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Stage T1 Bladder Cancer – Aspects of Diagnosis and Treatment

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Stage T1 Bladder Cancer
– Aspects of Diagnosis and Treatment

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Abstract: Bladder cancer is the most common malignant tumour in the urinary tract. Approximately 75% of the cases are non-muscle-invasive cancers that can be treated with transurethral resection of the bladder (TURB). One fourth of the patients present with muscle-invasive tumours and require radical cystectomy, occasionally in combination with chemotherapy. In stage T1 bladder cancer, the tumour is not muscle-invasive but has invaded the basal membrane and thus has a high potential to progress to muscle-invasive cancer. In these cases, a second-look resection should follow the primary TURB to ensure that all tumour tissue has been removed. Intravesical treatment with bacillus Calmette-Guérin (BCG) is recommended to prevent recurrence. Despite existing prognostic models based on clinical and morphological factors, especially in stage T1 bladder cancer, it is difficult to make correct assumptions regarding prognosis and optimal treatment. The aim of the research underlying this thesis was to elucidate various aspects of diagnosis and treatment of stage T1 bladder cancer.

The first study used tumours from a previously investigated cohort of 237 patients with bladder cancer of all stages that had previously been classified into five molecular subtypes (urobasal A and B, genomically unstable, squamous carcinoma cell like, and infiltrated), to develop a classifier based on immunohistochemistry (IHC). This IHC classifier could reproduce the molecular subtypes in 88% of the cases. The subtypes were shown to have different biological properties and varying potential to progress. In the second study, this IHC classifier was used to subtype primary stage T1 bladder cancer tumours in a population-based cohort of 167 patients. The risk of progression to muscle-invasive disease depended on the molecular pathological subtype. Outcome was even more precisely predicted by using the molecular pathological subtype in combination with clinical factors or with information on immune response. In the third study, the use of second-look resection was investigated in a population-based cohort including all 910 patients diagnosed with stage T1 bladder cancer in Sweden in 2008-2009, and the results revealed substantial differences in the use of this procedure in the six healthcare regions. Bladder cancer death was more common in patients with than in those without remaining T1 tumour in their second-look specimens, but was noted even more often in patients that did not undergo second-look resection. Differences in bladder cancer death between healthcare regions could not be explained solely by the observed disparities in the use of second-look resection. The fourth investigation included all 3,758 patients diagnosed with stage T1 bladder cancer in Sweden in 1997-2006. The use of BCG during the study period increased from 18% in 1997-2000 to 31% in 2004-2006. Patients younger than 75 years, patients treated at high-volume hospitals, patients with grade 3 tumours, and patients from the northern, southern, and Uppsala/Örebro healthcare regions were more frequently treated with BCG. Rates of overall and cancer-specific survival were higher in patients treated with BCG and lower in patients that were ≥75 years of age and those with grade 3 tumours. From a population-based perspective, BCG was found to be underused in Sweden, especially in patients aged ≥75 years and those treated at low-volume hospitals.

Key words: urothelial bladder cancer, tumour stage T1, molecular pathological classification, second-look resection, bacillus Calmette-Guérin.

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Contents

Abbreviations

BC	bladder cancer
BCG	bacillus Calmette-Guérin
CCNB1	cyclin B1
CCND1	cyclin D1
CD3	cluster of differentiation 3
CDH1	E-cadherin
CDKNB1	cyclin-dependent kinase inhibitor 1B
CDKN2A	cyclin-dependent kinase inhibitor 2A, isoform 1
CHT	chemohyperthermia
CIS	carcinoma in situ
CSS	cancer-specific survival
CUETO	Club Urológico Español de Tratamiento Oncológico
EAU	European Association of Urology
EMDA	electromotive drug administration
EORTC	European Organisation for Research and Treatment of Cancer
ERBB2	Her2 = Herceptin 2
E2F3	E2F transcription factor 3
FGFR3	fibroblast growth factor receptor 3
FOXA3	forkhead box A3
GATA3	GATA binding protein 3
GSTM1	glutathione S-transferase mu 1
GU	genomically instable (molecular subtype)
HRAS	Harvey rat sarcoma virus oncogene homolog
IHC	immunohistochemistry
KRAS	KRAS proto-oncogene
KRT	cytokeratin

LOH	loss of heterozygosity
LVI	lymphovascular invasion
MDT	multidisciplinary tumour conference
MMC	mitomycin C
MIBC	muscle-invasive bladder cancer
MDACC	MD Anderson Cancer Center
MKI67	monoclonal antibody Ki 67
NAT2	N-acetyltransferase 2
NMIBC	non-muscle-invasive bladder cancer
PIK3CA	phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform
PTEN	phosphatease and tensin homolog
PUNLMP	papillary urothelial neoplasm of low malignant potential
RB1	retinoblastoma 1
SCCL	squamous cell carcinoma-like (molecular subtype)
SNRUBC	Swedish National Registry for Urothelial Bladder Cancer
STAG3	stromal antigen 3
TCGA	The Cancer Genome Atlas
TCS	tumour cell score
TMA	tissue microarray
TNM	tumour, node, metastasis (classification)
TP	tumour protein
TURB	transurethral resection of the bladder
UNC	University of North Carolina at Chapel Hill
UPK3	uroplakin 3
Uro	urobasal (molecular subtype)
WHO	World Health Organisation

List of publications

This thesis is based on the following papers, which are referred to in the text by the Roman numerals I-IV:

I.   Sjödahl G, Lövgren K, Lauss M, **Patschan O**, Gudjonsson S, Chebil G, Aine M, Eriksson P, Månsson W, Lindgren D, Fernö M, Liedberg F, Höglund M. Toward a Molecular Pathologic Classification of Urothelial Carcinoma. *Am J Pathol* Sept 2013; 183:681–691.

II. **Patschan O***, Sjödahl G*, Chebil G, Lövgren K, Lauss M, Gudjonsson S, Kollberg P, Eriksson P, Aine M, Månsson W, Fernö M, Liedberg F, Höglund M. A Molecular Pathologic Framework for Risk Stratification of Stage T1 Urothelial Carcinoma. *Eur Urol* 2015;68:824–32. * These authors contributed equally to the manuscript.

III. **Patschan O**, Holmäng S, Rosell S, Hosseini A, Jancke G, Liedberg F, Ljungberg B, Malmström PU, Jahnson S. Second-look resection for primary stage T1 bladder cancer: a population-based study. Submitted to *Scand J Urol*.

IV. **Patschan O**, Holmäng S, Hosseini A, Liedberg F, Ljungberg B, Malmström PU, Rosell J, Jahnson S. Scand J Urol. Use of bacillus Calmette–Guérin in stage T1 bladder cancer: Long–term observation of a population–based cohort. *Scand J Urol* 2015;49:127–32.

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List of additional papers not included in the thesis

I.   Sjödahl G, Lauss M, Lövgren K, Chebil G, Gudjonsson S, Veerla S, **Patschan O**, Aine M, Ferno M, Ringner M, Månsson W, Liedberg F, Lindgren D, Hoglund M.: A molecular taxonomy for urothelial carcinoma. *Clin Cancer Res* 2012;18:3377-86.

II.   Lindgren D, Sjödahl G, Lauss M, Staaf J, Chebil G, Lövgren K, Gudjonsson S, Liedberg F, **Patschan O**, Månsson W, Fernö M, Höglund M: Integrated genomic and gene expression profiling identifies two major genomic circuits in urothelial carcinoma. *PLoS One* 2012;7:e38863.

III. Liedberg F, Lauss M, **Patschan O**, Aine M, Chebil G, Cwikiel M, Engilbertsson H, Gudjonsson S, Kollberg P, Sjödahl G, Månsson W, Lindgren D, Höglund M; The Lund Bladder Cancer Group: The Importance of Being Grade 3: WHO 1999 Versus WHO 2004 Pathologic Grading. *Eur Urol* 2012;62:620-3.

IV.   Lauss M, Aine M, Sjödahl G, Veerla S, **Patschan O**, Gudjonsson S, Chebil G, Lövgren K, Fernö M, Månsson W, Liedberg F, Ringnér M, Lindgren D, Höglund M: DNA methylation analyses of urothelial carcinoma reveal distinct epigenetic subtypes and an association between gene copy number and methylation status. *Epigenetics* 2012;7:858-67.

V.   Sjödahl G, Lövgren K, Lauss M, Chebil G, **Patschan O**, Gudjonsson S, Månsson W, Fernö M, Leandersson K, Lindgren D, Liedberg F, Höglund M: Infiltration of CD3+ and CD68+ cells in bladder cancer is subtype specific and affects the outcome of patients with muscle-invasive tumors. *Urol Oncol* 2014;32:791-7.

VI.   **Patschan O**, Sjödahl G, Chebil G, Lövgren K, Lauss M, Gudjonsson S, Kollberg P, Eriksson P, Aine M, Fernö M, Liedberg F, Höglund M. Reply from Authors re: Bas W.G. van Rhijn, Mark A. Behrendt, Kees Hendricksen, Theo H. van der Kwast. Toward Optimal Prediction of Prognosis in T1 Urothelial Carcinoma of the Bladder. *Eur Urol* 2015;68:824-32.

Introduction

In 2015, urinary bladder cancer (BC) was the fifth most common malignancy in men in Sweden, and a total of 2,560 patients with a median age of 73 years were diagnosed with the disease in this country[1]. Smoking is the most important risk factor, but the risk of BC is also higher after exposure to toxic agents, predominantly aromatic amines from the chemical, rubber, and dye industries[2]. Certain medical treatments (e.g, pelvic radiation[3], cyclophosphamide[4], or the diabetes medication pioglitazone[5]) have also shown to raise the risk. Furthermore, the risk of such malignancy is elevated in individuals with a genetically determined reduced ability to break down carcinogenic substances (GSTM1 null genotype), and particularly in smokers with the NAT2 slow acetylator genotype[6]. Hereditary bladder cancer is not known, with the exception of being part of the Lynch syndrome, in which a family history of non-polyposis colorectal cancer has been linked to an increased risk of cancers in the urinary tract, ovaries, uterus, and other organs[7].

The overwhelming majority of BCs arise from the urothelium and are thus defined as urothelial cancers. Hematuria is the most common symptom, but urge or recurrent urinary tract infections are also plausible presenting symptoms[8]. BC prognosis is heterogeneous and depends chiefly on the stage of disease at diagnosis. Approximately 25% of newly diagnosed BC patients have advanced disease, and in these cases the tumour has invaded the muscular layer of the bladder. These muscle-invasive BCs (MIBCs) are classified as stage T2 or higher, and the risk of metastasis to lymph nodes or to other organs is greater than 30%. Despite aggressive treatment with radical cystectomy and urinary diversion, with or without prior chemotherapy, approximately half of the patients die from the disease within 5 years of diagnosis. The remaining 75% of newly diagnosed BC patients have tumours confined to the urothelium or to the underlying subepithelial tissue, called the lamina propria. These cases of non-muscle-invasive BC (NMIBC) are treated with transurethral resection of the bladder with or without additional second-look resection followed either by surveillance or by intravesical treatment with a cytostatic drug or bacillus Calmette-Guérin (BCG). Prognosis is predominantly good in patients with tumours confined to the urothelium (stage Ta), but it is heterogeneous in patients with tumours that have invaded the lamina propria (stage T1): about 50% are cured by the above-mentioned treatment, 25% progress to muscle-invasive disease and require aggressive therapy with either radical cystectomy or radiation, and 25% ultimately die from the disease[9,10]. Reliable instruments for prognostication are lacking.

This present thesis is focused on aspects of the diagnosis and treatment of stage T1 bladder cancer. In the first study in the present research, an immunohistochemistry (IHC)-based molecular pathological classification of bladder cancer tumours was developed by using an existing RNA-expression-derived molecular taxonomy for BC as starting point. In the second study, this IHC-based molecular pathological classification was used for risk stratification of stage T1 BC. The third and forth studies used large population-based cohorts in Sweden to elucidate separate use of the two most important treatment options for stage T1 BC, namely, second-look resection and BCG.

Diagnosis of bladder cancer

Staging

The pathological diagnosis of BC is usually made by transurethral resection of the bladder (TURB). The goals of such surgery are as follows: to radically remove all tumour tissue, to acquire information regarding the clinical stage, and to obtain all tumour tissue for pathological evaluation and staging[11]. Correct staging is of fundamental importance to make the right treatment decisions, and this process includes palpation of the bladder to exclude extravesical tumour growth. A thorough staging also comprises description of tumour size and number, as well as reporting of the location and macroscopic appearance of the tumour. Pathological staging is performed according to the 2009 tumour, node, metastasis (TNM) classification system[12] (Table 1).

Table 1.
Pathological stages according to the TNM classification (2009).

T - Primary tumour		
TX		Primary tumour cannot be assessed
T0		No evidence of primary tumour
Ta		Non-invasive papillary carcinoma
Tis		Carcinoma in situ
T1		Tumour invades subepithelial connective tissue (lamina propria)
T2		Tumour invades muscle
	T2a	Tumour invades superficial muscle (inner half)
	T2b	Tumour invades deep muscle (outer half)
T3		Tumour invades perivesical tissue
	T3a	Microscopically
	T3b	Macroscopically (extravesical mass)
T4		Tumour invades any of the following: prostate, uterus, vagina, pelvic wall, abdominal wall
	T4a	Tumour invades prostate, uterus or, vagina
	T4b	Tumour invades pelvic wall or abdominal wall

Tumour stages Tis, Ta, and T1 are defined as NMIBCs and stages T2 to T4 as MIBCs. It is debatable whether this distinction between NMIBCs and MIBCs is optimal for prognostic purposes, because Tis- and T1-tumours have a higher malignant potential than the Ta tumours in the NMIBC group.

In stage T1 BC, additional information can be considered for prognostic purposes: There is growing evidence that substaging of T1 BC is of prognostic value, and various substaging systems have been proposed. In one approach, tumours with extensive growth, defined as reaching deeper than 0.5 mm beyond the basal membrane, have been shown to be associated with a worse prognosis than tumours found at a depth of less than 0.5 mm[13]. The same strategy but using 1.0 mm depth of invasion instead of 0.5 mm as cut-off value has been proposed by Patriarca et al.[14]. An anatomical method based on invasion of the muscularis mucosae is another commonly evaluated system that is hampered by the fact that the muscularis mucosae is absent in a considerable number of cases[15,16]. A large meta-analysis has confirmed the importance of T1 substaging for prognostic purposes[9]. Nevertheless, although it does seem to be agreed upon that substaging should be performed in stage T1 BC, so far there is no consensus regarding what aspects should be included in that process which is why such classification has not yet been incorporated into current BC staging systems[17,18]. A solid tumour pattern has been associated with an adverse prognosis in stage T1 BC[19], but this pattern has not been incorporated into the TNM-system due to the lack of prospective evaluations.

