al. (2002) determined that black Americans were twice as likely as white Americans to develop SCC of the bladder, with an overall annual incidence of 1.2 per 100,000 person-years (95% confidence interval [CI], 0.4–1.37) in blacks versus 0.6 per 100,000 person-years (95% CI, 0.57–0.64) in whites (Porter et al. 2002). This variability by race was observed in men and women of all age groups beyond 45 years, and, despite a slight decline in annual incidence over the 3 decades studied, remained relatively constant.

In the USA, although the incidence of urothelial carcinoma in men is ≥3 times than in women, less of a male predominance is noted in SCC. After compiling data from 915 patients in 10 series of SCC (Miller et al. 1969; Johnson et al. 1976; Rundle et al. 1982) Johansson and Cohen (1997) reported that the ratio of males to females was 1.4:1, which is consistent with ratios documented in other reports. As with urothelial carcinoma, women are more likely than men to present with advanced disease (Fleshner et al. 1996). After analyzing data from the Netherlands Cancer Registry, Mungan et al. (2000) confirmed that a higher incidence of T3 and T4 nonurothelial bladder cancer occurs in women (21.7% vs 14.5% in T3, and 14.5% vs 8.4% in T4).

22.1.2 Spinal Cord Injury

In the USA, patients with spinal cord injury represent the largest group of patients affected by SCC. This condition is thought to be owing to inflammation from chronic urinary tract irritation; SCC also is likely to occur in patients with chronic inflammatory disorders of the bladder, persistent calculi, chronic cystitis, and bladder diverticuli (Cohen et al. 2000).

It has been estimated that 10% of cases of SCC of the bladder occur in patients who have had an indwelling catheter for ≥10 years (Sene et al. 1990). Other studies have documented a 16–28-fold increased risk for SCC of the bladder in paraplegic individuals. Patients who perform clean intermittent self-catheterization are less likely to develop the disease (Sene et al. 1990; Zaidi et al. 1997).

The incidence of bladder cancer in patients with spinal cord injury was initially thought to be 2.3–10%, with most cases representing SCC (Locke et al. 1985; Bejany et al. 1987; Kaufman et al. 1977). A recent US Department of Veterans Affairs review (West et al. 1999) of admission data from 33,560 patients with spinal cord injury identified only 130 patients with bladder cancer, for an overall incidence of 0.39%. Some 42 patient records were available for review, including 23 (55%) with urothelial carcinoma, 14 (33%) with SCC, and 4 (10%) with adenocarcinoma. It is noteworthy that in 26 patients with indwelling catheters, the incidences of SCC and urothelial carcinoma were equal, implicating chronic inflammation caused by

an indwelling foreign body in the pathogenesis of SCC. The higher prevalence of urothelial carcinoma in this population may also reflect the higher prevalence of cigarette smoking in the US veteran population.

In another study of patients from 3 Louisiana medical centers, Bickel et al. (1991) reported that bladder cancer was diagnosed in 8 of 2,900 patients with spinal cord injuries, for an overall incidence of 0.32%. Only 2 of the 8 (25%) individuals were found to have SCC, neither of whom had an indwelling catheter.

Finally, in the largest study to date, Pannek (2002) reported results in 43,561 patients from Eastern Europe with spinal cord injury who completed a questionnaire that had been sent to all urologic departments involved in the management of spinal cord injury. In all, 48 patients with bladder cancer were identified, for an overall incidence of 0.11%. It is interesting to note that 7% of patients in this series had indwelling catheters, and only 19% had SCC. The authors concluded that the declining percentage of SCC and the declining incidence of bladder cancer may be a consequence of the reduced use of indwelling catheters.

Guidelines cannot be provided on the surveillance of patients with spinal cord injury for SCC. Initial reports, in which a high percentage of patients with spinal cord injury developed SCC, reveal a number of flaws, primarily related to the retrospective manner in which data were obtained. Although the true incidence of SCC in the spinal cord–injured population appears to be <1%, it is recommended that these patients should be monitored, particularly if they have indwelling catheters. Any history of hematuria should be evaluated. The appropriate frequency of surveillance and extent of diagnostic evaluation cannot be determined on the basis of the literature to date.

22.1.3 Smoking

The relation between SCC and cigarette smoking is not clear; however, Johansson and Cohen (1997) found a higher incidence of SCC in smokers. SEER data support a direct correlation between quantity of cigarettes smoked and relative risk of developing SCC (Kantor et al. 1988). Indirect evidence from the Swedish Cancer Registry, however, does not support an association between SCC and smoking. A review of this database (Thorn et al. 1997), which plotted incidence trends in bladder cancer in Sweden between 1960 and 1993, revealed that, despite a rising incidence of urothelial carcinoma in Swedish women, which correlated with an increased prevalence of smoking during those years, the incidence of SCC remained relatively constant.

22.1.4 Causes

Because urinary tract infection is more common in women, a relation among squamous metaplasia, leukoplakia, and the development of SCC was proposed by

Connery (1953) and by Holley and Mellinger (1961). In a series of 20 patients with long-standing leukoplakia, O'Flynn and Mullaney (1974) observed the development of five cases of SCC. Although SCC is not considered a premalignant lesion, it is often associated with squamous metaplasia (Cohen et al. 2000). Studies have confirmed the high prevalence of squamous metaplasia in the general population (Wiener et al. 1979).

