Urinary Bladder Carcinoma – Studies of Outcome of Current Management and Experimental Therapy

TRULS GÅRDMARK
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Abstract

The thesis concerns the epidemiology, current and possible future treatment of urothelial cancer of the urinary bladder. The Swedish National Quality Registry for Bladder Cancer 1997-2001 was used to explore epidemiology, current therapies and outcome. More common in men, the incidence for Ta and T1 tumours peaks in the age range 70-79 years. There were differences in treatment activity between the reporting regions. An increasing activity was seen. Older patients received less intravesical treatment, which was also a tendency for women. The five year relative survival for all stages (Ta-T4) was 70%; 93% for Ta and 75% for T1. For Ta or T1 survival did not differ significantly between regions. Because the registry has only been running since 1997 a long term follow-up (ten years) of 250 patients comparing Bacillus Calmette-Guérin and Mitomycin-C, was performed. No differences regarding complementary treatment, progression or survival (overall or disease specific) were shown. Looking for new drugs, gemcitabine was tried for intravesical instillations. Patients were randomised to one of three dose schedules. The effect on a marker tumour lesion was evaluated after nine weeks. The overall complete response rate was 31% (9/29). Side effects were more common in women but generally mild; the most common was nausea. One patient stopped instillations (nausea and fever). No patients were excluded due to pathological changes in laboratory parameters. For metastasised disease, over-expression of the growth factor receptor HER2 on urothelial cancer cells was explored in primary tumours and metastases, aiming at radionuclide target therapy. With a new antigen retrieval procedure and evaluation protocol 80% of primary tumours overexpressed the receptor and 72% remained so in the metastases. In conclusion current therapies were increasingly used by clinicians. Superiority for BCG could not be proven. Prerequisites for new therapies have been explored and the way has been paved for future studies.

Keywords: Bladder, Epidemiology, Administration, Intravesical, Radiotherapy

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II Ten Year Follow Up of a Randomized Prospective Study Comparing Mitomycin-C and Bacillus Calmette-Guerin in Patients with High Risk Bladder Cancer
Truls Gårdmark, Staffan Jahnson, Rolf Wahlquist, Hans Wijkström, Peter Wiklund, Per-Uno Malmström and members of the Swedish-Norwegian Bladder Cancer Study Group
Submitted

III A Randomized Phase II Marker Lesion Study Evaluating the Effect of Scheduling on Response to Intravesical Gemcitabine in Recurrent Ta Urothelial Cell Carcinoma of the Bladder
Truls Gårdmark, Malcolm Carringer, Eva Beckman, Per-Uno Malmström and members of the Intravesical Gemcitabine Study Group
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IV Analysis of HER2 Expression in Primary Urinary Bladder Carcinoma and Corresponding Metastases
Truls Gårdmark, Kenneth Wester, Manuel de la Torre, Jörgen Carlsson and Per-Uno Malmström
Letter to the editor, author reply
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# Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>MMC</td>
<td>Mitomycin-C</td>
</tr>
<tr>
<td>BCG</td>
<td>Bacillus Calmette Guerin</td>
</tr>
<tr>
<td>SIU</td>
<td>Societe International d’Urologie</td>
</tr>
<tr>
<td>EAU</td>
<td>European Association of Urology</td>
</tr>
<tr>
<td>TURBT</td>
<td>Transurethral resection of bladder tumours</td>
</tr>
<tr>
<td>TCC</td>
<td>Transitional Cell Carcinoma</td>
</tr>
<tr>
<td>HER</td>
<td>Human Epidermal Growth Factor Receptor</td>
</tr>
<tr>
<td>EORTC</td>
<td>European Organisation for the Research and Treatment of Cancer</td>
</tr>
<tr>
<td>UICC</td>
<td>International Union Against Cancer</td>
</tr>
<tr>
<td>AUA</td>
<td>American Urological Association</td>
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</table>
Introduction

The present thesis comprises four different aspects of cancer of the urinary bladder. It goes from an overview of the epidemiology of the disease in Sweden and the compliance to guidelines regarding treatment of the non muscle-invasive forms with subsequent outcome in paper I to an evaluation of the impact of the recommended treatments in the more controlled setting of a randomised trial in paper II. The need for development of new therapies can be concluded and a new drug, gemcitabine is explored for intravesical use in a phase II study in paper III. Once the cancer has evaded the efforts to eradicate it in the bladder and metastasised the prognosis is very poor. In the fourth paper the possibilities to hunt it down via an overexpressed HER2 receptor on the urothelial cancer cell surface is explored.
Background

Historical Overview

Early Urology and Why Alternatives to Surgery were Sought

Figure 1. Roman matronae Julia Domna, wife of the Roman emperor Septimius Severus. Picture by Ove Kaneberg, Museum of Mediterranean and Near Eastern Antiquities.