Pathological grading

The microscopic appearance of the tumour cells and their architecture allows grading of a BC tumour, which is used to judge the inherent aggressiveness of the lesion. Histological grading of BC tumours is performed using the World Health Organisation (WHO) classification system[20]. The European Association of Urology (EAU)[8] guidelines for NMIBC recommended that both the 1973 and 2004 WHO-classification systems be used in clinical practice (Table 2)[21]; the simultaneous use of the two systems is necessary, because the WHO 2004 system has not yet been incorporated into evaluated prognostic models.

In Sweden, the WHO 1999 grading has been the standard for more than a decade[22]. In the 1999 system, the excessively large G2 category in the WHO 1973 system has been reduced in size (Figure 1) by taking into consideration the architectural patterns and the distinction of variation of the cellular features. The G1 category in the WHO 1999 grading is the same as the low-grade category in WHO 2004. However, the high-grade group in the 2004 system was subdivided into G2 and G3 in the 1999 system (Figure 3), which caused a debate when the two-tiered WHO 2004 was launched. The proponents of the WHO 2004 system argued, that a two-tiered system would provide better reproducibility[23], but, for prognostic purposes, there is evidence that the distinction between G2 and G3 is

critical[24,25]. This may explain why the WHO 1999 system is still used by approximately 30% of pathologists in Europe[26]. The Swedish National Healthcare Programme for Bladder Cancer that was presented in 2013 recommends use of both the WHO 1999 and the WHO 2004 system[27].

Table 2.
WHO 1973, 1999, and 2004 pathological grading of bladder cancer.

| **WHO 1973 grading** |
| --- |
| *Urothelial papilloma* |
| Grade 1: well differentiated |
| Grade 2: moderately differentiated |
| Grade 3: poorly differentiated |
| **WHO 1999 grading** |
| *Urothelial papilloma* |
| Papillary urothelial neoplasm of low malignant potential (PUNLMP) |
| Low-grade urothelial carcinoma, Grade I |
| High-grade urothelial carcinoma, Grade II |
| High-grade urothelial carcinoma, Grade III |
| **WHO 2004/2016 grading system (papillary lesions)** |
| *Urothelial papilloma* |
| Papillary urothelial neoplasm of low malignant potential (PUNLMP) |
| Low-grade (LG) papillary urothelial carcinoma |
| High-grade (HG) papillary urothelial carcinoma |
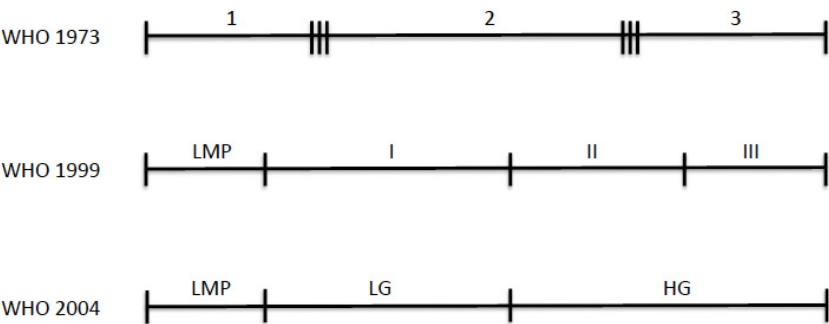
Figure 1.
Relationship between the WHO 1973, 1999, and 2004 classification systems.

An increased risk of progression to muscle invasion has also been reported in stage T1 BC cases with lymphovascular invasion (LVI)[28-30], although prospective evaluation of LVI is lacking. Some histological variants (e.g., micropapillary, sarcomatoid, plasmocytoid, microcystic and small cell) have been shown to have features associated with aggressive disease in univariate but not in multivariate analysis[31]. At least small cell and micropapillary cancer of the bladder might be considered for more aggressive treatment[32,33]. These variants are not included into the grading system, but they are taken into consideration when making individual treatment decisions[20].

Molecular grading

In order to reduce interobserver variability in the established WHO grading system for BC tumours, investigators have explored the use of "molecular grading"[34-36]. Such categorisation aims to estimate the malignant potential of a tumour and is achieved by using certain molecular markers either in addition to pathological grading or instead of any pathological grading at all. The most extensively investigated molecular markers used for this purpose are the mutation pattern of *fibroblast growth factor receptor 3* (*FGFR3*) and the expression patterns of monoclonal antibody Ki 67 (MKI67), tumour protein 53 (TP53), and cyclin-dependent kinase inhibitor B1 (CDKNB1). In a clinical evaluation conducted by van Rhijn and colleagues[35] *FGFR3* mutation status represented BC tumours with favourable prognosis, whereas tumours with adverse outcome were found to have aberrant expression patterns of MKI67, TP53, and CDKNB1. In a retrospective investigation reported by Otto and co-workers[37,38], MKI67 and cytokeratin 20 (KRT20) were identified as prognostic markers for recurrence-free and progression-free survival, respectively, in stage T1 BC, and this was in addition to WHO 1973 grading, which was predictive of cancer-specific survival. However, molecular grading has not been shown to consistently discriminate between high- and low-risk cases, and it does not capture the variability of inherent biological properties of BC tumours. When making individual treatment decisions, it is probably essential to know more about the molecular processes involved in tumour development.

Methods for gene expression analysis

It is possible that measuring gene expression in a tumour represents the most direct method of analysing the inherent potential of the lesion to recur, progress, or metastasise, or to be sensitive to specific treatments (e.g., chemotherapy or targeted therapy). Gene expression can be measured on the mRNA-level or the protein level. The methods used in the present research are described in this section.

Array-based gene expression analysis

DNA microarrays are created using systematically collected specific DNA spots that are attached to a microchip and arranged in a two-dimensional grid. These microarrays can be used to analyse DNA sequences, whole DNA strands, or mRNA[39].

Briefly, in DNA arrays, the mRNA of interest is extracted and subjected to quality-assessment. During a reverse transcription process in which an anti-sense DNA (cDNA) is synthesized, the cDNA is labelled with a fluorescent dye[39]. The labelled probes are subsequently hybridised to the microarray; the labelled cDNA-probes bind to the specific DNA sequences in the array. Thereafter, non-specifically binding probes are washed out, the grid is dried, and the intensity of the fluorescence of each spot is detected and quantified. Quantification is done in relation to the intensity of the other spots in the same sample. The fluorescence intensity of each spot corresponds to the abundance of the mRNA transcript that the spot sequence was directed towards. A possible shortcoming of microarray analysis is the limited dynamic range, which may lead to poor quantification of genes with very low and very high expression[40]. Also, when comparing array-based gene expression in different samples, it is necessary to be aware of a possible batch effect, that is, a technical bias that can be caused by aspects such as disparities in hybridisation procedures or storage or labelling of samples[41]. Knowledge about every step, from retrieval of specimens to analysis, is essential to handle the batch effect[42]. When analysing several batches, it is necessary to perform a labelling transformation step, in which the signal intensity of every batch is scaled in relation to the intensity of the other batches[42]. A general limitation of array-based experiments is that mRNA extracted from a tumour sample always includes mRNA from cells other than tumour cells (e.g., vessels,

and lymphatic cells), and hence it is likely that the genetic information that is obtained actually represents a mixture of several cell entities. This limitation is circumvented when cells are investigated by IHC.

IHC using a tissue microarray

IHC enables visualisation of protein expression in tissues. Briefly, this method involves use of a specific antibody that binds to the molecule of interest, and addition of a second antibody that is conjugated to an enzyme that catalyses a colour-producing reaction[43]. The expression of the colour correlates with the expression of the protein studied in the tissue and can be visualised under a microscope. Conventional IHC has been used for many decades and is well established in all pathology laboratories. Comparison of specimens collected at different time points might be biased by heterogeneous staining results due to differences in antigen retrieval, reagent concentrations, incubation times, and/or wash conditions.

To achieve a higher throughput of specimens and more homogeneous staining compared to conventional IHC, Kononen and co-workers[44] developed the tissue microarray (TMA) technique. With this method, up to 1,000 tumour biopsies with a diameter of 0.6 mm or 1.0 mm are embedded in a paraffin wax matrix (array). Depending on the type of tissue investigated it is possible to obtain up to 200 consecutive tissue sections with a thickness of 4-8 µm. After staining with the markers of interest, the sections can be scored either manually or automatically. The TMA technique allows analysis of large number of biomarkers and also enables the evaluation of the interactions of co-expressed biomarkers in the same tissue-area. It should be noted that application of this method might be limited in heterogeneous tumours or in small tumour-specimens (e.g., TURB-specimens)[45]. Compared to gene expression microarrays and quantitative PCR, the TMA technique is relatively inexpensive to use and easy to perform.

IHC differs from array-based mRNA expression analysis in that it measures gene expression on the protein level. Therefore, with IHC it is possible to specifically study gene expression in certain tumour cells, whereas array-based mRNA expression analysis investigates the entire tumour tissue, including cells other than tumour cells (e.g., stromal, blood vessel, and/or lymphatic cells).

Models of bladder cancer development

It is hypothesised that development of a malignant BC tumour from the normal urothelium is a process in which one or several fundamental cell functions are disrupted step by step, which in turn is leads to cell-growth being out of control[46]. Processes involved in cell proliferation and tumour spread include reaching sustainable proliferative signalling, evading growth suppressors, resisting cell-death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. These processes are influenced by genome instability and by inflammation.

It has been proposed that BC development is primarily driven either by an increase in genes responsible for proliferation (called oncogenes) or by a decrease in genes involved in tumour suppression[47]. Some of the most comprehensively investigated genetic alterations in BC include the tumour suppressor gene *TP53* and the *FGFR3*. *TP53* mutations are common in high-grade, high-stage BCs[48], and are rarely seen in low-grade, low-stage tumours. In contrast, *FGFR3* mutations are found in approximately 70-80% of low-grade, low-stage tumours and to an even greater extent in high-grade, high-stage BCs. Mutations in these two genes are rarely seen together, which is why it has been suggested that they represent two different pathways of tumour genesis in BC[49] (Figure 2). This model is consistent with the observation that the genetic alterations seen in dysplasia and carcinoma in situ (CIS) correspond to those detected in high-grade tumours. Therefore, it has been proposed that high-grade tumours evolve from dysplasia and CIS; low-grade tumours are believed to develop from benign mucosal hyperplasia, where indicated genetic aberrations are not found. Still, this model does not explain why some low-stage, low-grade tumours progress to muscle-invasive, high-grade tumours.
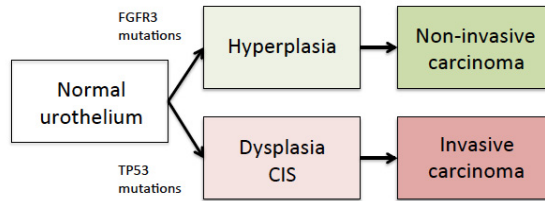
Figure 2.
Model of the two classic pathways of bladder cancer genesis.

A genomic alteration that is frequently seen in both hyperplasia and BC is the deletion of both arms of chromosome 9 (loss of heterozygosity = [LOH]). It has been hypothesised that LOH of *9p/9q* might predispose urothelial cells to more severe genomic alterations, because many such alterations are found on chromosome 9. However, it has not been shown that LOH of *9p/9q* can discriminate between high-risk and low-risk cases[49], nor has it been demonstrated that biallelic mutational inactivation of tumour suppressor genes on chromosome 9 is as common as LOH of *9p/9q*, and hence this alteration seems to indicate an early and important event in BC development[47].

Inactivating mutations of the tumour suppressor genes *TP53*, *RB1,* and *CDKN2A* are common in MIBC and are consequently associated with worse outcome[50]. Also, alterations of *ERBB2* and *PTEN* are most often seen in MIBC. In NMIBC, besides the *FGFR3* mutation status described above, mutations in *stromal antigen 2* (*STAG2*), *HRAS* and *PIK3CA* are frequently found[51]. Most progressing cases of high-grade NMIBC exhibit loss of *CDKN2A*, as found in MIBC (Figure 3). Even though the model of BC pathogenesis has been developed in recent decades, it still does not capture the biological variety among different tumours.
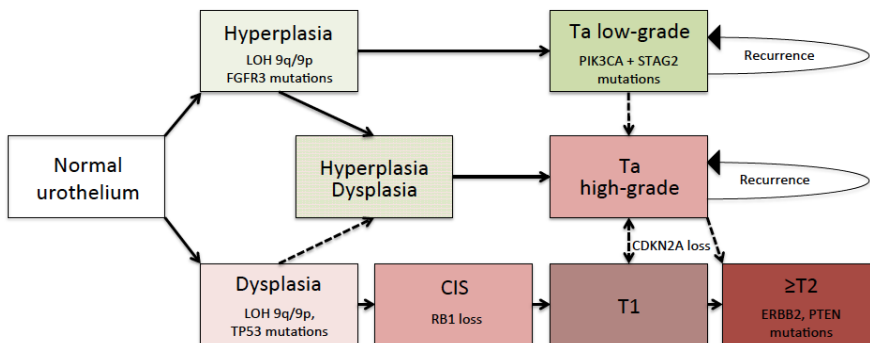

Figure 3.
Three pathways of bladder cancer development (modified from Knowles MA et al. Nat Rev Can 2015[51]).

Another model, suggested by Lindgren and co-workers[52], defines at least two genomic circuits. The first of these is the *FGFR3/CCND1* circuit, which exhibits a strong link between *FGFR3* hyperactivation and overexpression of cyclin D1 (CCND1). Within this circuit, *9q*-deletions, *1q*-gains, and *PIK3CA*-mutations are frequently found, along with two molecular phenotypes: one phenotype is associated with *CDKN2A* deletions, an increased number of genomic alterations, and a worse prognosis, suggesting progressing tumours rather than primarily high-risk tumours; and the other phenotype does not have these features. *PIK3CA*-mutations, similar to other mutations such as those in *HRAS* or *KRAS*, affect key proteins in mitogenic pathways, which in turn increases the levels of CCND1. The CCND1 protein is a driver of the cell cycle, and the *CCND1* gene is amplified in BC[53], and thus *CCND1* is considered to be a principal driver of early-stage BC. In the second circuit of the model, there are amplifications of the *E2F3* locus and reduced expression of RB1- and PTEN, as well as *10q* deletions and *5p* gains. This circuit represents a group of high-risk BC tumours. Lindgren et al. also described a third heterogeneous group with poor prognosis, in which the only distinct pattern is an amplification of *CCND1*.

Molecular classification systems for urothelial cancer

A molecular classification system for BC based on gene expression was first described by the Lund research group. This system emanated from the initial analysis of more than 300 cases of both MIBC and NMIBC tumours, which revealed the five subtypes: urobasal A (UroA), urobasal B (UroB), genomically unstable (GU), squamous cell carcinoma like (SCCL), and an infiltrated tumour class. The subtypes showed different expression patterns for genes governing the early and late cell cycle, immune regulation, cytokeratin expression, cell adhesion, and also those encoding possible drug targets[54]. Moreover, the subtypes had a prognostic impact[54]: UroA cases had a very good prognosis and mainly represented patients with low-grade Ta tumours that rarely progressed to MIBC; UroB cases had predominantly high-grade NMIBC that frequently progressed to muscle-invasive tumours; GU cases had a poor prognosis due to progressing NMIBC or MIBC; SCCL cases had the worst prognosis, almost exclusively with grade 3 MIBC tumours; the infiltrated subtype showed an intense immune response gene expression profile, which did not allow analysis of the expression patterns of tumour-related genes.