A few case reports have documented the association among cyclophosphamide, bacille Calmette-Guérin (BCG), human papillomavirus, and SCC of the urinary bladder (Westenend et al. 2001; Tatsura et al. 1995). Recent studies have revealed possible genetic and chromosomal changes related to SCC. Abnormalities of chromosomes 3, 8, 10, 13, and 17 have been detected in SCC (Fadl-Elmula et al. 1998). Studies on uroplakin II gene expression found a significant difference in expression between urothelial carcinoma and SCC, with expression being greater in SCC. Uroplakins are the major differentiation products of the urothelium that control the various pathways of urothelial differentiation (Serretta et al. 2000).

22.1.5 Clinical and Pathologic Features

Hematuria is the main clinical presentation in 63–100% of patients. Irritative bladder symptoms are seen in two thirds of patients; weight loss, back or pelvic pain, and frank obstructive symptoms are less common and are suggestive of advanced disease (Johnson et al. 1976; Rundle et al. 1982; Lopez 1994; Pl et al. 1974). A urinary tract infection is present in 30–93% of patients at the time of diagnosis (Johnson et al. 1976; Rundle et al. 1982; Lopez 1994; Jones et al. 1980; Quilty and Duncan 1986). Symptoms are often present for a protracted time before the diagnosis is reached (Johnson et al. 1976; Rundle et al. 1982; Serretta et al. 2000; Jones et al. 1980). Most patients present with no previous history of urologic tumor. Superficial SCC of the bladder is rare, and most tumors are muscle invasive at presentation.

Pure SCC of the bladder must be distinguished from urothelial carcinoma with squamous differentiation. Relative to the number of series addressing treatment in patients with urothelial carcinoma, few series conducted in Western countries have addressed pure SCC. Most of these are >10 years old and did not use current staging and grading systems.

Superficial tumors are almost never seen; patients are usually given the diagnosis of SCC at an advanced stage. Most patients have a large, solitary tumor that extensively involves the bladder wall. These tumors appear as sessile lesions, often with ulceration and areas of squamous metaplasia adjacent to the primary tumor. A predilection for the trigone has been noted, but SCC can arise anywhere within the bladder. It may extend locally into the ureters and urethra. It may occupy a bladder diverticulum and has been described in association with bladder calculi (Costello et al. 1984).

Debbagh et al. (1997) reported that 10 of 14 patients had a palpable tumor on rectal examination, and 11 had upper urinary tract obstruction. Pretreatment imaging studies demonstrate hydronephrosis in 33–59% of cases (Johnson et al. 1976; Rundle et al. 1982; Serretta et al. 2000). Of 114 patients with SCC of the bladder, 92% had T2 to T4 disease at the time of diagnosis, and most tumors were of high grade (Rundle et al. 1982). As with urothelial carcinoma, clinical understaging is seen in as many as 73% of patients (Rundle et al. 1982; Richie et al. 1976).

22.1.6 Treatment

Pure SCC of the bladder has a poor prognosis, with most patients dying within 1–3 years of diagnosis. Despite a variety of treatment regimens, including radiation, chemotherapy, and surgery, in a series of 120 patients from the Royal Marsden Hospital, the overall 5-year survival rate was 16%, with only 8% of patients developing metastatic disease (Jones et al. 1980). Therefore, failure to provide locoregional control appears to be the problem in managing these tumors.

22.1.6.1 Radiation

Reported results after treatment by definitive external irradiation are uniformly poor (Rundle et al. 1982; Pl et al. 1974). Radiation therapy in patients with SCC has been used as primary treatment or as an adjunct to surgery in the neoadjuvant setting. In a report by Quilty and Duncan (1986), 51 patients were treated with radical radiotherapy, delivered with a three-field beam-directed technique, covering the entire bladder, to a prescribed dose of 55 Gy, given in 20 fractions over 4 weeks. Patients were treated prone, immediately after they had emptied their bladder. Only four patients had T2 cancer, and the overall survival rate was 26.9% at 3 years, with a median survival time of 14.3 months. Of 48 patients treated with radiation therapy by Rundle et al. (1982) 5-year survival rates for patients with T2 and T3 disease were 16.7% and 4.8%, respectively, and no patient with T4 disease survived beyond 11 months. Similarly, in a series of 17 patients with T2 and T3 tumors who were treated with radiation therapy alone, Johnson et al. (1976) reported a 20% 5-year survival rate, which was not statistically lower than the 34.6% 5-year survival rate seen in seven patients in which preoperative radiation was followed by radical cystectomy.

22.1.6.2 Radical Cystectomy

Surgical treatment appears to provide a better therapeutic yield. Richie et al. (1976) reported a 5-year survival rate of 48% in 25 patients treated with radical

cystectomy. Investigators believed that this compared favorably with results in patients with urothelial carcinoma; however, they did not include in the analysis three patients (9%) who died during the perioperative period or an additional five patients in whom insufficient pathologic study or follow-up data were obtained. After adjustments are made for these cases, the 5-year survival rate is significantly lower than was reported. Tumor stage was identified as the most important predictor of outcome.

Serretta et al. (2000) reported on 19 patients with pure SCC of the bladder. All patients had a solitary locally advanced tumor and were treated with radical cystectomy. Of patients who were treated, 63% died of locally recurrent bladder cancer during a mean follow-up period of 52 months. Distant metastases were observed in only one patient.

Johnson et al. (1976) used integrated preoperative radiation therapy followed by cystectomy and reported a 5-year survival rate of 34%. Swanson et al. (1990) reported their results with the same approach. Patients with T2 disease showed the highest survival figures. Furthermore, results were better in patients whose tumors were downstaged by preoperative irradiation than in those who showed no downstaging. However, no conclusions can be drawn about the efficacy of preoperative irradiation plus cystectomy for nonbilharzial SCC because too few patients treated in this way have been reported (Ghoneim 1994). Because the tumor is uncommon, only a few cases are available for study. It would be extremely difficult to conduct well-controlled prospective studies that would achieve objective conclusions.