In a recent paper on the history of urology[1] a Roman physician named Pliny the Elder (23/24 - 79 A.D.) is quoted:

"The experience of time has concluded that the disease causing the sharpest agony is strangury from stone in the bladder; next comes disease of the stomach, and after that pain produced by diseases of the head".

In conclusion he maintains that these are

"about the only diseases that are responsible for suicides".
Other urological diseases also included were urethral stenosis, prostatic hypertrophy and perhaps bladder and prostate cancers, which were not only very common diseases at that time, but also frequently led patients to commit suicide.

Another physician active just before Pliny, Celsus (14-37 AD), mentioned surgery as a last resort but after the first half of the first century AD surgery was not recommended. According to the authors of the quoted paper this was most likely due to the fact that Roman “matronae” had started employing renowned physicians (and not barbers) to perform castrations on their male slave lovers as a means of contraception. The procedure was well paid for and the greed for money led to a widespread practice. Even the prestigious surgeon Heliodorus is said to have been involved. At this stage the best medical schools took to changing the Hippocratic Oath, inserting the line

"I will not use the knife, not even, verily, on sufferers from stone, but will give place to such as are craftsmen therein".

After this physicians were forced to try the drugs available. The authors conclude:

"…apart from some light diuretics (like white charlock, celery, parsley, asparagus, orpine, kale, carrot, costus and pine kernels); the only drugs which could be useful were garden cress (a good diuretic), leek (somehow useful against gravel, dysuria and anuria) and white mustard (an anti-inflammatory agent for the urinary tracts). As to pains, Celsus could do very little. Some of his drugs had an analgesic power (like saffron, water mint, the roots of spike-nard, poppy and garden thyme), this is true, but it was so light that we can be sure that a patient with any urological disease had only three alternatives: either to die, or to commit suicide, or to put himself into the hands of a surgeon and - hope in a God. But generally Gods were not so quick to answer his prayers!"

So the first attempts at treating disorders of the urinary bladder both with and without surgery were not so successful…
Chemotherapy Introduced
As illustrated on the front cover surgery and intravesical treatments were used in what is now Turkey but it was not until the beginning of the 20th century that the use of chemotherapeutic agents begun, making JG Connolly [2] state that:

"Almost any promising chemotherapeutic or antitumor agent, providing it is not too toxic, will be used to treat superficial bladder cancer".

Table 1. Some chemotherapeutic agents tried for intravesical use during the 20th century [2]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver nitrate</td>
<td>1903</td>
</tr>
<tr>
<td>Trichloroacetic acid</td>
<td>1919</td>
</tr>
<tr>
<td>Podophyllin</td>
<td>1948</td>
</tr>
<tr>
<td>Thiopeta</td>
<td>1961</td>
</tr>
<tr>
<td>Actinomycin C</td>
<td>1965</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>1965</td>
</tr>
<tr>
<td>Mannitol myleran</td>
<td>1966</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>1966</td>
</tr>
<tr>
<td>Mitomycin-C</td>
<td>1967</td>
</tr>
<tr>
<td>Etoposicid (Epodyl)</td>
<td>1973</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>1977</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>1977</td>
</tr>
<tr>
<td>Epipodophyllotoxin (VM-26)</td>
<td>1978</td>
</tr>
</tbody>
</table>

The mere possibility to use an agent does however not automatically mean that is efficient and it was not until the introduction of Thiotepa in the early 1960’s that the use of intravesical instillations became more widespread.

Currently derivatives of antibiotics, Mitomycin-C and Epirubicin/Doxorubicin are the most widely used. Mitomycin-C functions as a bifunctional or trifunctional alkylation agent inducing inter- and intrastrand DNA crosslinks. It is also active in degrading and inhibiting DNA synthesis. Doxorubicin and Epirubicin are anthracycline antibiotics binding DNA base pairs and acting as intercalating agents inhibiting Topoisomerase II.