Independently from the system developed in Lund, other groups have described molecular classification systems based on analysis of gene expression datasets from MIBC cases. Specifically, these approaches comprise the following

developed in the United States: the two-tiered system from the University of North Carolina (UNC)[55], the three-tiered system from MD Anderson Cancer Center (MDACC) in Texas[56], and the four-tiered system from "The Cancer Genome Atlas" (TCGA) project[57]. The research groups underlying these systems have argued that MIBC should be considered as a separate tumour entity in BC, because it is of greater clinical importance than NMIBC. They have also noted that the molecular subtypes involved in MIBC genesis are rarely found in NMIBC[58]. Furthermore, it was suggested that the subtypes described in MIBC have the same molecular features as the breast cancer luminal and basal subtypes, which hypothetically might make it possible to assign treatment algorithms used in breast cancer also in BC[59]. Figure 4 illustrates the tiered classification approaches, which can be briefly summarised as follows: the UNC system includes a basal subtype with poor prognosis and a luminal subtype with moderate prognosis[55]; the MDACC system has divided the luminal subtype further into TP53-like and luminal, the latter two with similar prognosis; the four-tiered TCGA system added a fourth cluster to the basal (or "squamous") cluster. All three of these classifications were developed exclusively on the basis of unsupervised clustering and without taking biological aspects into consideration.

Another attempt at molecular classification of BC was made by the research group in Aarhus, Denmark, with exclusive focus on the event of tumour progression and investigated cases of NMIBC[60,61]. These researchers also found luminal and basal subtypes in progressing BC cases, and they identified a class 1 subtype that is almost identical to the UroA subtype in the Lund taxonomy.
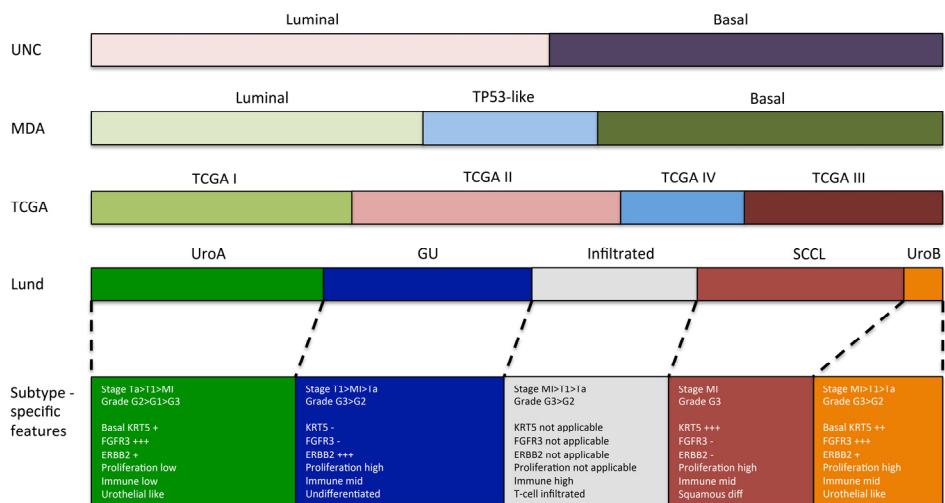
UNC | Luminal | Basal

MDA | Luminal | TP53-like | Basal

TCGA | TCGA I | TCGA II | TCGA IV | TCGA III

Lund | UroA | GU | Infiltrated | SCCL | UroB

Subtype-specific features

	UroA	GU	Infiltrated	SCCL	UroB
Stage	Stage Ta>T1>MI	Stage T1>MI>Ta	Stage MI>T1>Ta	Stage MI	Stage MI>T1>Ta
Grade	Grade G2>G1>G3	Grade G3>G2	Grade G3>G2	Grade G3	Grade G3>G2
	Basal KRT5 +	KRT5 -	KRT5 not applicable	KRT5 +++	Basal KRT5 ++
	FGFR3 +++	FGFR3 -	FGFR3 not applicable	FGFR3 -	FGFR3 +++
	ERBB2 +	ERBB2 +++	ERBB2 not applicable	ERBB2 -	ERBB2 +
	Proliferation low	Proliferation high	Proliferation not applicable	Proliferation high	Proliferation high
	Immune low	Immune mid	Immune high	Immune mid	Immune mid
	Urothelial like	Undifferentiated	T-cell infiltrated	Squamous diff	Urothelial like

Figure 4.
Schematic representation of overlapping of the different BC subtype classification systems (modified from Aine et al Eur Urol 2016[62]) and subtype-specific features of the Lund molecular classification. For each classification system, the subtype distribution is proportional to classification results. GU = genomically unstable; MDA = MD Anderson Cancer Center; SCCL = squamous cell carcinoma like; TCGA = The Cancer Genome Atlas; UNC = University of North Carolina; UroA = urobasal A; UroB = urobasal B.

Although the classification systems described above do seem to show similarities, comparison at high resolution also reveals substantial differences between these strategies[62]. Thus far, only the Lund group has included classification of BC cases of all stages, based on the assumption that BC genesis is driven by dynamic and complex processes that are neither stage nor grade specific[62]. The tumour stage merely reflects a single morphological aspect of the many inherent characteristics of a tumour that cannot be captured solely by studying a certain stage (Figure 5). The Lund classification is also alone in having included a thorough biological analysis of the different molecular subtypes after having performed clustering of more than 300 BC cases[54]. Through this approach, seven molecular subtypes could be merged to arrive at a total of five biologically relevant subtypes. Most importantly, the resulting molecular taxonomy represents a framework that enables virtually all published biomarkers of recurrence and progression to be allocated to the different molecular subtypes, and it puts these biomarkers into a biological context.
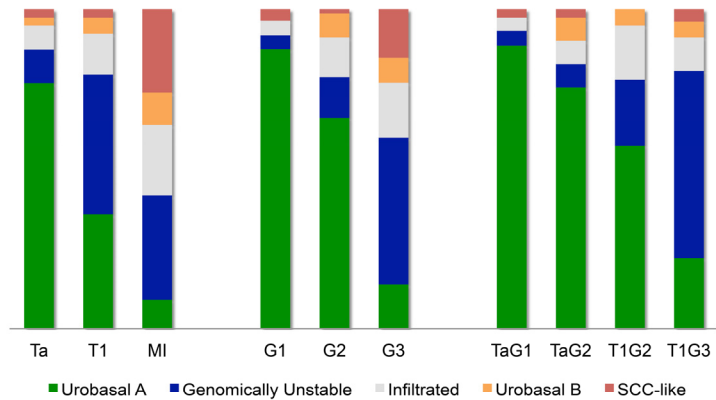
Figure 5.
Distribution of molecular subtypes in different tumour stages, tumour grades, and compositions of tumour stages and grades in NMIBC (modified from Sjödahl et al Clin Can Res 2012[54]).

Despite the overlapping between the UNC, TCGA, MDACC, and Lund classification methods, there is still no consensus between these systems with regard to molecular subtyping. The only subtype that is similar in all these classification systems is a group of tumours with high expression of KRT5/6 and KRT14, and almost no expression of FOXA1 and GATA3, which corresponds to the SCCL-group in the Lund taxonomy and was re-named basal/SCC-like by Lerner et al.[63]. Before any consensus can be reached regarding other molecular subtypes of BC, it will be necessary to investigate larger cohorts that include all stages of BC.

Treatment of stage T1 bladder cancer

Transurethral resection and second-look resection

In primary stage T1 BC treated with transurethral resection (TURB) alone, the risk of recurrence is high, occurring in approximately 20-30% of patients within the first year of primary treatment[64,65,66]. It is impossible to know whether these tumours are true recurrences, or if they arise from residual tumour tissue left after the primary TURB. It appears that the quality of the primary TURB is of outmost importance for recurrence[67], but determining whether this intervention has been radically performed is difficult to objectivise. The presence of detrusor muscle in the specimens from the primary TURB has been proposed as a surrogate marker of good resection quality[68]. Notwithstanding, even if detrusor muscle is detectable in the specimens from the primary resection, residual tumour is found in more than 50% of the cases at second-look resection, and the fraction of remaining T1 tumour is at its best one in four[69]. Another factor that influences the quality of TURB seems to be the surgeon`s level of experience, as indicated by a study reported by Jancke et al.[70] in which it was observed that the risk of recurrence in BC patients was lower when the surgery was performed by specialists than when it was carried out by residents. Therefore, it is plausible that, at least in high-risk cases, TURB should be performed exclusively by specialists.

Residual tumour tissue after primary TURB is common in stage T1 BC, found in 33-71% of the specimens retrieved at second-look resection performed within a few weeks of the primary operation[11,69]. Upstaging to MIBC at second-look resection is also common for tumours initially staged as T1 BC, as observed in one of five cases[71]. Retrospective trials have supported the evidence that the risk of recurrence in NMIBC is lower after second-look resection compared to after primary TURB alone[69,72]. Notably, one of the cited trials also showed a lower risk of progression in patients that had undergone second-look resection for stage T1 BC, but only in cases in which no muscle was apparent in the specimens from the primary TURB[69]. A randomised clinical trial evaluating stage T1 BC has confirmed that TURB combined with second-look resection is superior to TURB alone for treatment of such disease in terms of recurrence- and progression-free survival[11], unfortunately, the patients in that trial were not treated with BCG, which is the recommended postoperative treatment for this stage of disease. However, based on the existing evidence, it is recommended that a routine second-look resection be conducted in all cases of high-grade NMIBC[8].

Postoperative instillation therapy

Intravesical instillation therapy with one dose of chemotherapy (i.e., epirubicin or mitomycin C [MMC]) immediately after TURB has been advocated for almost two decades. The rationale for this treatment is to reduce seeding of free-floating tumour cells and re-implantation of such cells at the vulnerable site of resection[73,74]. The recommendation for this approach is based on several randomised trials[75-77] and on a meta-analysis showing that recurrence-free survival in NMIBC patients was improved by TURB with postoperative instillation therapy compared to TURB alone[76-78]. This chemotherapy procedure prevents recurrences in low- and intermediate-risk NMIBC and has few side effects[76,77], and it has been calculated, that 8.5 instillations can prevent one recurrence. Based on these analyses, postoperative instillation therapy was recommended for all cases of NMIBC in earlier versions of the EAU-guidelines[79]. However, two randomised clinical trials carried out in Sweden questioned the effectiveness of performing postoperative instillation therapy in all cases of NMIBC[80,81]. In one of those investigations, Gudjonsson et al.[80] showed that the benefit of instillation therapy is minimal in NMIBC patients at intermediate and high risk of recurrence. The other study, conducted by Berrum-Svennung et al.[81], corroborated the efficacy of postoperative instillation therapy, but also provided evidence that instillation therapy only prevents small recurrences that can easily be fulgurated in an outpatient setting. Based on these objections and a recent meta-analysis, the updated EAU guidelines for NMIBC from 2016 differ from the earlier versions as follows[82]: immediate chemotherapy after TURB is now recommended for patients with low-risk and previous intermediate-risk NMIBC tumours, but with the same number of recurrences or less than one recurrence per year and an EORTC score of <5[8,82]. Recently, a randomised study comparing postoperative saline irrigation for 15 hours with one immediate postoperative instillation of mitomycin C, showed no differences between the two treatments with respect to recurrence-free and progression-free survival[83], but those findings must be confirmed in further trials before they can be used to influence guidelines recommendations.

Intravesical bacillus Calmette-Guérin (BCG)

Intravesical instillation therapy with BCG is usually started 2 to 4 weeks after transurethral surgery for high-risk NMIBC. BCG is a solution that contains a strain of the bacterium *Mycobacterium bovis,* and it is administered into the bladder through a catheter at 1-week intervals for at least 6 weeks in what is called the "induction course"[84]. The mechanism of action for BCG is still not completely

understood, although it seems to involve both cytotoxic and immune-stimulating properties that increase anti-tumour immunity in the urothelium and the bladder mucosa[85]. Consequently, BCG is not indicated in patients suffering from diseases that weaken the immune system, such as AIDS or leukaemia. Brausi et al.[86] found that 3-years maintenance therapy with BCG was associated with side effects in about 70% of patients noted as follows: the majority (two thirds) were local side effects such as urgency and hematuria, and about one third were systemic side effects such as chills and fever. Due to these side effects, it is necessary to interrupt BCG treatment in seven out of every hundred patients[86]. It has also been reported that an attempt to reduce side effects by giving BCG in one third of the standard dose was not successful[87].

BCG treatment has been shown to lower the risk of recurrence not only compared to TURB alone, but also compared to intravesical MMC and epirubicin[88-91]. It is still a matter of debate whether BCG can also decrease the risk of progression in stage T1 BC. There is evidence that the effect of BCG on progression and on cancer-specific survival is age dependent, with lower efficacy in patients older than 70 years[92], and it has been suggested that this age-related deterioration of the effect is a consequence of immunosenescence[93]. The effect of BCG is also dose-dependent and fades gradually after about 6 months[94], which is why different maintenance schedules for such treatment have been propagated[87,92,95]. The optimal duration and schedule of BCG treatment is still a matter of debate, although it has been demonstrated that a maintenance course is superior to an induction course and that maintenance schedules are needed to achieve better results when using BCG, similar to MMC[96]. The BCG strain is also important for the effect of treatment in terms of recurrence, progression, and survival. A recently published retrospective analysis of more than 2,000 patients showed that the BCG strain had no influence on progression or cancer-specific survival[97]. However, in that study, BCG Connaught was found to be better at preventing recurrences when no maintenance was used, and BCG TICE was superior to BCG Connaught when used in maintenance treatment. According to the EAU-guidelines[8], adjuvant BCG therapy is the recommended strategy for stage T1 BC treated with TURB and second-look resection. The treatment should be given for 1 to 3 years and include an induction course of six weekly treatments and three weekly treatments on months 3, 6, and 12 after primary treatment[8].