In another more contemporary series with a mean follow-up of 42 months, 9 of 14 patients (37%) with SCC who underwent radical cystectomy were still alive. Only one of the patients who underwent cystectomy received preoperative radiation therapy; four received neoadjuvant chemotherapy with M-VAC (methotrexate, vinblastine, doxorubicin [Adriamycin; Bedford Laboratories, Bedford, OH], and cisplatin), and no objective response was noted. In this series of 19 patients, 12 died of locoregional disease, and only one patient died of documented metastases. All three patients with ileal neobladder developed recurrence at the anastomosis between the neobladder and the urethra. It was not specified whether frozen section biopsies of the bladder neck or urethra were performed intraoperatively. This contrasts with no anastomotic recurrences in five female patients with SCC who underwent orthotopic urinary diversion in a series by Stenzl et al. (2001). In this series, negative intraoperative frozen section biopsy specimens of the bladder neck were obtained before orthotopic reconstruction was performed.

Several large contemporary cystectomy series in the literature have compared results in patients with urothelial carcinoma with those in patients with nonurothelial bladder cancer. In a large series from Japan (Nishiyama et al. 2004), no significant difference was observed in 5-year postcystectomy survival for patients with urothelial carcinoma (68.0%; $n = 1{,}042$) and with nonurothelial carcinoma (60.8%; $n = 89$). Multivariate analysis determined that nonurothelial carcinoma was not an independent prognostic factor in survival.

22.1.6.3 Chemotherapy

The role of neoadjuvant or adjuvant chemotherapy in the treatment of patients with pure SCC of the bladder is uncertain. Chemotherapy usually is not recommended because of the low chemosensitivity of SCC of the bladder. SCC is considerably less responsive to standard chemotherapy regimens used for urothelial carcinoma (Khaled et al. 2000; Loehrer et al. 1992); neoadjuvant M-VAC has been tried with no objective response (Sternberg et al. 1985). An effective chemotherapy protocol against this disease has not yet been found, although newer combination regimens consisting of agents such as gemcitabine, paclitaxel, and docetaxel, when combined with a platinum compound, may yield sustained disease remission in up to 50% of cases; these treatments hold promise for the future (Raghavan 2003).

It is interesting to note that SCC has a low incidence of distant metastasis, ranging from 8% to 10% (Wishnow and Dmochowski 1988). Still, the prognosis of SCC of the bladder is dismal.

Most patients die of locoregional failure within 3 years. Distant metastasis is more often the cause of death in patients with urothelial carcinoma than in those with SCC. Therefore, pelvic control for SCC is more important and adds incentive to attempt methods of treatment targeted at reducing the incidence of pelvic recurrence (El-Bolkainy et al. 1972).

22.1.6.4 Prevention and Early Detection

Several screening protocols have been advocated in an attempt to diagnose these tumors earlier, thereby improving outcomes. Broecker et al. (1981) recommended annual cystoscopy and urine cytology in patients with long-term paraplegia. Others have suggested routine random bladder biopsies every 1–2 years. Navon et al. (1997) did not routinely use urine cytology or random biopsies, except in patients with spinal cord injuries lasting 10 years and in those with recurrent or chronic urinary tract infection. Celis et al. (1996) showed that psoriasin (a calcium-binding protein expressed by squamous epithelia) is a potential marker of SCC. Other biomarkers, such as SCC antigen and bcl-2 and p53 oncoproteins, may have a possible role in early diagnosis (Tsukamoto et al. 1992). However, the exact role of these new markers in the early detection and follow-up of bladder SCC requires further study for validation.

22.1.7 Conclusion

In summary, nonbilharzial SCC is an uncommon form of bladder cancer. It has a poor prognosis, and death most often is related to locoregional failure – not to metastasis. The current literature, although limited, supports cystectomy as the treatment of choice (Abrams 2004).

22.2 Squamous Cell Carcinoma in the Bilharzial Bladder

22.2.1 Epidemiology

This type of cancer is prevalent where urinary bilharziasis is endemic. The highest incidence of SCC of the bilharzial bladder occurs in Egypt. In a recent report by Ghoneim et al. (1997), SCC accounted for 59% of 1,026 cystectomy specimens. A high incidence of SCC is also found in Iraq, the Jizan region in southern Saudi Arabia, Yemen, and Sudan. In Africa, the disease has been reported in the Gold Coast region and in South Africa.

However, the incidence in these countries is lower because bilharziasis is less endemic and less severe (El-Bolkainy 1998). The mean age of patients is 10–20 years younger than that seen with nonbilharzial cancer (El-Bolkainy et al. 1972); the median age is 46 years. Some 80% of cancer specimens have showed histologic evidence of bilharzial infestation (Abrams 2004). A lag period of approximately 30 years has been reported between bilharzial infection and subsequent development of the disease. The male-to-female ratio is 5:1 (El-Sebai et al. 1974). This male predominance is thought to be related to increased exposure to bilharzial infestation, in that men work more often in the fields and stay in contact longer with water that is contaminated with the infective parasite.