Immunostimulation, Bacillus Calmette-Guerin
In 1882 the German scientist Robert Koch discovered the bacterial cause of tuberculosis. From 1908 to 1921 the two scientists Albert Calmette and Camille Guérin worked at the French Pasteur Institute to develop a vaccine, a less virulent strain of bacteria, which would protect from tuberculosis. It became the Bacillus Calmette-Guerin, BCG. The use of this vaccine as an immunostimulant active against neoplastic cells via a delayed hypersensitiv-
ity reaction was investigated and in 1976 dr A. Morales published the first nine cases of bladder cancer treated with intravesical instillations of BCG [3].

Present Day

Staging and Grading

Decisions on treatment of cancer of the urinary bladder are based on the clinical findings (which will be described in detail later) together with histological evaluation by the pathologists. The categories for different levels of differentiation of the urothelial cells and the depth of invasion into the bladder wall have been revised during the years.

For grading the history of the development of different grading systems has been described by Busch et al [4]. It began in the 1920s when the proportion of undifferentiated tumour areas was described up to the 1973 WHO system based on cellular anaplasia, grades 1, 2 and 3, and the 1986 Malmstrom modified Bergkvist system based on architectural pattern and object-related features. Most studies published so far have used the WHO 1973 system. The latest step has been the synthesis of two new systems; the WHO/ISUP 1998 and the WHO 1999 to what is now called the WHO 2004 (WHO/ISUP 1998). The system is meant to avoid the overdiagnosis of cancer and create better criteria for the grades. It distinguishes:

- Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP),
- Low grade
- High grade.

The TNM Staging System was developed beginning in the 1940’s by Pierre Denoix, France and in the 1950’s a Committee on Tumour Nomenclature and Statistics was appointed by the UICC, the International Union against Cancer. In 1958 the first recommendations were published for the clinical stage classifications of cancers of the breast and larynx and for the presentation of the results. The present edition is number 6 (TNM 2002). The classifications for carcinomas of the urinary bladder of the AJCC/UICC have not changed since the fifth edition (1997) and are shown in figure 2 and table 2.
Figure 2. Stages according to the 1997/2002 TNM classification. Picture by Jana Howe of the Uppsala Regional Oncological Centre.
<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX</td>
<td>Primary tumour cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumour</td>
</tr>
<tr>
<td>Ta</td>
<td>Non-invasive papillary carcinoma</td>
</tr>
<tr>
<td>Tis</td>
<td>Carcinoma in situ: “flat tumour”</td>
</tr>
<tr>
<td>T1</td>
<td>Tumour invades subepithelial connective tissue</td>
</tr>
<tr>
<td>T2</td>
<td>Tumour invades muscle</td>
</tr>
<tr>
<td>T2a</td>
<td>Tumour invades superficial muscle (inner half)</td>
</tr>
<tr>
<td>T2b</td>
<td>Tumour invades deep muscle (outer half)</td>
</tr>
<tr>
<td>T3</td>
<td>Tumour invades perivesical tissue</td>
</tr>
<tr>
<td>T3a</td>
<td>Microscopically</td>
</tr>
<tr>
<td>T3b</td>
<td>Macroscopically (extravesicular mass)</td>
</tr>
<tr>
<td>T4</td>
<td>Tumour invades any of the following: prostate, uterus, vagina, pelvic wall, and abdominal wall</td>
</tr>
<tr>
<td>T4a</td>
<td>Tumour invades prostate or uterus or vagina</td>
</tr>
<tr>
<td>T4b</td>
<td>Tumour invades pelvic wall or abdominal wall</td>
</tr>
<tr>
<td>NX</td>
<td>Regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>No regional metastasis</td>
</tr>
<tr>
<td>N1</td>
<td>Single &lt; 2 cm</td>
</tr>
<tr>
<td>N2</td>
<td>Single ≥ 2 cm to 5 cm, multiple ≤ 5 cm</td>
</tr>
<tr>
<td>N3</td>
<td>≥ 5 cm</td>
</tr>
<tr>
<td>MX</td>
<td>Distant metastases cannot be assessed</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastasis</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis</td>
</tr>
</tbody>
</table>

By AJCC/UICC convention, the designation “T” refers to a primary tumour that has not been previously treated.
The symbol “p” refers to the pathologic classification of the TNM as opposed to the clinical classification, and is based on gross and microscopic examination.
pT entails a resection of the primary tumour or biopsy adequate to evaluate the highest pT category;
pN entails removal of nodes adequate to validate lymph node metastasis; and pM implies microscopic examination of distant lesions.
Clinical classification (cTNM) is usually carried out by the referring physician before treatment during initial evaluation of the patient or when pathologic classification is not possible.
The suffix “m” should be added to the appropriate T category to indicate multiple tumours.
The suffix “is” may be added to any T to indicate the presence of associated carcinoma in situ.