Sequential and device-assisted instillation therapy

In an attempt to reduce side effects and increase the efficacy of BCG treatment, several studies have evaluated sequential therapy schedules that combine the impact of BCG with the effect of chemoresection achieved by intravescial chemotherapy[98-101]. One of those investigations was conducted by Osterlinck et al.[98], and patients that were included received six weekly MMC instillations followed by six weekly BCG instillations, or were given six weekly BCG instillations followed by three weekly BCG instillations and thereafter maintenance BCG. In another of the studies, Kaasinen and co-workers[99] randomised patients to six weekly instillations with MMC followed by monthly instillations alternating between BCG and MMC, or to the same schedule but using BCG alone. In both those investigations, toxicity was higher in the arms receiving sequential treatment. In a randomised clinical trial described by Solsona and co-workers[102], BCG (6+3 cycles) was compared with MMC given the day before the BCG-instillations, and the results showed worse toxicity but also a better disease-free interval in the sequential treatment arm[102,103]. An approach that can probably decrease toxicity without the loss of efficacy was discovered in a small phase I trial performed by Svatek and colleagues in which the MMC is administered immediately prior to the BCG and not the day before[91].

The aim of device-assisted instillation therapy is to increase the diffusion capability of the chemotherapeutic drug[104]. Electromotive drug administration (EMDA) has been investigated in a randomised clinical trial in which maintenance BCG was compared with sequential BCG alternated with electromotive MMC in patients with high-grade NMIBC including CIS[105]. In that study, treatment with sequential EMDA was superior in terms of disease-free, progression-free, overall, and cancer-specific survival[105]. However, analysis of the subgroups in that investigation demonstrated that such treatment did not benefit patients with T1G3 BC and that it might only be favourable for patients with CIS, although it should be noted that studies confirming these results are lacking. Another therapeutic option is chemohyperthermia (CHT), which uses microwaves applied through a computer-delivered intravesical irrigation system. Colombo et al.[106] compared MMC with CHT combined with MMC and found that the latter approach was superior with respect to disease-free survival in NMIBC. In a recent multicentre study published by Arends and colleagues, patients with NMIBC were randomised to maintenance BCG or CHT combined with MMC[107], and both recurrence-free survival and progression-free survival were better in the group with CHT after 2-year follow-up. Nevertheless, the number of high-risk patients in that study was low (n=15), and thus it is not possible to draw any conclusions concerning stage T1 BC[108]. Compared to BCG therapy, CHT treatment is labour intensive and hence not easy to implement in daily clinical practice, which is reflected by the

fact that it took 9 years for eleven centres to recruit the 190 patients in that study[106]. Nevertheless, without any solid evidence, it seems reasonable to assume that CHT treatment might be a suitable addition in therapeutic strategies for BCG-refractive high-risk NMIBC-patients that are not fit for radical surgery, or for use as an alternative treatment under circumstances involving a worldwide shortage of BCG[109].

Radiation therapy

A treatment option for MIBC is external radiation therapy, which was compared with BCG or surveillance after TURB in stage T1 BC in a prospective randomised clinical trial conducted in Great Britain[110]. The results of that investigation showed that radiotherapy is more expensive, more toxic, and more harmful to the patients, without offering any advantage in terms of reduction in the risks of recurrence and progression. Therefore, radiotherapy is not recommended for this stage of disease.

Radical cystectomy

Radical cystectomy for NMIBC is a drastic treatment that includes the definitive removal of the whole urinary bladder along with the adjacent organs, such as the uterus and the anterior vaginal wall in females and the prostate in males, as well as the loco-regional lymph nodes. Urinary reconstruction is necessary after this procedure, which represents the first-line surgical treatment for MIBC[8]. Radical cystectomy is potentially curative, but it is associated with a high risk of short- and long-term complications, and consequently entails a considerable reduction in the quality of life[111].

The rationale for performing a radical cystectomy in stage T1 BC is to cure the patient by removing a potentially life-threatening tumour before any local or distant progression. The prognosis for patients that undergo primary radical cystectomy for stage T1 BC is usually good, with 5-year cancer-specific survival rates of 85%[112]. It has been reported that the outcome after radical cystectomy is worse for high-risk NMIBC that has progressed to muscle-invasive disease than for high-risk NMIBC treated with radical cystectomy prior to progression[113]. However, this survival benefit was not confirmed in a small retrospective analysis performed by Sternberg and co-workers, in which patients treated with early radical cystectomy for stage T1 BC less than 3 months after second-look resection, exhibited cancer-specific survival comparable to that observed in patients treated

with deferred cystectomy[112]. Several retrospective trials have confirmed the finding that stage T1 BC upstaged to muscle-invasive disease at radical cystectomy has a worse prognosis than stage T1 BC detected in the cystectomy specimen[114-117]. Radical cystectomy might be considered as primary treatment in stage T1 BC involving any of the following: massive infiltration of the lamina propria, lymphovascular invasion, or aggressive variant histology (e.g., micropapillary cancers)[9,30,32]. Radical cystectomy is also recommended for patients with stage T1 BC that progresses to muscle-invasive disease or patients with recurrence despite maintenance BCG treatment, if organ-confined disease is suspected and the patient is fit for major surgery[8].

Prognostic models of bladder cancer

A number of clinical aspects are measured and used to describe prognosis after primary treatment of BC. The time from TURB until the first intravesical recurrence is called recurrence-free survival. NMIBC cases recurring with a higher tumour stage or grade than present at initial diagnosis have, per definition, progressed. However, in clinical practice, progression is most often simply defined as progression from non-muscle invasive stages (Tis, Ta, T1) to muscle-invasive (MIBC) stages (T2-4) only. Due to this lack of clarity, the International Bladder Cancer Group[118] has recently proposed that progression be specified as an increase in T-stage from Tis or Ta to T1, or to T2 or higher, or to lymph node metastasis or distant metastasis, or an increase in grade from low to high. The time from primary diagnosis to progression is defined as progression-free survival. The time from diagnosis to death from the cancer being studied is defined as cancer-specific survival (CSS).

The EORTC and CUETO risk tables

Two models for prognostication of NMIBC are frequently used and have been thoroughly evaluated. One of these was constructed by the European Organisation for Research and Treatment of Cancer (EORTC)[119] based on a study population of 2,596 patients with either stage Ta or T1 BC. Second-look resection was not performed, nor was maintenance BCG given, and 78% received intravesical treatment predominantly with chemotherapy. Factors affecting the EORTC risk score are shown in Table 3. The EORTC scoring system categorises patients into four risk groups for recurrence and progression, respectively (Table 4).

The other model was developed by the Club Urológico Español de Tratamiento Oncológico (CUETO) through calculation of a risk-assessment tool based on 1,062 patients treated with maintenance BCG. The patients in the CUETO cohorts did not undergo second-look resection[95]. Factors in the CUETO model include sex, age, prior recurrence status, number of tumours, T stage, concurrent CIS, and tumour grade.

Using the CUETO and EORTC scoring systems in clinical practice requires knowledge about the underlying populations. Inasmuch as BCG is considered to be a more effective treatment than intravesical chemotherapy, and considering that the CUETO score is attributed to the effects of BCG, the calculated probability of

recurrence is probably lower in the CUETO scoring system than in the EORTC system.

Table 3.
Weighting used to calculate the EORTC risk score.

Factor	Recurrence	Progression
Number of tumours		
Single	0	0
2-7	3	3
> 8	6	3
Tumour 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
Concurrent CIS		
No	0	0
Yes	1	6
Grade		
G1	0	0
G2	1	0
G3	2	5
Total score	0-17	0-23

Table 4.
Probability of recurrence and progression according to total EORTC risk score.

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

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

CI = confidence interval

A clinical evaluation of both of these risk-assessment tools has shown that the models overestimate the probabilities of recurrence and progression in high-risk NMIBC cases[120-122].

It should also be noted that, especially in stage T1 BC, the existing prognostic models do not take into consideration other factors that might have prognostic relevance such as the following: the location of tumours in the bladder[123], the tumour status at second-look resection[71], recurrence status at 3 months[124], tumours in bladder diverticula[125], concomitant CIS in either the bladder or the prostatic urethra[126], previously mentioned histological variants[127,128], and lymphovascular invasion and growth pattern[19]. However, according to the EAU guidelines[8], both the CUETO and the EORTC risk table should be used for risk stratification of patients with NMIBC.

Biomarkers

In addition to using clinical and morphological factors used for prognostication, biomarkers have been employed to refine histological assessment, which has improved estimation of the risk of recurrence or progression for individual

tumours (Table 5a and 5b). Investigations of these biomarkers have contributed substantially to clarifying the changes in cell function during cancer development. Some of the markers have even been shown to be of independent prognostic value when assessed in multivariate models that at the least included tumour grade and stage[36,129]. Notwithstanding, none of the biomarkers have been consistently proven to reliably predict the course of disease in an individual patient. Also, it has to be considered unlikely that the complexity of cancer could be reflected by a single biomarker. Briefly, none of the known markers is currently in routine clinical use or recommended in contemporary treatment guidelines[8].

Molecular classifications

Molecular classification systems for BC, such as the Lund taxonomy for urothelial cancer described above, represent a framework that enables virtually all published biomarkers of recurrence and progression to be allocated to the different molecular subtypes, and makes it possible to put these biomarkers into a biological context[54]. Thus existing biomarkers are simply substitutes for the different molecular subtypes[54]. The inherent biological potential of a tumour to progress might well be reflected by the molecular subtype of the lesion[54,62].

Table 5a.
Selected biomarkers of recurrence of non-muscle-invasive bladder cancer.

Marker	High/Low	Univariate/Multivariate p	References
CCND1	low	0.003/0.03	Sgambato. Int J Cancer 2002[130]
CD24	high	<0.001/-	Liu. Oncol Lett 2013[131]
CDH1	abnormal	0.005/0.02	Mahnken. Oncol Rep 2005[132]
CDKN1A	low	0.005/0.03	Behnsawy. Urol Oncol. 2011[48]
CDKN1B	low	0.001-0.006/0.05-0.005	Sgambato. Cancer Res 1999[133]
CDKN2A	low	0.01/0.007	Yurakh. Eur Urol. 2006[134]
COX2	low	<0.001-0.05/<0.001,NS	Tadin. Diagn Path 2012[135]
ESRß	low	0.021/NS	Han World J Urol 2012[136]
FGFR3	low	0.04/-	Maeng. Kor J Urol 2010[137]
KRT20	abnormal	0.001/0.01	Bertz. Eur Urol 2014[38]
MKI67	high	0.003-0.04/0.0005	Quintero. J Clin Pathol 2006[138]
RBX1	high	<0.001/0.04	Wang. J Surg Oncol 2013[139]
SOX2	high	0.001/0.029	Ruan. Med Oncol 2013[140]
SPINK1	low	0.02/NS	Patschan. World J Urol 2012[141]
TP53	abnormal	-/0.004-0.06	Rev. in Malats. Lancet Oncol 2005[142]

Table 5b.
Selected biomarkers of progression of non-muscle-invasive bladder cancer.

Marker	High/Low	Univariate/Multivariate p	References
AQP3	low	0.02/0.03	Otto. BMC Cancer 2012[129]
CCND1	low	<0.001-0.005/0.05	Yurakh.Eur Urol 2006[134]
C16orf74	low	<0.001/0.001	Kim. PLoS ONE 2010[143]
CDKN2A	loss	0.018/0.009	Krüger. Eur Urol. 2005[144]
CTAG2	high	0.001/0.02	Dyrskjøt. Br J Cancer 2012[145]
EGFR	high	<0.0001-0.01/0.004	Mellon. J Urol 1995[146]
ERBB4	high	0.001/-	Puerta-Gil. Am J Pathol 2012[147]
ESRß	high	0.002/-	Miyamoto. BJUI 2012[148]
GATA3	high	0.05/0.05	Miyamoto. Hum Pathol 2012[149]
HYAL1	high	0.001/<0.001	Kramer. Eur Urol 2010[150]
KPNA2	high	<0.001/0.001	Jensen. Eur Urol 2011[151]
MKI67	high	<0.01-0.002/0.0005-0.002	Liukkonen. Eur Urol 1999[152]
RBX1	high	0.005/0.03	Wang. J Surg Oncol 2013[139]
S100A8	high	0.001/0.004	Ha. Kor J Urol 2010[153]
SERPINB5	low	0.001/NS	Fristrup. Am J Pathol 2012[154]
TP53	abnormal	-/0.06-0.0005	Rev. in Malats. Lancet Oncol 2005[142]

Specific aims of the present research

The aims of the present studies on diagnosis and treatment of BC (reported in Paper I-IV) were as follows:

Paper I

To apply IHC for molecular pathological characterisation of a BC cohort that had previously been classified by gene expression array profiling into the subtypes UroA and B, GU, and SCCL, and also to develop a simple IHC-based molecular pathological classifier that can reproduce the three molecular subtypes in histological specimens and is easy to use in clinical practice.

Paper II

To use the IHC-based molecular pathological classifier (described in Paper I) to categorise a cohort of primary stage T1 BC tumours into the three subtypes Uro, GU, and SCCL, and to investigate whether such classification, together with existing risk-stratification tools, can provide improved information on tumour progression.

Paper III

To evaluate the use of second-look resection in primary stage T1 BC in a population-based cohort in Sweden, and to investigate the impact of that surgery on cancer-specific survival.

Paper IV

To analyse the use of bacillus Calmette-Guérin (BCG) for treatment of primary stage T1 BC and the effect of such treatment on overall and cancer-specific survival in a population-based cohort in Sweden.

Patients and methods

Paper I

The first study included a cohort of 308 patients representing all stages of organ-confined BC, which had previously been subjected to a whole-genome expression analysis to classify the tumours into five molecular subtypes: Uro A and B, GU, infiltrated, and SCCL[54]. In the present investigation, 42 patients with the infiltrated subtype and 29 with insufficient tissue for the TMA were excluded, and the paraffin-embedded urothelial carcinoma specimens from the remaining 237 patients were used to construct a TMA sampling the invasive tumour front. Sections from the TMA blocks were mounted on slides and stained with the IHC markers of interest. Due to the limited amount of tumour tissue in the TMA, it was necessary to use a selection of predominantly high-quality markers to develop the IHC-classifier. The functions of the chosen markers represented relevant biological processes. The labelling intensity of 17 of the 20 markers was recorded, and the tumour cell score (TCS) was calculated as the intensity multiplied by the fraction of positive tumour cells. For cyclin B1 (CCNB1), MKI67, and tumour protein 63 (TP63), only the fraction of positive tumour cell nuclei was measured. The following was also noted: tumour stage and grade, and whether an invasive growth pattern, urothelial-like histology, and/or histological variants were apparent. Staining with α-smooth muscle actin (α-SMA) was performed to visualise the intact tumour-stroma interface.

Using the findings of the IHC analysis, an IHC classifier was developed to identify the molecular pathological subtypes (Uro, SCCL, and GU) in the specimens from the previously investigated cohort. Briefly, the results of the molecular pathological classification were correlated with the classification based on whole-genome gene expression and with cancer-specific survival.