22.2.2 Causes

Good evidence from animal models suggests that the biogenesis of bladder cancer is a multistage process. It involves initiation by carcinogens, followed by promotion of tumor growth (Hicks 1980). Bilharzial bladder cancer may be initiated by exposure to an environmentally or locally produced chemical carcinogen that is excreted in urine. This carcinogen reacts with the mucosal surface of the bladder to produce irreversible and potentially carcinogenic changes in the DNA of some urothelial cells. Chronic bacterial infection, which commonly complicates urinary bilharziasis, has been implicated in the production of nitrosamines, which are well-known potent carcinogens derived from precursors in the urine, and in the secretion of the β-glucuronidase enzyme, which may split conjugated carcinogens to yield free carcinogenic products (Hicks et al. 1977; El-Merzabani and El-Aaser 1979). The possibility that carcinogenic products may be of parasitic origin is not supported by recent investigation (El-Bolkainy 1998). However, local mechanical irritation by schistosoma eggs appears to be an important promoting factor (El-Merzabani and El-Aaser 1979). Vitamin A deficiency may explain the high frequency of squamous metaplasia of the bladder epithelium and the predominance of SCC in patients with bilharziasis.

22.2.3 Clinical and Pathologic Features

Patients usually present with symptoms of cystitis, including painful micturition, frequency, and hematuria. An extensive irregular filling defect is usually detected on cystogram. Computed tomography (CT) scanning or magnetic resonance imaging (MRI) is helpful for diagnosis and staging. The diagnosis depends on cystoscopy, biopsy, and careful bimanual examination under anesthesia (Ghoneim 1994). Urine cytology is also a valuable diagnostic tool for SCC in bilharzial patients (El-Bolkainy et al. 1974). Cytokeratin shedding in urine has been used as a biologic marker for the early detection of SCC (Basta et al. 1988).

Most patients present for treatment at an advanced stage, and 25% of cases are inoperable when first seen (El-Bolkainy et al. 1972). This is so because of the overlap of symptoms of simple bilharzial cystitis with early malignant cystitis. When clinical staging was compared with pathologic findings, a clinical error of 37% was found, with a tendency toward understaging (Ghoneim et al. 1974). In a study of 608 patients with SCC of bilharzial bladder, pT1 disease was found in 2.6%, pT2 in 10.5%, pT3 in 80.0%, and pT4 in 6.9% (Ghoneim et al. 1997). Grading of the tumor in the same study showed grade A in 49.7%, grade B in 33.2%, and grade C in 17.1%. Lymph nodes were involved in only 18.7% of cystectomy specimens. The prevalence of low-grade disease and the intensive mural fibrosis associated with bilharziasis may explain the low incidence of lymph node positivity (Ghoneim 1994).

Grossly, tumors are generally of the nodular, fungating type and are located in the dome or posterior or lateral walls of the bladder. Five gross types have been identified: nodular (60%), ulcerative (23%), verrucous (7%), papillary (7%), and diffuse (3%). (El-Bolkainy 1998) A variety of atypical changes in the bladder mucosa, including metaplasia, dysplasia, and, rarely, carcinoma in situ, may be associated with the disease (Kafagy et al. 1972).

22.2.4 Treatment

22.2.4.1 Endoscopic Resection

In view of the tumor bulk and its advanced stage, transurethral resection appears to be unfeasible for definitive treatment, and no reports have described results when the procedure is used in bilharzial bladder malignancy. Endoscopic resection should be used only for obtaining a biopsy specimen for histopathologic diagnosis.

22.2.4.2 Segmental Resection (Partial Cystectomy)

Segmental resection is an attractive alternative to circumvent the physiologic and social inconvenience of urinary diversion and the possible loss of sexual potency, but local resection is feasible only in select conditions. Solitary tumors must not

involve the trigone, and their size must allow resection with an adequate safety margin; the rest of the bladder mucosa must be free of any associated precancerous lesions. These strict criteria are met in only a minority of cases. El-Hammady et al. (1975) found resectable bladder cancer in only 19 of 190 (10%) patients. Augmentation cystoplasty was required in five patients to increase residual bladder capacity. The 5-year survival rate was 26.5%. Patients with low-grade tumor had roughly twice the survival rate of those with high-grade disease. On the other hand, less favorable results were reported by Omar (1969) in a series of 22 cases. All patients except one developed tumor recurrence within 2 years. This different outcome may be related to the wide variability of selection criteria.

22.2.4.3 Radical Cystectomy

In view of the clinical and pathologic characteristics and the natural history of the disease, radical cystectomy with urinary diversion provides a logical treatment approach for patients with respectable tumor (Ghoneim and Awaad 1980; Ghoneim et al. 1979). In men, the operation entails removal of the bladder, perivesical fat, peritoneal covering, prostate, seminal vesicles, and endopelvic lymph nodes. In women, the bladder, urethra (if is not used for orthotopic bladder substitution), uterus, upper vagina with pelvic fatty tissue, and aforementioned lymph nodes are removed.

In a series of 138 cases, Ghoneim et al. (1979) reported a high postoperative mortality rate of 13.7%. This was due to peritonitis, adhesive intestinal obstruction, and hepatic failure. Cardiopulmonary complications were uncommon among this relatively young group of patients. In this old series, overall the 5-year survival rate was 32.6%; it was 43% in pT1 and pT2, and 30% in pT3 and pT4. Low-grade tumors showed 46% survival; high-grade disease survival was rated at 21%. Lymph node involvement reduced the 5-year survival rate to 20% (Ghoneim and Awaad 1980). In a recent report of results in 1,026 patients from an endemic area of schistosomiasis who underwent cystectomy, 59% of tumors were SCC. Bilharzial ova were identifiable in 88% of specimens. Extravesical extension was not significantly different among patients with SCC or urothelial carcinoma (13.5% and 14.9%, respectively). Overall, the 5-year survival rate with SCC was 50.3%. Only tumor stage, grade, and lymph node involvement had independent significant effects on survival. The latter halved the survival rate (Ghoneim et al. 1997).