**Treatment Guidelines**
The foundation of treatment of cancer of the urinary bladder is transurethral resection, TURBT, aiming at complete removal of the affected tissue. Unfortunately this is sufficient only in cases where the neoplasm is restricted to
part of the bladder, limited in depth and dedifferentiation, and visible. If the growth is intramuscular or widespread the operation becomes just diagnostic, not curative.

Muscle invasive forms are today mostly treated by complete removal of the urinary bladder and local lymphoglandulae, cystectomy. Radiation treatment with curative intent is also possible but not so commonly used. Adjuvant or neoadjuvant treatment with chemotherapy is currently used but still the five year survival for this patient group is disappointing. Once the cancer cells have infiltrated beyond the bladder itself and metastasised, the prognosis is dismal.

The non muscle-invasive forms can be subdivided into risk groups based on the findings at the initial treatment and follow-up. The major urological societies have published recommendations for the treatment of these tumours but chosen to make the subdivision in different ways. The level of evidence for the recommendations is declared even though different terminologies are used.

The EAU have introduced the following subgroups:

- Low risk tumours: single, TaG1, ≤3cm diameter
- High risk tumours: T1G3, multifocal or highly recurrent, CIS
- Intermediate risk: all other tumours, Ta-1, G1-2, multifocal, >3cm diameter

For the low risk group a thorough and complete transurethral resection followed by an immediate instillation in the bladder of a chemotherapeutic drug within six hours is considered standard.

For the intermediate group the same is considered standard but a second operation after four to six weeks can be performed (optional). Adjuvant intravesical therapy with chemotherapeutic drugs or BCG is also recommended but there is no consensus regarding the optimal drug or scheme.

For the high risk group a second resection is recommended and BCG instillations including a maintenance schedule for at least one year should be performed. In these cases an immediate cystectomy is considered optional and recommended if the disease fails to respond to BCG [5].

In the USA recommendations (graded as standard, guidelines or optional) were published by the AUA in 1999[6]. The following subgroups, index patients, are identified:

- Patients who present with abnormal growth of the urothelium but have not yet been diagnosed with bladder cancer
- Patients with established cancer of any grade, stage Ta or T1, without CIS but no prior intravesical therapy
• Patients with CIS or high grade T1 cancer and treated with at least one course of intravesical therapy

For the patient in the process of getting a diagnosis the importance of getting proper material for a histological diagnosis is stressed. For the patients with a diagnosis but no prior treatment the importance of complete eradication of all tumour tissue is underlined. The use of intravesical treatment in patients with low grade Ta is set as optional unless the tumours are rapidly recurring. For CIS, T1 and high grade Ta tumours instillations of BCG or MMC are considered a guideline. Up front cystectomy is considered optional and may be performed if the tumours are large, high grade, poorly accessible diffusely growing or infiltrating lymphatic or vascular spaces or the prostatic urethra.

For patients in the third group the risk of progression is considered substantial and cystectomy may be considered (optional). Further intravesical therapy including chemotherapy or a second induction course of BCG is also considered optional and may be used in the case of a late recurrence after a previous complete response.

The SIU published a set of recommendations from the International Consultation on Urologic Disease in 2005. Recommendations were graded from Grade A= highest down to D= no recommendation possible. The recommendations were made for:

• Low-grade Ta[7]
• High-grade Ta and CIS[8]
• T1[9]

For Ta of low grade a complete resection is important followed by immediate instillation of chemotherapy. There is a possibility for secondary intravesical chemotherapy if there are multiple recurrences at the first follow-up cystoscopy.

For high-grade Ta tumours an immediate post operative instillation of chemotherapy is recommended (Grade A). A second resection with mapping biopsies should be performed after 2-4 weeks also with immediate instillation of single dose chemotherapy (Grade B). If the diagnosis is confirmed a 6-week induction course of BCG should be started followed by maintenance treatment for 1 to 3 years (Grade A). If BCG fails cystectomy can be considered if high-grade T1 or CIS is found (Grade B). For CIS the use of fluorescence cystoscopy should be considered because of its higher sensitivity than white light (Grade B) and if positive cytology random biopsies including the prostatic urethra should be taken (Grade B). Radical cystectomy will mean over-treatment in 50% of the cases (Grade A). BCG treatment includ-
ing maintenance should be used (Grade A). If a complete response has not been achieved in 6 months radical cystectomy is recommended (Grade B).