Paper II

The second study included a population-based cohort of 254 patients in southern Sweden with primary stage T1 BC. All pathological samples were re-evaluated (using both WHO 1999 and 2004 grading) to verify tumour stage and grade and the presence of histological variants, lymphovascular invasion, lymphocyte infiltration (quantified using CD3-positive cells), and the depth of lamina propria invasion were investigated. Eighty-seven cases were excluded due to clinical and technical factors, which left 167 cases for inclusion in the analysis. Progression risks were calculated according to the EORTC risk tables, but based on the WHO 1999 grading. A TMA was constructed using tumour tissue from the invasive

tumour front. The IHC-classifier (described in Paper I) was used to classify tumours as Uro, GU, or SCCL. Additional markers were employed to ensure that the classifier reliably resembled the different subtypes. The prognostic effect that these molecular pathological subtypes in combination with the EORTC (WHO 1999) score or with tumour infiltration with CD3-positive cells had on the time to progression was analysed. Progression was defined as development of MIBC, nodal or distant metastasis, or an adverse event that required radical cystectomy.

Paper III

The third study included all 1,043 patients diagnosed with primary stage T1 BC in Sweden in 2008 and 2009, identified in the Swedish National Registry for Urothelial Bladder Cancer (SNRUBC). In all, 133 patients were excluded due to either higher or lower tumour stage, metastatic disease, or primary treatment with radical cystectomy. The data from the SNRUBC were re-evaluated with regard to TNM stage and WHO 1999 grade, primary treatment, patient age, hospital volume, healthcare region, and whether a second-look resection had been performed within 8 weeks after the primary transurethral resection. Date and cause of death were acquired in 2014 from the National Cause of Death Registry and provided by the National Board of Health and Welfare.

Paper IV

The fourth study included all 4,319 patients that were diagnosed with primary stage T1 BC in Sweden between 1997 and 2006, identified in the SNRUBC. In all, 561 patients were excluded, because they had been treated with radical cystectomy, intravesical chemotherapy, radiation therapy, or more than one treatment option, or had not been adequately treated. Thus 3,758 patients remained for inclusion in the analysis. Patient age, gender, tumour grade (WHO 1973 and later WHO 1999), hospital volume, healthcare region, and primary treatment with BCG within 3 months of diagnosis were recorded. Date and cause of death were acquired in 2012 from the National Cause of Death Registry and provided by the National Board of Health and Welfare.

Results and conclusions

Paper I

According to the previously performed classification based on whole-genome expression analysis[54], the 237 cases with urothelial cancer were classified as follows: 118 as UroA, 20 as UroB, 25 as SCCL, and 74 as GU. The mRNA expression levels of the selected 20 markers, with the exception of UPK3 and CDH1 (E-Cad), and the corresponding IHC-based tumour cell scores or fractions of positive cells were compared and found to show good concordance (r = 0.42 – 0.73)(Figure 6). Almost all UroA tumours were NMIBC and had grade 1 or 2, and they exhibited high expression of FGFR3 and consistent expression of CCND1, greater than expression of CCNB1 and MKI67. CCNB1 expression was limited to the suprabasal cell-layer. KRT5 expression was observed only in the basal layer of UroA tumours. Fifty per cent of the UroB tumours were MIBC and grade 2 or 3. Similar to the UroA lesions, the UroB tumours showed high expression of FGFR3 and CCND1, as well as basal expression of KRT5 in a few cases.
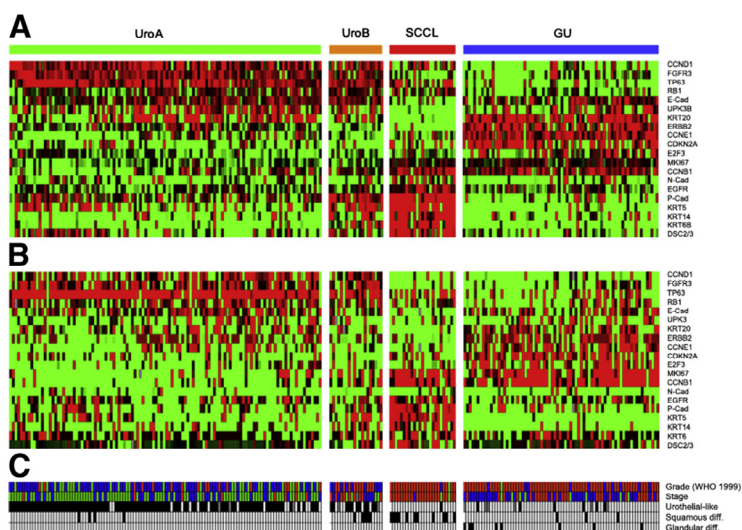

Figure 6.
(A) Heat map of mRNA expression levels in 237 patients with BC. Red = high expression; green = low expression. **(B)** Heat map of corresponding IHC-based TCS or fractions of positive cells. Red = high TCS/fractions of positive cells; green = low TCS/fractions of positive cells. **(C)** WHO 1999 grade, stage, growth pattern, and squamous and glandular differentiation. Green = stage Ta or grade G1; blue = T1 or G2; red = ≥T2 or G3; black = Urothelial-like growth pattern including signs of squamous or glandular differentiation; grey = missing data. (Figure reprinted from Sjödahl et al. 2013[155]; with kind permission from Elsevier).

Compared to UroA tumours, UroB tumours showed a larger fraction of positive cells with the proliferation markers CCNB1 and MKI67. SCCL tumours were often MIBC and almost exclusively grade 3. SCCL tumours were negative for CCND1 and displayed a high expression of CCNB1 and MKI67 throughout the tumour tissue, which reflects substantial proliferative activity. Expression of KRT5 was markedly increased throughout the entire urothelium. GU tumours were typically stage T1 or MIBC and had a high grade, and expression of FGFR3, CCND1, and TP63 was either low or absent. Expression of ERBB2 was higher in GU tumours than in SCCL tumours, and KRT5 was essentially not observed in GU lesions. Also, proliferative activity was elevated in GU cases, resulting in high expression of CCNB1 and MKI67.

The molecular classification is performed in a stepwise manner, and hence development of the IHC classifier was based on the same principle. The first step involved discrimination between UroA/B and GU/SCCL tumours. Low expression of CCNB1, a urothelial-like histology, and WHO 1999 grade 1 or 2 were considered indicative of a UroA/B tumour. KRT5 was used to differentiate between GU and SCCL (Figure 7): a high KRT5 tumour cell score indicated an SCCL tumour, whereas KRT5 was absent in GU tumours. There was good overall concordance between the molecular classification and the IHC classifier, with an overall accuracy of 0.88. However, the IHC classifier could not discriminate between UroA and UroB tumours, and thus such tumours were merged into one Uro subtype in the IHC classifier.
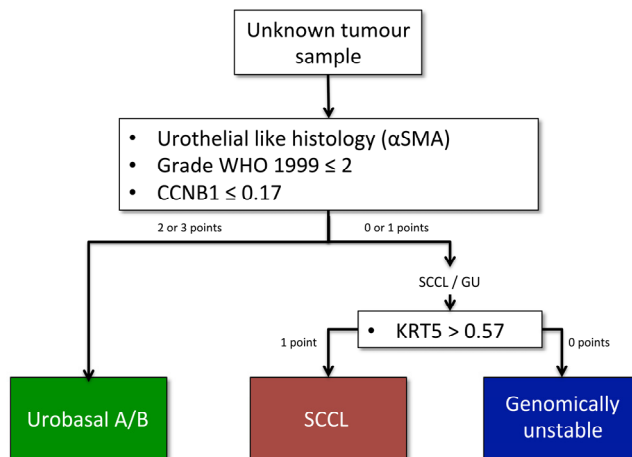

Figure 7.
IHC classifier that allows stratification of histological bladder cancer specimens into the molecular pathological subtypes urobasal A/B, genomically unstable (GU), and squamous carcinoma cell-like (SCCL).

Paper II

Of the 167 cases with primary stage T1 BC, 149 could be classified as Uro (n=48), GU (n=87), and SCCL (n=14); 18 cases were inconclusive and were not included into the analysis. Additional markers confirmed that the expression patterns of the different subtypes were in concordance with earlier findings, thus corroborating the IHC classifier. Compared to Uro tumours, GU tumours were more often multiple and showed deeper infiltration with lymphovascular invasion or concomitant CIS. The pattern of marker expression was similar to that observed in the previous study (Paper I). Patients with a high EORTC (WHO 1999) score (>12) were almost all GU/SCCL cases. A high CD3 score was associated with the following: a GU/SCCL subtype, a greater risk of progression within 3 years, and an increased risk of having deeper infiltrating tumours (≥T1b). Three-year progression-free survival was higher in patients with the GU/SCCL subtype and a low EORTC (WHO 1999) or CD3 (GU/SCCL low) score than in patients with the GU/SCCL subtype and a high EORTC (WHO 1999) or CD3 (GU/SCCL high) score. Also, progression-free survival was best in Uro cases with a low EORTC (WHO 1999) or CD3 (urobasal) score (Figure 8). The group of patients with Uro and a high EORTC (WHO 1999) or CD3 score was very small (n=4) and was therefore omitted from the analysis. Classification of stage T1 BC into subtypes, together with EORTC (WHO 1999) and CD3 score, improved risk stratification and clarified the differential expression of biomarkers and the differences in risk factors and progression rates.
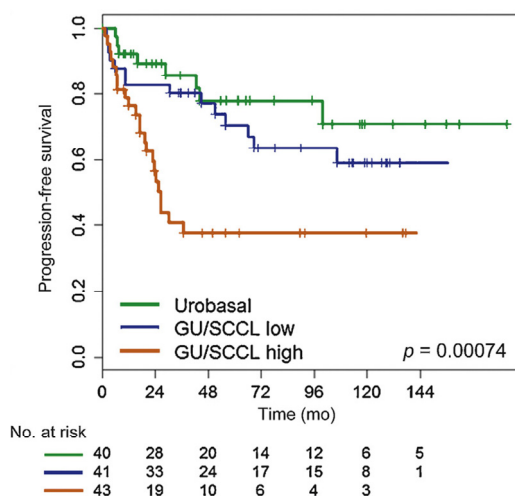

Figure 8.
Kaplan-Meier analysis with progression-free survival as endpoint. Grouping variables: urobasal, GU/SCCL low EORTC/CD3, and GU/SCCL high EORTC/CD3 (Figure reprinted from Patschan et al. 2015[156]; with kind permission from Elsevier).

Paper III

The third study included 910 patients with a mean age of 74 years, and a majority (77%) of these subjects were men. The mean follow-up time was 45 months. Second-look resection was performed in 51% of the patients, but the use of second-look resection differed substantially between the six Swedish healthcare regions (from 16% in the western region to 78% in the south-eastern region). In all, 48% of the patients received multiple adjuvant intravesical instillation therapy, with either BCG or chemotherapy. One-third of the patients were discussed at a multidisciplinary tumour conference (MDT).

Second-look resection was associated with intravesical instillation, age <74 years, grade 3 tumours, discussion at a MDT, and treatment at a high-volume hospital. Bladder cancer specific survival was lower in patients characterised by the following: no second-look resection (p < 0.001), age >74 years, discussed at a MDT, a grade 3 tumour, and no intravesical treatment. Patients treated in the Uppsala/Örebro and western healthcare regions had a lower CSS compared to those treated in the Stockholm region. Only 38% of the patients that underwent second-look resection had a tumour-free specimen at re-resection. The 5-year CSS differed depending on the tumour stage at second-look resection: CSS was 88% if there was no remaining tumour tissue, 90% when there was a Ta tumour, and 82% when T1 tumour was found. The 5-year CSS for patients with no second-look resection was 76%, which is statistically significantly lower than the CCS of patients with T0 at second-look resection. However, a selection bias and other unknown factors may have affected these findings.

Paper IV

Out of 3,758 patients included in this investigation, only 24% (896) were treated with BCG after the primary TURB. During the study period, the use of BCG increased from 18% in 1997-2000 to 31% in 2004-2006. Also, BCG was given more often to patients that were <75 years of age, treated at high-volume hospitals, had grade 3 tumours, and were treated in the northern, southern, and Uppsala/Örebro healthcare regions. Overall mortality and cancer-specific mortality were lower in patients receiving BCG (HR 0.76, [95% CI 0.68-0.85], p<0.001 and HR 0.79, [95% CI 0.66-0.96], p=0.015, respectively). Overall mortality was lower in female than in male patients, but there were no gender differences in BC mortality. Patients aged ≥75 years and those with grade 3 tumours had both higher overall mortality and higher BC mortality. Taken together, and from a population-based perspective, these findings suggest that BCG is underused in Sweden, particularly in patients aged 75 years or older and in those treated at low-volume hospitals. Considering that BCG treatment was associated with lower BC-specific mortality, such therapy should be applied more often.

Discussion and future perspectives

The results of the first two studies demonstrated that a molecular pathological classification can be used to categorise BC tumours based on a recently developed molecular taxonomy. This translation of the use of the molecular pathological classification to the highly relevant clinical setting of primary stage T1 BC is the first of its kind in BC. In these investigations, it became evident that a single tumour stage contains all of the molecular subtypes, thus reflecting its biological heterogeneity. The results showed that the molecular pathological subtypes could discriminate between different risk groups of stage T1 BC when combined with EORTC (WHO 1999) score or infiltration of tumours with CD3-positive cells, and this represents an approach that can enhance understanding of the underlying tumour biology. Bearing this in mind, it seems reasonable that not only the grade and stage, but also the molecular subtype of tumours be used in future clinical trials to assess aspects such as chemosensitivity or BCG resistance in BC.

The molecular pathological classification enables clinicians to analyse formalin-fixed, paraffin-embedded specimens in daily practice and to refine their diagnostic work-up. It is important that other research groups use the Lund taxonomy and confirm the results. Relevant issues to investigate in a prospective setting are whether molecular subtypes can improve stratification of high- and low-risk BC, and determining which molecular subtypes best predispose patients to respond to targeted therapy or certain chemotherapeutic agents. Another important issue is to find a common nomenclature for the different molecular classification systems that exist today (UNC, MDACC, TCGA, Aarhus, Lund). In the near future, RNA array techniques may reduce the cost of performing routine molecular classification of tumours, which would accelerate the implementation of such approaches and eventually provide the possibility to evaluate more individualised treatment plans.

The third and fourth studies evaluated the treatment of stage T1 BC with second-look resection and BCG, respectively, and both showed that these treatment options have been underused on a population level in Sweden. Concerning second-look resection, it is remarkable that this procedure has been used to such varying degrees in Sweden, and this is actually the case even if many factors affecting the use of second-look resection could not be analysed in this population-based study. This observation is clearly thought provoking. Second-look resection should definitely be the given choice for stage T1 BC when considering the following: the high malignant potential of this stage of the disease; the difficulties in defining whether a TURB has or has not been radically performed; the fact that over 40%

of the current second-look-treated patients had remaining T1 tumour in their second-look specimens; the clear recommendations in national[27] and international guidelines[8]. It is difficult not to reflect on the idea that the decision not to undertake a second-look resection in stage T1 BC should be exclusively reserved either for patients planned for primary radical cystectomy or for those managed with non-curative intent only.