These clinical trials provide evidence that cystectomy alone, despite the fact that it is a radical treatment, is inadequate to deal with the extent of local disease. An adjuvant treatment directed to the pelvis might improve survival. Preoperative radiation therapy has been proposed as adjuvant therapy.

22.2.5 Radiation

The growth characteristics of carcinoma of the bilharzial bladder have been studied with the goal of evaluating its potential radioresponsiveness (Awaad et al. 1979a).

Two growth features were noted: (1) high cell mitotic rate with a potential doubling time of 6 days, and (2) an extensive cell loss factor. Tumors with such growth characteristics were expected to exhibit a radiation response (Denekamp 1972). Nevertheless, early experiences with external beam radiation therapy for definitive control of these tumors were disappointing (Awaad 1958). Factors that interfered with the efficiency of radiation treatment in these cases included coexisting bilharzial urologic lesions, which interfere with local tissue tolerance, and considerable tumor bulk, which reduces local tumor control. Furthermore, the presence of radioresistant hypoxic tumor cells is suspected in light of the capillary vascular pattern of this cancer (Omar et al. 1975).

22.2.5.1 Neoadjuvant Radiation

The aim of preoperative radiation is to eradicate smaller cell burdens in deeply infiltrating portions of the tumor and in microextensions into the perivesical tissues and lymphatics. These small tumor foci are expected to have a better radiation response because they are oxygenated and are composed of a relatively small number of cells with high mitotic indices. These biologic factors and the pattern of treatment failure due to local pelvic recurrence have justified the use of preoperative radiotherapy.

Awaad et al. (1979b) compared the results of cystectomy after preoperative administration of 40 Gy with outcomes in a control group treated with cystectomy only. Reported 2-year survival rates were significantly improved in the irradiated group. Ghoneim et al. (1985) compared the results of cystectomy after preoperative irradiation using 20 Gy with those of cystectomy alone. Investigators treated 92 patients, divided into two groups, and followed them for 60 months. Although patients who received preoperative radiation had better survival rates, this improvement did not approach statistical significance. In low-stage tumors, regardless of grade, survival was not influenced by preoperative irradiation, as it was in high-stage tumors.

The presence of a large proportion of hypoxic cells within bulky tumors could explain the modest improvement that was observed after this regimen. To enhance the therapeutic value of irradiation, misonidazole, a hypoxic cell sensitizer, was given before the radiation regimen was delivered. The trial group was divided into three arms: cystectomy only, 20 Gy of preoperative radiation followed by cystectomy, and preoperative radiation followed by cystectomy with misonidazole added as a radiosensitizer. The addition of misonidazole did not provide any additional survival benefit to patients who were given preoperative radiotherapy (Ghoneim et al. 1985; Denekamp et al. 1980).

22.2.5.2 Chemotherapy

In the management of unresectable SCC of the bilharzial bladder, several chemotherapeutic agents have been tried by Gad-el-Mawla et al. (1989) at the National

Cancer Institute of Cairo University. All trials were phase two studies in which a single agent was used. The most promising results were obtained with epirubicin. Neoadjuvant and adjuvant epirubicin chemotherapy were used in a prospective, randomized study involving 71 patients with invasive cancer in bilharzial bladders. Two thirds of treated patients had SCC. Disease-free survival rates were 73.5% and 37.9%, favoring the chemotherapy group (Gad-el-Mawla et al. 1991). Additional long-term follow-up results have not been published.

In a recent multicenter study that consisted of 120 patients treated with neoadjuvant cisplatin and gemcitabine (Khalid et al. 2003), patients with SCC showed no survival benefit over those who underwent cystectomy alone.

22.2.5.3 Prevention and Early Detection

Bilharzial bladder cancer is a preventable malignant disease. Primary prevention entails control of bilharziasis through snail control (the intermediate host of the parasite) and mass treatment of the rural population with oral antibilharzial drugs such as praziquantel (El-Bolkainy 1998). Secondary prevention includes early detection with urine cytology and selective screening of the population at risk. The yield of a single screening study done in a rural area in Egypt was 2 per 1,000 individuals (El-Bolkainy et al. 1974). Such a detection rate would not justify regular screening.

22.2.5.4 Conclusion

In summary, bilharzial SCC is the most common form of bladder cancer in endemic areas. It most often presents at an advanced stage but with low-grade cells. Cystectomy is the standard treatment, but long-term survival remains disappointing. Limited evidence supports a potential role of neoadjuvant chemotherapy and radiation therapy but is not sufficient to facilitate a recommendation.

22.3 Adenocarcinoma

22.3.1 Overview

Adenocarcinoma of the bladder is the third most common histologic type of bladder carcinoma. It accounts for 0.5–2.0% of all bladder tumors (Jacobo et al. 1977; Bennett et al. 1984). Adenocarcinoma has the unique distinction of being the most common tumor arising in the bladder of patients with exstrophy. These patients carry a 4% lifetime risk of developing adenocarcinoma (Smeulders and Woodhouse 2001). Adenocarcinoma of the bladder may also occur in association with schistosomiasis,

endometriosis, bladder augmentation, and other irritative conditions of the urinary bladder (Makar 1962; Anderstrom et al. 1983).

A study from the USA (Kantor et al. 1988) used SEER data to identify only 32 patients (0.7%) with adenocarcinoma from 4,045 patients with newly diagnosed bladder cancer over a 1-year period from 1977 to 1978. Similarly to urothelial carcinoma, adenocarcinoma shows a male predominance. In a total of 11 series comprising 247 patients (Johansson and Cohen 1997), the sex ratio of males to females was 2.7:1.