Future research will have to determine if the surgical concept of en-bloc resection can enable urologists and pathologists to better judge whether a TURB has been radically performed[157,158]. Another question that needs to be addressed is whether en-bloc resection also has the potential to lower the risk of residual tumour at second-look resection for stage T1 BC.

Regarding BCG treatment, the fourth study clearly demonstrated that patients aged 75 years or older should be more frequently treated with BCG. The use of BCG in stage T1 BC increased in Sweden during the present study period, but, despite that, two thirds of the patients did not receive this recommended treatment. Even after identifying the need for better adherence to guidelines and more frequent use of BCG, a limiting factor might be a continued global shortage of BCG in the future. BCG production is technically advanced and so far conducted in only a few locations, and thus it might be difficult to meet increasing BCG demands[109]. It is essential to create alternative options that can reduce the need for intravesical BCG treatment, such as sequential intravesical chemotherapy.

Populärvetenskaplig sammanfattning

Urinblåsecancer är den vanligaste cancerformen i urinvägarna. Den kan ha väldigt olika förlopp: I de flesta fall växer urinblåsecancer inte igenom ytskiktet av blåsväggen (tumörstadium Ta) och kan behandlas med s.k. hyvling igenom urinröret (transuretral resektion av blåsan, kort: TURB). I en fjärde del av fallen växer urinblåsecancer dock in i blåsmuskeln (tumörstadium T2-4). Då måste den behandlas mycket aggressivt med borttagande av blåsan, vilket ofta kombineras med konventionell cellgiftsbehandling.

När tumören vuxit precis igenom det tunna membran som skiljer blåsans ytskikt ifrån kärl- och bindvävsskiktet (tumörstadium T1), är det oftast svårt att avgöra hur sjukdomen kommer att utvecklas och hur man bäst ska behandla urinblåsecancer. För att vara säker på att man opererat bort tumören i sin helhet rekommenderas en s.k. "andra titt" eller "second-look" resektion. I syfte att förhindra återfall i tumören ska man också ge upprepade behandlingar direkt i blåsan med bacillus Calmette-Guérin (BCG), ett ämne som är toxiskt och tros aktivera immunsystemet i blåsan.

För att kunna förutse sjukdomsförloppet, bedömer man idag tumörens utseende och uppbyggnad i mikroskop, dvs. man tittar på tumörstadium, tumörgrad och histologi. Dessutom bedömer man kliniska faktorer, såsom antal och storlek av tumörer, återfallsfrekvens och patientens ålder. Dessa faktorer är dock otillräckliga för att kunna ge en säker prognos. Tidigare forskning har visat att man med molekylär klassificering, dvs. genom att analysera tumörens arvsmassa, kan dela in urinblåscancer tumörer i fem olika undergrupper. Dessa undergrupper har olika biologiska och kliniska egenskaper som ger mer information avseende prognos än ovan nämnda faktorer. Dock är molekylär klassificering f.n. för dyr och för komplicerad för att kunna användas i klinisk vardag. Immunhistokemiska analysmetoder, dvs. att man gör infärgningar av vävnadssnitt inför mikroskopi, är däremot utbredda och relativt enkla att genomföra.

I det första arbetet av denna avhandling utvecklades en modell som skulle kunna göra molekylär klassificering lättare att använda i kliniken. Modellen använder sig av immunhistokemi för att kunna avgöra vilken molekylär undergrupp en tumör tillhör. I de undersökta 237 blåscancerfallen kunde man med denna immunhistokemiska modell återfinna de molekylära undergrupperna i 88% av fallen, vilket talar för att modellen fungerar bra. Detta arbete lade grunden till det andra delarbetet, där det studerades om immunhistokemisk klassning kunde ge bättre information avseende sjukdomsförloppet hos patienter med urinblåsecancer i stadium T1, än befintliga kliniska och mikroskopiska faktorer. I de studerade 167

fall med nyupptäckt urinblåsecancer med stadium T1 kunde man klassa tumörerna i de beskrivna undergrupperna. Patienternas sjukdomsförlopp var beroende av undergrupp. Ännu bättre kunde sjukdomsförloppet förutsägas om man kombinerade undergrupp med kliniska data eller information avseende immunsystemets svar på tumörväxten.

I det tredje arbetet undersöktes användandet av second-look resektion hos alla 910 patienter som fick diagnos urinblåsecancer med tumörstadium T1 i Sverige 2008-2009. Det visade sig att second-look resektion används i cirka hälften av alla fall med nyupptäckt blåscancer stadium T1, och att användandet skiljer sig mycket åt i de olika sjukvårdsregionerna i landet, från 16 till 78%. Patienter som hade kvarvarande T1 tumörvävnad i preparaten från second-look operationen hade lägre 5-års blåscancerspecifik överlevnad än de som inte hade kvarvarande tumörvävnad (82% vs. 88%). Men patienter som inte genomgick en second-look resektion hade ännu lägre 5-års blåscancerspecifik överlevnad (76%). Studien visade också att second-look resektion gjordes oftare hos patienter som var yngre än 74 år, hos de som hade tumörgrad 3, som fick behandling med BCG eller cellgift i blåsan, hos patienter som diskuterades på en tumörkonferens och hos patienter som behandlades på ett hög-volym sjukhus. Det fanns skillnader i blåscancerdödlighet mellan sjukvårdsregionerna som dock inte enbart kunde förklaras med skillnader i användande av second-look resektion.

I det fjärde delarbetet undersöktes behandling med BCG hos alla 3.758 patienter med nyupptäckt urinblåsecancer med tumörstadium T1 i Sverige som var diagnosticerade 1997-2006. Studien visade att BCG användandet ökade i Sverige under observationstiden från 18% till 31%. Patienter yngre än 75 år, de som behandlades på hög-volym sjukhus, som hade grad 3 tumörer och som behandlades i norra, södra och Uppsala/Örebro sjukvårdsregionen fick BCG i större omfattning än patienter från de övriga regionerna. Patienter som behandlades med BCG hade bättre överlevnad, medan patienter äldre än 75 år och patienter med grad 3 tumörer hade kortare cancerspecifik och allmän överlevnad. Ur ett populationsbaserat perspektiv användes således BCG behandling för lite i Sverige, framförallt hos patienter äldre 75 år och hos patienter som behandlas på låg-volym sjukhus.

Acknowledgements

References

1. Nationellt kvalitetsregister för urinblåsecancer - Kvalitetsregisterrapport, diagnosår 2015. http://wwwcancercentrumse/globalassets/cancerdiagnoser/urinvagar/urinblase--och-urinrorscancer/rapport2015finalpdf.

2. Cumberbatch MG, Windsor-Shellard B, Catto JW. The contemporary landscape of occupational bladder cancer within the United Kingdom: a meta-analysis of risks over the last 80 years. BJU Int 2016.

3. Nieder AM, Porter MP, Soloway MS. Radiation therapy for prostate cancer increases subsequent risk of bladder and rectal cancer: a population based cohort study. The Journal of urology 2008; **180**(5): 2005-9; discussion 9-10.

4. Monach PA, Arnold LM, Merkel PA. Incidence and prevention of bladder toxicity from cyclophosphamide in the treatment of rheumatic diseases: a data-driven review. Arthritis and rheumatism 2010; **62**(1): 9-21.

5. Tuccori M, Filion KB, Yin H, Yu OH, Platt RW, Azoulay L. Pioglitazone use and risk of bladder cancer: population based cohort study. BMJ (Clinical research ed) 2016; **352**: i1541.

6. Garcia-Closas M, Malats N, Silverman D, et al. NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. Lancet 2005; **366**(9486): 649-59.

7. Sijmons RH, Kiemeney LA, Witjes JA, Vasen HF. Urinary tract cancer and hereditary nonpolyposis colorectal cancer: risks and screening options. The Journal of urology 1998; **160**(2): 466-70.

8. Babjuk M, Bohle A, Burger M, et al. EAU Guidelines on Non-Muscle-invasive Urothelial Carcinoma of the Bladder: Update 2016. Eur Urol 2016.

9. Martin-Doyle W, Leow JJ, Orsola A, Chang SL, Bellmunt J. Improving selection criteria for early cystectomy in high-grade t1 bladder cancer: a meta-analysis of 15,215 patients. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2015; **33**(6): 643-50.

10. Herr HW. Tumour progression and survival in patients with T1G3 bladder tumours: 15-year outcome. British journal of urology 1997; **80**(5): 762-5.

11. Divrik RT, Sahin AF, Yildirim U, Altok M, Zorlu F. Impact of routine second transurethral resection on the long-term outcome of patients with newly

diagnosed pT1 urothelial carcinoma with respect to recurrence, progression rate, and disease-specific survival: a prospective randomised clinical trial. Eur Urol 2010; **58**(2): 185-90.

12.   Greene FL, Sobin LH. A worldwide approach to the TNM staging system: collaborative efforts of the AJCC and UICC. Journal of surgical oncology 2009; **99**(5): 269-72.

13.   van Rhijn BW, van der Kwast TH, Alkhateeb SS, et al. A new and highly prognostic system to discern T1 bladder cancer substage. Eur Urol 2012; **61**(2): 378-84.

14.   Patriarca C, Hurle R, Moschini M, et al. Usefulness of pT1 substaging in papillary urothelial bladder carcinoma. Diagnostic pathology 2016; **11**: 6.

15.   Holmang S, Hedelin H, Anderstrom C, Holmberg E, Johansson SL. The importance of the depth of invasion in stage T1 bladder carcinoma: a prospective cohort study. The Journal of urology 1997; **157**(3): 800-3; discussion 4.

16.   Angulo JC, Lopez JI. Re: The importance of the depth of invasion in stage T1 bladder carcinoma: a prospective cohort study. The Journal of urology 1997; **158**(5): 1922.

17.   Pan CC. Does muscularis mucosae invasion in extensively lamina propria-invasive high-grade papillary urothelial carcinoma provide additional prognostic information? The American journal of surgical pathology 2013; **37**(3): 459-60.

18.   Hu Z, Mudaliar K, Quek ML, Paner GP, Barkan GA. Measuring the dimension of invasive component in pT1 urothelial carcinoma in transurethral resection specimens can predict time to recurrence. Annals of diagnostic pathology 2014; **18**(2): 49-52.

19.   Andius P, Johansson SL, Holmang S. Prognostic factors in stage T1 bladder cancer: tumor pattern (solid or papillary) and vascular invasion more important than depth of invasion. Urology 2007; **70**(4): 758-62.

20.   Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part B: Prostate and Bladder Tumours. Eur Urol 2016; **70**(1): 106-19.

21.   Epstein JI, Amin MB, Reuter VR, Mostofi FK. The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. Bladder Consensus Conference Committee. The American journal of surgical pathology 1998; **22**(12): 1435-48.

22.   Busch C, Algaba F. The WHO/ISUP 1998 and WHO 1999 systems for malignancy grading of bladder cancer. Scientific foundation and translation to

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one another and previous systems. Virchows Archiv : an international journal of pathology 2002; **441**(2): 105-8.

23. Miyamoto H, Miller JS, Fajardo DA, Lee TK, Netto GJ, Epstein JI. Non-invasive papillary urothelial neoplasms: the 2004 WHO/ISUP classification system. Pathology international 2010; **60**(1): 1-8.

24. Liedberg F, Lauss M, Patschan O, et al. The importance of being grade 3: WHO 1999 versus WHO 2004 pathologic grading. Eur Urol 2012; **62**(4): 620-3.

25. Pellucchi F, Freschi M, Moschini M, et al. Oncological predictive value of the 2004 World Health Organisation grading classification in primary T1 non-muscle-invasive bladder cancer. A step forward or back? BJU Int 2015; **115**(2): 267-73.

26. Lopez-Beltran A, Algaba F, Berney DM, et al. Handling and reporting of transurethral resection specimens of the bladder in Europe: a web-based survey by the European Network of Uropathology (ENUP). Histopathology 2011; **58**(4): 579-85.

27. Cancer i urinblåsa, njurbäcken, urinledare och urinrör - Regional tillämpning av nationellt vårdprogram 2015. https://wwwcancercentrumse/globalassets/cancerdiagnoser/urinvagar/urin blase--och-urinrorscancer/vardprogram/regvpurinblasa_2016-03-01pdf.

28. Kim HS, Kim M, Jeong CW, Kwak C, Kim HH, Ku JH. Presence of lymphovascular invasion in urothelial bladder cancer specimens after transurethral resections correlates with risk of upstaging and survival: a systematic review and meta-analysis. Urologic oncology 2014; **32**(8): 1191-9.

29. Cho KS, Seo HK, Joung JY, et al. Lymphovascular invasion in transurethral resection specimens as predictor of progression and metastasis in patients with newly diagnosed T1 bladder urothelial cancer. The Journal of urology 2009; **182**(6): 2625-30.

30. Tilki D, Shariat SF, Lotan Y, et al. Lymphovascular invasion is independently associated with bladder cancer recurrence and survival in patients with final stage T1 disease and negative lymph nodes after radical cystectomy. BJU Int 2013; **111**(8): 1215-21.

31. Xylinas E, Rink M, Robinson BD, et al. Impact of histological variants on oncological outcomes of patients with urothelial carcinoma of the bladder treated with radical cystectomy. European journal of cancer (Oxford, England : 1990) 2013; **49**(8): 1889-97.

32. Willis DL, Fernandez MI, Dickstein RJ, et al. Clinical outcomes of cT1 micropapillary bladder cancer. The Journal of urology 2015; **193**(4): 1129-34.

33. Mukesh M, Cook N, Hollingdale AE, Ainsworth NL, Russell SG. Small cell carcinoma of the urinary bladder: a 15-year retrospective review of treatment and survival in the Anglian Cancer Network. BJU Int 2009; **103**(6): 747-52.

34. Burger M, van der Aa MN, van Oers JM, et al. Prediction of progression of non-muscle-invasive bladder cancer by WHO 1973 and 2004 grading and by FGFR3 mutation status: a prospective study. Eur Urol 2008; **54**(4): 835-43.

35. van Rhijn BW, Vis AN, van der Kwast TH, et al. Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2003; **21**(10): 1912-21.

36. van Rhijn BW, Catto JW, Goebell PJ, et al. Molecular markers for urothelial bladder cancer prognosis: toward implementation in clinical practice. Urologic oncology 2014; **32**(7): 1078-87.

37. Otto W, Denzinger S, Fritsche HM, et al. Introduction and first clinical application of a simplified immunohistochemical validation system confirms prognostic impact of KI-67 and CK20 for stage T1 urothelial bladder carcinoma: single-center analysis of eight biomarkers in a series of three hundred six patients. Clinical genitourinary cancer 2013; **11**(4): 537-44.

38. Bertz S, Otto W, Denzinger S, et al. Combination of CK20 and Ki-67 immunostaining analysis predicts recurrence, progression, and cancer-specific survival in pT1 urothelial bladder cancer. Eur Urol 2014; **65**(1): 218-26.