Adenocarcinoma of the urinary bladder is classified according to its site of origin as primary adenocarcinoma, urachal adenocarcinoma, or secondary (metastatic) adenocarcinoma, the latter of which represents the local extension of primary colon, prostate, or ovarian cancer (Wheeler and Hill 1954). Primary, urachal, exstrophy-associated, and metastatic adenocarcinoma are discussed below. Primary Adenocarcinoma

22.3.1.1 Epidemiology

El-Bolkainy et al. (1972) reported an incidence of primary adenocarcinoma of 8.1% in a series of 229 cases of bladder cancer. Between 1970 and 1995, 1,870 cystectomies were performed in the Urology and Nephrology Center of Mansoura, Egypt. Of these, 185 cases (9.9%) proved on histopathologic examination to be primary nonurachal adenocarcinoma of the bladder (El-Mekresh et al. 1998).

22.3.1.2 Clinical Features

In most patients, primary adenocarcinoma of the bladder presents with hematuria, which may be associated with irritative voiding symptoms and, occasionally, passage of mucus in the urine (Khalid et al. 2003; Jacobo et al. 1977; El-Mekresh et al. 1998; Kramer et al. 1979; Dandekar et al. 1997; Gill et al. 1989). Cystoscopically, the tumor is usually sessile, but it may be papillary (Dandekar et al. 1997). It can arise anywhere along the lateral walls, trigone, dome, and anterior wall of the bladder (Khalid et al. 2003; El-Mekresh et al. 1998; Dandekar et al. 1997; Gill et al. 1989; Grignon et al. 1991). Multiple tumors are present approximately 50% of the time (Khalid et al. 2003; El-Mekresh et al. 1998). Adenocarcinoma is virtually always invasive; only 1 series documented 2 tumors of 27 that were Ta or T1 (Grignon et al. 1991). It is interesting to note that both patients were alive at 51 and 61 months after treatment with transurethral resection alone.

Primary adenocarcinoma of the bladder has a poor prognosis, regardless of the modalities used for treatment. The 5-year survival rates range from 0% to 31%; the small number of patients in each series precludes individual comparisons on the basis of treatment provided. In a retrospective series of 48 patients treated for primary adenocarcinoma of the bladder, stage was the only factor that was highly predictive of outcome.

22.3.1.3 Pathologic Features

For a diagnosis of primary adenocarcinoma of the bladder to be made, it must be distinguished from urothelial carcinoma with areas of glandular metaplasia. The pathogenesis of primary nonurachal adenocarcinoma is based on the ability of the urothelium to undergo metaplastic changes (Choi et al. 1984). Mostafi (1954) proposed that the metaplastic potential of the urothelium has two distinct patterns. Progressive invagination of hyperplastic epithelial buds into the lamina propria (von Brunn's nests) leads to the formation of cystitis cystica. Subsequently, metaplasia of the urothelial lining of these cysts to columnar mucin-producing cells results in the production of cystitis glandularis, which is a premalignant lesion. Follow-up is necessary (Allen and Henderson 1965). Alternatively, cuboidal or columnar metaplasia of the surface epithelium may occur with no downward invagination. Chronic vesical irritation and infection are the predisposing factors for these changes (Choi et al. 1984; Kittredge et al. 1964). This explains, at least in part, the higher incidence of these tumors among patients with bilharzial cystitis.

Histologically, adenocarcinoma may be non-mucin producing or mucin producing. Most of these tumors are mucin secreting, but the passage of mucus during micturition is uncommon (Mostofi et al. 1955). In a large series in Egypt, two thirds of tumors were mucin secreting, and, in most, the site of deposition was extracellular (interstitial). Less commonly, mucin is secreted within the lumen of the acini and, infrequently, excessive intracellular mucin displaces the nucleus to a peripheral crescent, giving the cells a signet ring appearance. It is generally believed that this variety has a poor prognosis (El-Sebai 1961; Blute et al. 1989).

No grading system for adenocarcinoma of the bladder has been uniformly accepted. On the basis of histopathologic findings, Anderstrom et al. (1983) classified vesical adenocarcinoma into five patterns: glandular with columnar, sometimes enteric-appearing, cells; colloid carcinoma; papillary adenocarcinoma; signet ring cell carcinoma; and clear cell carcinoma. Several other histologic subtypes have been described, including mucinous; enteric (colonic); adenocarcinoma not otherwise specified; clear cell; hepatoid; and mixed type (Gill et al. 1989). Unfortunately, no clear data have been gathered on whether these different varieties have an impact on survival or indicate prognosis, although signet ring cell carcinoma appears to impart a rapid course, resulting in death in most patients within 6 months of diagnosis (Grignon et al. 1991).

22.3.1.4 Treatment

Several treatment modalities have been used in the management of primary adenocarcinoma of the bladder. The therapeutic yield after transurethral resection with or without radiotherapy has been shown to be poor; Kramer et al. (1979) reported a 5-year survival rate of 19%.

Partial cystectomy for localized disease in mobile parts of the bladder has been used by several investigators. Analysis of the published data indicates that results attained with this procedure are dismal (Jacobo et al. 1977; Fiter et al. 1993; Thomas et al. 1971; Abenoza et al. 1987). On the other hand, Anderstrom et al. (1983) reported a 5-year survival rate of 54% among 15 patients treated with partial cystectomy.

Adenocarcinoma is not a radioresponsive disease. Reported 5-year survival is < 20% in patients treated with external irradiation alone. The addition of preoperative irradiation did not improve survival in two studies of 34 and 25 patients (Makar 1962; Fiter et al. 1993).