39. Mocellin S, Provenzano M, Rossi CR, Pilati P, Nitti D, Lise M. DNA array-based gene profiling: from surgical specimen to the molecular portrait of cancer. Annals of surgery 2005; **241**(1): 16-26.

40. Wang Y, Li Y, Liu S, et al. Study on the dynamic behavior of a DNA microarray. Journal of nanoscience and nanotechnology 2005; **5**(8): 1249-55.

41. Viljoen KS, Blackburn JM. Quality assessment and data handling methods for Affymetrix Gene 1.0 ST arrays with variable RNA integrity. BMC genomics 2013; **14**: 14.

42. Lauss M, Visne I, Kriegner A, Ringner M, Jonsson G, Hoglund M. Monitoring of technical variation in quantitative high-throughput datasets. Cancer informatics 2013; **12**: 193-201.

43. Ramos-Vara JA. Principles and methods of immunohistochemistry. Methods in molecular biology (Clifton, NJ) 2011; **691**: 83-96.

44. Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nature medicine 1998; **4**(7): 844-7.

45. Kramer MW, Merseburger AS, Hennenlotter J, Kuczyk M. Tissue microarrays in clinical urology--technical considerations. Scandinavian journal of urology and nephrology 2007; **41**(6): 478-84.

46. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; **144**(5): 646-74.

47. Hoglund M. The bladder cancer genome; chromosomal changes as prognostic makers, opportunities, and obstacles. Urologic oncology 2012; **30**(4): 533-40.

48. Bakkar AA, Wallerand H, Radvanyi F, et al. FGFR3 and TP53 gene mutations define two distinct pathways in urothelial cell carcinoma of the bladder. Cancer research 2003; **63**(23): 8108-12.

49. Wu XR. Urothelial tumorigenesis: a tale of divergent pathways. Nature reviews Cancer 2005; **5**(9): 713-25.

50. Mitra AP, Hansel DE, Cote RJ. Prognostic value of cell-cycle regulation biomarkers in bladder cancer. Seminars in oncology 2012; **39**(5): 524-33.

51. Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. Nature reviews Cancer 2015; **15**(1): 25-41.

52. Lindgren D, Sjodahl G, Lauss M, et al. Integrated genomic and gene expression profiling identifies two major genomic circuits in urothelial carcinoma. PloS one 2012; **7**(6): e38863.

53. Bringuier PP, Tamimi Y, Schuuring E, Schalken J. Expression of cyclin D1 and EMS1 in bladder tumours; relationship with chromosome 11q13 amplification. Oncogene 1996; **12**(8): 1747-53.

54. Sjodahl G, Lauss M, Lovgren K, et al. A molecular taxonomy for urothelial carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research 2012; **18**(12): 3377-86.

55. Damrauer JS, Hoadley KA, Chism DD, et al. Intrinsic subtypes of high-grade bladder cancer reflect the hallmarks of breast cancer biology. Proceedings of the National Academy of Sciences of the United States of America 2014; **111**(8): 3110-5.

56. Choi W, Czerniak B, Ochoa A, et al. Intrinsic basal and luminal subtypes of muscle-invasive bladder cancer. Nature reviews Urology 2014; **11**(7): 400-10.

57. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature 2014; **507**(7492): 315-22.

58.   McConkey DJ, Choi W, Dinney CP. New insights into subtypes of invasive bladder cancer: considerations of the clinician. Eur Urol 2014; **66**(4): 609-10.

59.   Choi W, Porten S, Kim S, et al. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer cell 2014; **25**(2): 152-65.

60.   Hedegaard J, Lamy P, Nordentoft I, et al. Comprehensive Transcriptional Analysis of Early-Stage Urothelial Carcinoma. Cancer cell 2016; **30**(1): 27-42.

61.   Dyrskjot L, Reinert T, Novoradovsky A, et al. Analysis of molecular intra-patient variation and delineation of a prognostic 12-gene signature in non-muscle invasive bladder cancer; technology transfer from microarrays to PCR. British journal of cancer 2012; **107**(8): 1392-8.

62.   Aine M, Eriksson P, Liedberg F, Hoglund M, Sjodahl G. On Molecular Classification of Bladder Cancer: Out of One, Many. Eur Urol 2015; **68**(6): 921-3.

63.   Lerner SP, McConkey DJ, Hoadley KA, et al. Bladder Cancer Molecular Taxonomy: Summary from a Consensus Meeting. Bladder cancer (Amsterdam, Netherlands) 2016; **2**(1): 37-47.

64.   Sfakianos JP, Kim PH, Hakimi AA, Herr HW. The effect of restaging transurethral resection on recurrence and progression rates in patients with nonmuscle invasive bladder cancer treated with intravesical bacillus Calmette-Guerin. The Journal of urology 2014; **191**(2): 341-5.

65.   Cookson MS, Herr HW, Zhang ZF, Soloway S, Sogani PC, Fair WR. The treated natural history of high risk superficial bladder cancer: 15-year outcome. The Journal of urology 1997; **158**(1): 62-7.

66.   Hemdan T, Malmstrom PU, Jahnson S, Segersten U. Emmprin Expression Predicts Response and Survival following Cisplatin Containing Chemotherapy for Bladder Cancer: A Validation Study. The Journal of urology 2015; **194**(6): 1575-81.

67.   Mariappan P, Finney SM, Head E, et al. Good quality white-light transurethral resection of bladder tumours (GQ-WLTURBT) with experienced surgeons performing complete resections and obtaining detrusor muscle reduces early recurrence in new non-muscle-invasive bladder cancer: validation across time and place and recommendation for benchmarking. BJU Int 2012; **109**(11): 1666-73.

68.   Mariappan P, Zachou A, Grigor KM. Detrusor muscle in the first, apparently complete transurethral resection of bladder tumour specimen is a surrogate marker of resection quality, predicts risk of early recurrence, and is dependent on operator experience. Eur Urol 2010; **57**(5): 843-9.

69.   Gontero P, Sylvester R, Pisano F, et al. The impact of re-transurethral resection on clinical outcomes in a large multicentre cohort of patients with T1 high-grade/Grade 3 bladder cancer treated with bacille Calmette-Guerin. BJU Int 2016; **118**(1): 44-52.

70.   Jancke G, Rosell J, Jahnson S. Impact of surgical experience on recurrence and progression after transurethral resection of bladder tumour in non-muscle-invasive bladder cancer. Scandinavian journal of urology 2014; **48**(3): 276-83.

71.   Dalbagni G, Vora K, Kaag M, et al. Clinical outcome in a contemporary series of restaged patients with clinical T1 bladder cancer. Eur Urol 2009; **56**(6): 903-10.

72.   Schwaibold HE, Sivalingam S, May F, Hartung R. The value of a second transurethral resection for T1 bladder cancer. BJU Int 2006; **97**(6): 1199-201.

73.   Kaasinen E, Rintala E, Hellstrom P, et al. Factors explaining recurrence in patients undergoing chemoimmunotherapy regimens for frequently recurring superficial bladder carcinoma. Eur Urol 2002; **42**(2): 167-74.

74.   Solsona E, Iborra I, Ricos JV, Monros JL, Casanova J, Dumont R. Effectiveness of a single immediate mitomycin C instillation in patients with low risk superficial bladder cancer: short and long-term followup. The Journal of urology 1999; **161**(4): 1120-3.

75.   Ali-el-Dein B, Nabeeh A, el-Baz M, Shamaa S, Ashamallah A. Single-dose versus multiple instillations of epirubicin as prophylaxis for recurrence after transurethral resection of pTa and pT1 transitional-cell bladder tumours: a prospective, randomized controlled study. British journal of urology 1997; **79**(5): 731-5.

76.   Oosterlinck W, Kurth KH, Schroder F, Bultinck J, Hammond B, Sylvester R. A prospective European Organization for Research and Treatment of Cancer Genitourinary Group randomized trial comparing transurethral resection followed by a single intravesical instillation of epirubicin or water in single stage Ta, T1 papillary carcinoma of the bladder. The Journal of urology 1993; **149**(4): 749-52.

77.   Rajala P, Kaasinen E, Raitanen M, Liukkonen T, Rintala E. Perioperative single dose instillation of epirubicin or interferon-alpha after transurethral resection for the prophylaxis of primary superficial bladder cancer recurrence: a prospective randomized multicenter study--FinnBladder III long-term results. The Journal of urology 2002; **168**(3): 981-5.

78.   Sylvester RJ, Oosterlinck W, van der Meijden AP. A single immediate postoperative instillation of chemotherapy decreases the risk of recurrence in patients with stage Ta T1 bladder cancer: a meta-analysis of published results of randomized clinical trials. The Journal of urology 2004; **171**(6 Pt 1): 2186-90, quiz 435.

79.   Babjuk M, Oosterlinck W, Sylvester R, et al. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder, the 2011 update. Eur Urol 2011; **59**(6): 997-1008.

80.   Gudjonsson S, Adell L, Merdasa F, et al. Should all patients with non-muscle-invasive bladder cancer receive early intravesical chemotherapy after transurethral resection? The results of a prospective randomised multicentre study. Eur Urol 2009; **55**(4): 773-80.

81.   Berrum-Svennung I, Granfors T, Jahnson S, Boman H, Holmang S. A single instillation of epirubicin after transurethral resection of bladder tumors prevents only small recurrences. The Journal of urology 2008; **179**(1): 101-5; discussion 5-6.

82.   Sylvester RJ, Oosterlinck W, Holmang S, et al. Systematic Review and Individual Patient Data Meta-analysis of Randomized Trials Comparing a Single Immediate Instillation of Chemotherapy After Transurethral Resection with Transurethral Resection Alone in Patients with Stage pTa-pT1 Urothelial Carcinoma of the Bladder: Which Patients Benefit from the Instillation? Eur Urol 2016; **69**(2): 231-44.

83.   Onishi T, Sugino Y, Shibahara T, Masui S, Yabana T, Sasaki T. Randomized controlled study of the efficacy and safety of continuous saline bladder irrigation after transurethral resection for the treatment of non-muscle-invasive bladder cancer. BJU Int 2016.

84.   Lamm DL, Thor DE, Harris SC, Reyna JA, Stogdill VD, Radwin HM. Bacillus Calmette-Guerin immunotherapy of superficial bladder cancer. The Journal of urology 1980; **124**(1): 38-40.

85.   Redelman-Sidi G, Glickman MS, Bochner BH. The mechanism of action of BCG therapy for bladder cancer--a current perspective. Nature reviews Urology 2014; **11**(3): 153-62.

86.   Brausi M, Oddens J, Sylvester R, et al. Side effects of Bacillus Calmette-Guerin (BCG) in the treatment of intermediate- and high-risk Ta, T1 papillary carcinoma of the bladder: results of the EORTC genito-urinary cancers group randomised phase 3 study comparing one-third dose with full dose and 1 year with 3 years of maintenance BCG. Eur Urol 2014; **65**(1): 69-76.

87.   Oddens J, Brausi M, Sylvester R, et al. Final results of an EORTC-GU cancers group randomized study of maintenance bacillus Calmette-Guerin in intermediate- and high-risk Ta, T1 papillary carcinoma of the urinary bladder: one-third dose versus full dose and 1 year versus 3 years of maintenance. Eur Urol 2013; **63**(3): 462-72.

88. Malmstrom PU, Sylvester RJ, Crawford DE, et al. An individual patient data meta-analysis of the long-term outcome of randomised studies comparing intravesical mitomycin C versus bacillus Calmette-Guerin for non-muscle-invasive bladder cancer. Eur Urol 2009; **56**(2): 247-56.

89. Sylvester RJ, van der MA, Lamm DL. Intravesical bacillus Calmette-Guerin reduces the risk of progression in patients with superficial bladder cancer: a meta-analysis of the published results of randomized clinical trials. The Journal of urology 2002; **168**(5): 1964-70.

90. Sylvester RJ, Brausi MA, Kirkels WJ, et al. Long-term efficacy results of EORTC genito-urinary group randomized phase 3 study 30911 comparing intravesical instillations of epirubicin, bacillus Calmette-Guerin, and bacillus Calmette-Guerin plus isoniazid in patients with intermediate- and high-risk stage Ta T1 urothelial carcinoma of the bladder. Eur Urol 2010; **57**(5): 766-73.

91. Shelley MD, Mason MD, Kynaston H. Intravesical therapy for superficial bladder cancer: a systematic review of randomised trials and meta-analyses. Cancer treatment reviews 2010; **36**(3): 195-205.

92. Oddens JR, Sylvester RJ, Brausi MA, et al. The effect of age on the efficacy of maintenance bacillus Calmette-Guerin relative to maintenance epirubicin in patients with stage Ta T1 urothelial bladder cancer: results from EORTC genito-urinary group study 30911. Eur Urol 2014; **66**(4): 694-701.

93. Pfister G, Savino W. Can the immune system still be efficient in the elderly? An immunological and immunoendocrine therapeutic perspective. Neuroimmunomodulation 2008; **15**(4-6): 351-64.

94. Kim JC, Steinberg GD. The limits of bacillus Calmette-Guerin for carcinoma in situ of the bladder. The Journal of urology 2001; **165**(3): 745-56.

95. Fernandez-Gomez J, Madero R, Solsona E, et al. Predicting nonmuscle invasive bladder cancer recurrence and progression in patients treated with bacillus Calmette-Guerin: the CUETO scoring model. The Journal of urology 2009; **182**(5): 2195-203.

96. Bohle A, Bock PR. Intravesical bacille Calmette-Guerin versus mitomycin C in superficial bladder cancer: formal meta-analysis of comparative studies on tumor progression. Urology 2004; **63**(4): 682-6; discussion 6-7.

97. Witjes JA, Dalbagni G, Karnes RJ, et al. The efficacy of BCG TICE and BCG Connaught in a cohort of 2,099 patients with T1G3 non-muscle-invasive bladder cancer. Urologic oncology 2016.

98. Oosterlinck W, Kirkali Z, Sylvester R, et al. Sequential intravesical chemoimmunotherapy with mitomycin C and bacillus Calmette-Guerin and with

bacillus Calmette-Guerin alone in patients with carcinoma in situ of the urinary bladder: results of an EORTC genito-urinary group randomized phase 2 trial (30993). Eur Urol 2011; **59**(3): 438-46.

99.   Kaasinen E, Wijkstrom H, Malmstrom PU, et al. Alternating mitomycin C and BCG instillations versus BCG alone in treatment of carcinoma in situ of the urinary bladder: a nordic study. Eur Urol 2003; **43**(6): 637-45.

100.   Bono AV, Hall RR, Denis L, Lovisolo JA, Sylvester R. Chemoresection in Ta-T1 bladder cancer. Members of the EORTC Genito-Urinary Group. Eur Urol 1996; **29**(4): 385-90.

101.   Bono AV, Lovisolo JA, Saredi G. Transurethral resection and sequential chemo-immunoprophylaxis in primary T1G3 bladder cancer. Eur Urol 2000; **37**(4): 478-83.