Experience in the use of chemotherapy for the treatment of patients with bladder adenocarcinoma is limited. From results observed with gastrointestinal adenocarcinoma, combination chemotherapy based on 5-fluorouracil has been attempted by several investigators. Most published series involve small numbers, and the response is universally unsatisfactory (Nocks et al. 1983; Nevin et al. 1974; Logothetis et al. 1985).

Radical cystectomy with or without adjuvant therapy has been reported by several authors. Most published reports are based on few patients and involve short-term follow-up. Reported 5-year disease-free survival rates range from 0% to 80% (Makar 1962; Fiter et al. 1993; Thomas et al. 1971; Abenoza et al. 1987; Hatch and Fuchs 1989; Burrett et al. 1991).

In an Egyptian series, 5-year survival after cystectomy was 55%, and no difference was noted between cases of urothelial or squamous cell origin (Tsukamoto et al. 1992; Raitanen et al. 1993). Cox regression analysis proved that stage, grade, and lymph node involvement were all independent prognostic factors. No histologic varieties, regardless of cell type or site of mucin deposition, were shown to be independent prognostic factors (Wheeler and Hill 1954).

22.3.2 Conclusion

In summary, the treatment of patients with adenocarcinoma varies according to subclassification. Primary adenocarcinoma is poorly responsive to radiation and chemotherapy, and patients should be treated with radical cystectomy. Urachal adenocarcinoma should be treated with en bloc resection of the urachus and umbilicus with partial cystectomy. The incidence of adenocarcinoma is much higher in patients with exstrophy. Any patient with bladder exstrophy who has retained his or her bladder should be closely followed, although an exact regimen cannot be prescribed on the basis of currently available evidence. Patients with metastatic adenocarcinoma involving the bladder should undergo complete resection of the involved portion of the bladder, with partial cystectomy with verified negative margins or with the use of radical cystectomy.

22.4 Small Cell Carcinoma

22.4.1 Epidemiology

Small cell carcinoma is a neuroendocrine tumor that most commonly arises in the lungs. Extrapulmonary small cell carcinoma may occur in multiple locations, including the urinary bladder. Primary small cell carcinoma of the urinary bladder is exceedingly rare, with only 286 cases reported in the English language literature (Sved et al. 2004). Evidence is limited and is provided primarily in the form of small case series and case reports.

Two prior reviews have shown that small cell carcinoma accounts for 0.48–0.7% of all cases of primary bladder tumor (Walther 2002; Trias et al. 2001; Blomjour et al. 1989; Holmang et al. 1995). One review of 243 cases of small cell carcinoma of the bladder revealed that 62% were pure small cell carcinoma and 38% were combined carcinomas, most frequently with urothelial carcinoma, adenocarcinoma, or SCC (Abbas et al. 1995). It is interesting to note that the reverse (32% pure small cell carcinoma, 68% other histologic types) was seen in a recent multiinstitutional review by Cheng et al. (2004).

22.4.2 Clinical Features

- An analysis of the characteristics of 238 patients with small cell carcinoma of the bladder has been reported by Sved et al. (2004). Mean patient age was 67.8 years, with a range of 20–91 years, and 80% of patients were male. Similarly, a review of 64 patients by Cheng et al. (2004) showed a male-to-female ratio of 3.3:1 and a mean age of 66 years (range, 36–85 years). Hematuria was the presenting symptom in 88–90% of patients in the two reviews. Rarely, paraneoplastic syndromes herald the diagnosis (Abbas et al. 1995; Kanat et al. 2003). The initial workup is the same as for any patient with hematuria and a suspected bladder tumor. Small cell carcinoma cannot be distinguished from urothelial carcinoma on cystoscopy, but when a pathologist identifies this lesion, the patient should undergo a full metastatic workup (Blomjour et al. 1989). The vast majority of patients (94% of 183 patients on whom this information is available) present with muscle-invasive disease. Metastatic disease is reported in 67% of cases, most commonly to lymph nodes, liver, bone, lung, and brain (Sved et al. 2004).

22.4.3 Pathologic Features

The tumor is composed of a population of relatively uniform cells with scant cytoplasm and hyperchromatic nuclei. Frequent mitotic figures and extensive necrosis

are common (Grignon 1997). The origin of small cell carcinoma is uncertain. It may be derived from neuroendocrine cells or multipotent stem cells of the bladder (Blomjour et al. 1989). Diagnosis is usually made with hematoxylin and eosin; however, special stains to confirm the neuroendocrine origin may be required (Helpap 2002).

A retrospective analysis of 29 urine specimens from patients in whom small cell carcinoma was diagnosed showed that 56% could be diagnosed by urinary cytopathology. The other cases were interpreted as high-grade urothelial carcinoma. Five patients had both small cell carcinoma and urothelial carcinoma, and three of these were identified as small cell carcinoma on cytologic examination (Van Hoeven and Artymyshyn 1996).

22.4.4 Treatment

The most common site for small cell carcinoma is the lung. High-quality studies have been performed and treatment regimens are well defined for primary small cell lung carcinoma. Small cell carcinoma of the lung is treated as a systemic disease because, as with primary small cell carcinoma of the bladder, fewer than one third of patients present with organ-confined disease. Chemotherapy is the mainstay of management. Patients with early-stage small cell carcinoma of the lung are most commonly treated with cisplatin plus etoposide or with alternating cyclophosphamide, doxorubicin, and vincristine (Sandler 2003). The median survival time for these patients is 10–14 months (Ihde 1992). Radiation confers an additional survival advantage in early-stage disease but is not helpful in patients with advanced disease (Erridge and Murray 2003). Surgical resection has not been shown to be beneficial (Sandler 2003). Patients with advanced disease have a uniformly poor prognosis.

The small number of reports on patients with small cell carcinoma of the bladder suggests that it behaves similarly to small cell carcinoma of the lung. Overall, local treatment yields poor survival rates, and systemic therapy provides improvement. In the review by Sved et al. (2004), all the seven patients who underwent cystectomy alone died between 1 and 25 months after surgery. The dismal prognosis of patients treated with radical surgery invited the use of neoadjuvant and adjuvant chemotherapy regimens.

Adding chemotherapy to the regimen appears to enhance survival. In 5 small series reviewed by Sved et al. (Sved et al. 2004; Grignon et al. 1992; Oesterling et al. 1990; Nejat et al. 2001; Cheng et al. 1995), 13 of 18 patients who were treated with cystectomy plus chemotherapy were alive at a mean of 27 months. In addition, Walther (2002) reported favorable response rates in 7 patients treated with systemic etoposide and cisplatin in neoadjuvant and adjuvant protocols.

Similarly, in a recent retrospective review from the M. D. Anderson Cancer Center, at the University of Texas, Houston, median survival and 5-year disease-free survival were significantly improved in patients who received preoperative chemotherapy (Siefker-Radtke et al. 2004). Of 25 patients who underwent cystectomy with or

without postoperative chemotherapy, median cancer-specific survival was 23 months, and the 5-year disease-free survival rate was only 36%. Conversely, of 21 patients who were given preoperative chemotherapy, the median cancer-specific survival rate had not yet been determined at the time of the report, and the 5-year disease-free survival rate was 78%. Only four cancer-related deaths occurred in these 21 patients, all before 2 years. The preoperative chemotherapy regimen determined response; only 2 of 12 patients who received a regimen directed toward small cell carcinoma (etoposide and cisplatin or ifosfamide and doxorubicin) had residual small cell tumors in their cystectomy specimen. Six of nine patients who were given a regimen directed toward urothelial carcinoma (M-VAC, or taxol, methotrexate, and cisplatin) had residual small cell carcinoma on cystectomy (Siefker-Radtke et al. 2004).

Reports of probable cure do exist. One patient with small cell carcinoma metastatic to the pelvic lymph nodes who received M-VAC before undergoing radical cystoprostatectomy was alive at 9 years (Cheng et al. 1995). Another patient treated with neoadjuvant M-VAC before radical cystoprostatectomy had no evidence of disease in the specimen and remained disease free 3 years after surgery (Bastus et al. 1999).

Radiation therapy alone has not been successful for treating patients with small cell carcinoma; mean survival of <8 months has been reported in 40 cases, but long-term survival has been reported in patients treated with radiation therapy and chemotherapy. Of 5 patients who had complete remission after receiving chemotherapy and radiation, all were alive after 4 years, and only 1 required cystectomy for local recurrence (Sved et al. 2004).

Of 12 patients who received partial cystectomy with chemotherapy or radiation therapy, three remained alive at the time of reporting, with a median survival of 34.9 months. Management with transurethral resection of the bladder tumor (TURBT) alone has resulted in uniformly poor results, with a mean survival time of <8 months in most small series (Sved et al. 2004).

22.5 Conclusion

In summary, small cell carcinoma of the bladder is an aggressive disease that often presents at advanced stages. Because of the rarity of this lesion, evidence is limited. Cure is most likely with aggressive multimodal therapy and a combined local and systemic approach.

22.5.1 SCC

1. A surveillance schedule for SCC in patients with spinal cord injury cannot be determined from the currently available evidence.
2. Cystectomy is the best primary therapy for SCC, whether bilharzial or nonbilharzial.

22.5.2 Adenocarcinoma

1. Patients with bladder exstrophy who have retained their bladders should be closely followed, but no particular regimen can be recommended on the basis of currently available evidence.
2. Patients with primary adenocarcinoma should be treated with radical cystectomy.
3. Those with urachal adenocarcinoma should be treated with en bloc excision of the urachus and umbilicus with partial cystectomy.
4. Patients with metastatic adenocarcinoma involving the bladder should undergo complete resection of the involved portion of the bladder, with partial cystectomy with verified negative margins or with the use of radical cystectomy.

22.5.3 Small Cell Carcinoma

1. When small cell carcinoma is identified on a TURBT specimen, the patient should undergo a full metastatic workup, including CT of the abdomen and pelvis, bone scan, chest x-ray, and a neurologic examination.
2. Patients with small cell carcinoma of the urinary bladder require aggressive combination therapy, such as combined chemotherapy and radical cystectomy or chemotherapy and radiation therapy, to achieve cure.

22.5.4 Bladder Sarcoma

1. Patients with bladder sarcoma should be treated with radical cystectomy with negative margin resections.
2. Those with metastatic sarcoma should be treated with a multimodality protocol.

22.5.5 Carcinosarcoma and Sarcomatoid Tumors

1. Carcinosarcoma and sarcomatoid tumors have a poor prognosis, and surgical management is inadequate. Multimodality therapy is recommended.

22.5.6 Paraganglioma and Pheochromocytoma

- The standard treatment for patients with paraganglioma and pheochromocytoma is partial cystectomy with pelvic lymph node dissection, with the same precautions taken as for any other pheochromocytoma with adrenergic blockade.

22.5.7 Bladder Pseudotumor

- When the diagnosis of a bladder pseudotumor is clear, transurethral resection or partial cystectomy is the appropriate treatment. If necessary, to exclude sarcoma, radical cystectomy may be performed.

22.5.8 Melanoma

- Patients with primary bladder melanoma should be treated with radical surgery, but the prognosis remains poor.

22.5.9 Lymphoma

- Those with primary lymphoma of the bladder should be treated with local irradiation.

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Index

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