102.   Solsona E, Madero R, Chantada V, et al. Sequential combination of mitomycin C plus bacillus Calmette-Guerin (BCG) is more effective but more toxic than BCG alone in patients with non-muscle-invasive bladder cancer in intermediate- and high-risk patients: final outcome of CUETO 93009, a randomized prospective trial. Eur Urol 2015; **67**(3): 508-16.

103.   Svatek RS, Zhao XR, Morales EE, et al. Sequential intravesical mitomycin plus Bacillus Calmette-Guerin for non-muscle-invasive urothelial bladder carcinoma: translational and phase I clinical trial. Clinical cancer research : an official journal of the American Association for Cancer Research 2015; **21**(2): 303-11.

104.   Di Stasi SM, Vespasiani G, Giannantoni A, Massoud R, Dolci S, Micali F. Electromotive delivery of mitomycin C into human bladder wall. Cancer research 1997; **57**(5): 875-80.

105.   Di Stasi SM, Giannantoni A, Giurioli A, et al. Sequential BCG and electromotive mitomycin versus BCG alone for high-risk superficial bladder cancer: a randomised controlled trial. Lancet Oncol 2006; **7**(1): 43-51.

106.   Colombo R, Salonia A, Leib Z, Pavone-Macaluso M, Engelstein D. Long-term outcomes of a randomized controlled trial comparing thermochemotherapy with mitomycin-C alone as adjuvant treatment for non-muscle-invasive bladder cancer (NMIBC). BJU Int 2011; **107**(6): 912-8.

107.   Arends TJ, Nativ O, Maffezzini M, et al. Results of a Randomised Controlled Trial Comparing Intravesical Chemohyperthermia with Mitomycin C Versus Bacillus Calmette-Guerin for Adjuvant Treatment of Patients with Intermediate- and High-risk Non-Muscle-invasive Bladder Cancer. Eur Urol 2016; **69**(6): 1046-52.

108. Oddens JR, Sylvester RJ. Chemohyperthermia with Mitomycin-C Compared with Bacillus Calmette-Guerin: A "Hot" Topic. Eur Urol 2016; **69**(6): 1053-4.

109. Mostafid AH, Palou Redorta J, Sylvester R, Witjes JA. Therapeutic options in high-risk non-muscle-invasive bladder cancer during the current worldwide shortage of bacille Calmette-Guerin. Eur Urol 2015; **67**(3): 359-60.

110. Harland SJ, Kynaston H, Grigor K, et al. A randomized trial of radical radiotherapy for the management of pT1G3 NXM0 transitional cell carcinoma of the bladder. The Journal of urology 2007; **178**(3 Pt 1): 807-13; discussion 13.

111. Parekh DJ, Bochner BH, Dalbagni G. Superficial and muscle-invasive bladder cancer: principles of management for outcomes assessments. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2006; **24**(35): 5519-27.

112. Sternberg IA, Keren Paz GE, Chen LY, Herr HW, Dalbagni G. Role of immediate radical cystectomy in the treatment of patients with residual T1 bladder cancer on restaging transurethral resection. BJU Int 2013; **112**(1): 54-9.

113. Herr HW, Sogani PC. Does early cystectomy improve the survival of patients with high risk superficial bladder tumors? The Journal of urology 2001; **166**(4): 1296-9.

114. May M, Bastian PJ, Brookman-May S, et al. Pathological upstaging detected in radical cystectomy procedures is associated with a significantly worse tumour-specific survival rate for patients with clinical T1 urothelial carcinoma of the urinary bladder. Scandinavian journal of urology and nephrology 2011; **45**(4): 251-7.

115. Fritsche HM, Burger M, Svatek RS, et al. Characteristics and outcomes of patients with clinical T1 grade 3 urothelial carcinoma treated with radical cystectomy: results from an international cohort. Eur Urol 2010; **57**(2): 300-9.

116. Huguet J, Crego M, Sabate S, Salvador J, Palou J, Villavicencio H. Cystectomy in patients with high risk superficial bladder tumors who fail intravesical BCG therapy: pre-cystectomy prostate involvement as a prognostic factor. Eur Urol 2005; **48**(1): 53-9; discussion 9.

117. Gupta A, Lotan Y, Bastian PJ, et al. Outcomes of patients with clinical T1 grade 3 urothelial cell bladder carcinoma treated with radical cystectomy. Urology 2008; **71**(2): 302-7.

118. Lamm D, Persad R, Brausi M, et al. Defining progression in nonmuscle invasive bladder cancer: it is time for a new, standard definition. The Journal of urology 2014; **191**(1): 20-7.

119. Sylvester RJ, van der Meijden AP, Oosterlinck W, et al. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol 2006; **49**(3): 466-5; discussion 75-7.

120. Xylinas E, Kent M, Kluth L, et al. Accuracy of the EORTC risk tables and of the CUETO scoring model to predict outcomes in non-muscle-invasive urothelial carcinoma of the bladder. British journal of cancer 2013; **109**(6): 1460-6.

121. Fernandez-Gomez J, Madero R, Solsona E, et al. The EORTC tables overestimate the risk of recurrence and progression in patients with non-muscle-invasive bladder cancer treated with bacillus Calmette-Guerin: external validation of the EORTC risk tables. Eur Urol 2011; **60**(3): 423-30.

122. Hernandez V, De La Pena E, Martin MD, Blazquez C, Diaz FJ, Llorente C. External validation and applicability of the EORTC risk tables for non-muscle-invasive bladder cancer. World journal of urology 2011; **29**(4): 409-14.

123. Jancke G, Rosell J, Jahnson S. Residual tumour in the marginal resection after a complete transurethral resection is associated with local recurrence in Ta/T1 urinary bladder cancer. Scandinavian journal of urology and nephrology 2012; **46**(5): 343-7.

124. Palou J, Rodriguez-Rubio F, Millan F, et al. Recurrence at three months and high-grade recurrence as prognostic factor of progression in multivariate analysis of T1G2 bladder tumors. Urology 2009; **73**(6): 1313-7.

125. Golijanin D, Yossepowitch O, Beck SD, Sogani P, Dalbagni G. Carcinoma in a bladder diverticulum: presentation and treatment outcome. The Journal of urology 2003; **170**(5): 1761-4.

126. Palou J, Sylvester RJ, Faba OR, et al. Female gender and carcinoma in situ in the prostatic urethra are prognostic factors for recurrence, progression, and disease-specific mortality in T1G3 bladder cancer patients treated with bacillus Calmette-Guerin. Eur Urol 2012; **62**(1): 118-25.

127. Beltran AL, Cheng L, Montironi R, et al. Clinicopathological characteristics and outcome of nested carcinoma of the urinary bladder. Virchows Archiv : an international journal of pathology 2014; **465**(2): 199-205.

128. Gofrit ON, Yutkin V, Shapiro A, et al. The Response of Variant Histology Bladder Cancer to Intravesical Immunotherapy Compared to Conventional Cancer. Frontiers in oncology 2016; **6**: 43.

129. Otto W, Rubenwolf PC, Burger M, et al. Loss of aquaporin 3 protein expression constitutes an independent prognostic factor for progression-free survival: an

immunohistochemical study on stage pT1 urothelial bladder cancer. BMC cancer 2012; **12**: 459.

130.   Sgambato A, Migaldi M, Faraglia B, et al. Cyclin D1 expression in papillary superficial bladder cancer: its association with other cell cycle-associated proteins, cell proliferation and clinical outcome. International journal of cancer 2002; **97**(5): 671-8.

131.   Liu C, Zheng S, Shen H, et al. Clinical significance of CD24 as a predictor of bladder cancer recurrence. Oncology letters 2013; **6**(1): 96-100.

132.   Mahnken A, Kausch I, Feller AC, Kruger S. E-cadherin immunoreactivity correlates with recurrence and progression of minimally invasive transitional cell carcinomas of the urinary bladder. Oncology reports 2005; **14**(4): 1065-70.

133.   Sgambato A, Migaldi M, Faraglia B, et al. Loss of P27Kip1 expression correlates with tumor grade and with reduced disease-free survival in primary superficial bladder cancers. Cancer research 1999; **59**(13): 3245-50.

134.   Yurakh AO, Ramos D, Calabuig-Farinas S, et al. Molecular and immunohistochemical analysis of the prognostic value of cell-cycle regulators in urothelial neoplasms of the bladder. Eur Urol 2006; **50**(3): 506-15; discussion 15.

135.   Tadin T, Krpina K, Stifter S, Babarovic E, Fuckar Z, Jonjic N. Lower cyclooxygenase-2 expression is associated with recurrence of solitary non-muscle invasive bladder carcinoma. Diagnostic pathology 2012; **7**: 152.

136.   Han B, Cui D, Jing Y, Hong Y, Xia S. Estrogen receptor beta (ERbeta) is a novel prognostic marker of recurrence survival in non-muscle-invasive bladder cancer potentially by inhibiting cadherin switch. World journal of urology 2012; **30**(6): 861-7.

137.   Maeng YH, Eun SY, Huh JS. Expression of fibroblast growth factor receptor 3 in the recurrence of non-muscle-invasive urothelial carcinoma of the bladder. Korean journal of urology 2010; **51**(2): 94-100.

138.   Quintero A, Alvarez-Kindelan J, Luque RJ, et al. Ki-67 MIB1 labelling index and the prognosis of primary TaT1 urothelial cell carcinoma of the bladder. Journal of clinical pathology 2006; **59**(1): 83-8.

139.   Wang W, Qiu J, Liu Z, et al. Overexpression of RING box protein-1 (RBX1) associated with poor prognosis of non-muscle-invasive bladder transitional cell carcinoma. Journal of surgical oncology 2013; **107**(7): 758-61.

140.   Ruan J, Wei B, Xu Z, et al. Predictive value of Sox2 expression in transurethral resection specimens in patients with T1 bladder cancer. Medical oncology (Northwood, London, England) 2013; **30**(1): 445.

141.   Patschan O, Shariat SF, Chade DC, et al. Association of tumor-associated trypsin inhibitor (TATI) expression with molecular markers, pathologic features and clinical outcomes of urothelial carcinoma of the urinary bladder. World journal of urology 2012; **30**(6): 785-94.

142.   Malats N, Bustos A, Nascimento CM, et al. P53 as a prognostic marker for bladder cancer: a meta-analysis and review. Lancet Oncol 2005; **6**(9): 678-86.

143.   Kim WT, Yun SJ, Park C, et al. Identification of C16orf74 as a marker of progression in primary non-muscle invasive bladder cancer. PloS one 2010; **5**(12): e15260.

144.   Kruger S, Mahnken A, Kausch I, Feller AC. P16 immunoreactivity is an independent predictor of tumor progression in minimally invasive urothelial bladder carcinoma. Eur Urol 2005; **47**(4): 463-7.

145.   Dyrskjot L, Zieger K, Kissow Lildal T, et al. Expression of MAGE-A3, NY-ESO-1, LAGE-1 and PRAME in urothelial carcinoma. British journal of cancer 2012; **107**(1): 116-22.

146.   Mellon K, Wright C, Kelly P, Horne CH, Neal DE. Long-term outcome related to epidermal growth factor receptor status in bladder cancer. The Journal of urology 1995; **153**(3 Pt 2): 919-25.

147.   Puerta-Gil P, Garcia-Baquero R, Jia AY, et al. miR-143, miR-222, and miR-452 are useful as tumor stratification and noninvasive diagnostic biomarkers for bladder cancer. The American journal of pathology 2012; **180**(5): 1808-15.

148.   Miyamoto H, Yao JL, Chaux A, et al. Expression of androgen and oestrogen receptors and its prognostic significance in urothelial neoplasm of the urinary bladder. BJU Int 2012; **109**(11): 1716-26.

149.   Miyamoto H, Izumi K, Yao JL, et al. GATA binding protein 3 is down-regulated in bladder cancer yet strong expression is an independent predictor of poor prognosis in invasive tumor. Human pathology 2012; **43**(11): 2033-40.

150.   Kramer MW, Golshani R, Merseburger AS, et al. HYAL-1 hyaluronidase: a potential prognostic indicator for progression to muscle invasion and recurrence in bladder cancer. Eur Urol 2010; **57**(1): 86-93.

151.   Jensen JB, Munksgaard PP, Sorensen CM, et al. High expression of karyopherin-alpha2 defines poor prognosis in non-muscle-invasive bladder cancer and in patients with invasive bladder cancer undergoing radical cystectomy. Eur Urol 2011; **59**(5): 841-8.

152.   Liukkonen T, Rajala P, Raitanen M, Rintala E, Kaasinen E, Lipponen P. Prognostic value of MIB-1 score, p53, EGFr, mitotic index and papillary status in

primary superficial (Stage pTa/T1) bladder cancer: a prospective comparative study. The Finnbladder Group. Eur Urol 1999; **36**(5): 393-400.

153.   Ha YS, Kim MJ, Yoon HY, et al. mRNA Expression of S100A8 as a Prognostic Marker for Progression of Non-Muscle-Invasive Bladder Cancer. Korean journal of urology 2010; **51**(1): 15-20.

154.   Fristrup N, Ulhoi BP, Birkenkamp-Demtroder K, et al. Cathepsin E, maspin, Plk1, and survivin are promising prognostic protein markers for progression in non-muscle invasive bladder cancer. The American journal of pathology 2012; **180**(5): 1824-34.

155.   Sjodahl G, Lovgren K, Lauss M, et al. Toward a molecular pathologic classification of urothelial carcinoma. The American journal of pathology 2013; **183**(3): 681-91.

156.   Patschan O, Sjodahl G, Chebil G, et al. A Molecular Pathologic Framework for Risk Stratification of Stage T1 Urothelial Carcinoma. Eur Urol 2015; **68**(5): 824-32; discussion 35-6.

157.   Kramer MW, Rassweiler JJ, Klein J, et al. En bloc resection of urothelium carcinoma of the bladder (EBRUC): a European multicenter study to compare safety, efficacy, and outcome of laser and electrical en bloc transurethral resection of bladder tumor. World journal of urology 2015; **33**(12): 1937-43.

158.   Ukai R, Hashimoto K, Iwasa T, Nakayama H. Transurethral resection in one piece (TURBO) is an accurate tool for pathological staging of bladder tumor. International journal of urology : official journal of the Japanese Urological Association 2010; **17**(8): 708-14.

Bladder cancer is the most common cancer in the urinary tract. Patients with stage T1 bladder cancer have a heterogeneous prognosis: about 50% can be cured by transurethral resection, second-look resection, and intravesical BCG; 25% will progress to muscle-invasive disease and require aggressive therapy with either total removal or radiation of the bladder; and 25% will ultimately die from the disease. Reliable instruments for prognostication are lacking, and choosing the right treatment for patients with this stage of the disease remains a challenge. This thesis elucidates different aspects of diagnosis and treatment of stage T1 bladder cancer.

STAGE T1 BLADDER CANCER

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