For the T1 tumours the immediate instillation is recommended (Grade A). Multiple random biopsies are indicated (Grade C). A second TURBT within 1 to 4 weeks is suggested (Grade B). Both cystectomy and instillation therapy are acceptable primary therapies. Intravesical BCG should be considered if the tumour has been completely resected, the patient is satisfied with the bladder function and can tolerate BCG (Grade C). Maintenance therapy should be given (Grade A). If there is a recurrence of high grade disease at 6 months the patient should be offered a cystectomy (Grade C).

In Sweden national guidelines have been available since the 1990’s via the National Board of Health and Welfare. The recommendations are not as detailed as those described previously but it is stated that intravesical treatment should be considered for patients with high risk for recurrence or progression. Immediate post operative instillation of chemotherapy is discussed but no distinct recommendation is made. For CIS BCG is the therapy of choice. If intravesical therapy fails cystectomy must be considered.

The above recommendations are based on data indicating that, apart from MMC, BCG is superior to intravesical chemotherapy with respect to the effect on recurrence of papillary tumours and carcinoma in situ (CIS). The effect on progression and survival is less clear. Several meta-analyses have been performed to address this matter [10-12]. The efforts to combine data from different studies for meta-analysis have been hampered by several confounding factors such as different treatment schedules regarding both dosage and duration of treatment and short observation times (usually three years or less).
The Swedish National Quality Registry for Bladder Cancer

Since 1958 all new cases of cancer have been reported to the Swedish Cancer Registry, run by the Swedish National Board of Health and Welfare. It is compulsory for every health care provider to report newly detected cancers to the registry. The reports come from both pathologists and clinicians and include Personal identification number, sex, age, place of residence, site of tumour, histological type, stage (since 2004), basis of diagnosis, date, reporting hospital and department, reporting pathology department and identification number for the tissue specimen. Date of death or migration and cause of death are also recorded. The overall reporting to the registry is estimated to be 96% of all diagnosed cases.

The information within this register was considered insufficient for deeper analyses of epidemiology, changes in patterns of treatment and outcome and during the 1990’s several extensions of it were started.

The Swedish National Quality Registry for Bladder Cancer is such a national population based registry and was started in 1997. It covers all the urological centres in Sweden and includes on average 94% of all new cases of bladder cancer (as compared to the Cancer Registry). The physicians treating the patients report clinical and pathological data and initial treatment
within three months (Stockholm six months) after diagnosis to the regional oncological centre of the health care region. Anonymous data from the six centres:

- Northern (N) 883 215
- Uppsala-Örebro (U-Ö) 1 914 055
- Stockholm-Gotland (S-G) 1 896 294
- Western (W) 1 658 526
- South-Eastern (S-E) 974 884
- Southern (S) 1 582 154

are gathered in a national database (the population of the respective regions is per 2001-12-31).

The reports include age, gender, residency, stage and grade. For the studied time period in paper I WHO 1973 was used except for the Stockholm region which used the modified Bergquist-Moberger system, equivalent to WHO-99.

Registered treatments are transurethral resections (TURBT), intravesical chemo- or immunotherapy, cystectomy and radiation. Summaries and analyses of the data from the registry are published annually and meetings are held for continuous improvement and adaptation.

### Development of New Drugs and Treatment Modalities

#### New Chemotherapeutic Agents - Gemcitabine

The development of new chemotherapeutic agents is ongoing and several new drugs have been introduced in the last decade. For systemic use in the treatment of advanced urothelial cancer new combinations with cisplatinum are available; the nucleoside analogue 2,2-difluorodeoxycytidine (dFdC), gemcitabine, has shown a more beneficial toxicity profile compared to the previously used MVAC regimen[13] while maintaining efficacy.

The gemcitabine molecule is metabolised intracellularly by nucleoside kinases to the active di- (dFdCDP) and trifostate (dFdCTP) nucleosides, primarily killing cells undergoing DNA-synthesis, i.e. in the S-phase, and blocking the progression through G1/S-phase.

1. dFdCDP inhibits ribonucleotide reductase, causing a reduction in the concentration of all naturally occurring deoxynucleotide triphosphates, especially deoxycytidine triphosphate (dCTP). This increases the chances that the dFdCTP will be incorporated into the growing DNA strands instead of dCTP - “self-potentiation”
2. DNA polymerase epsilon is unable to remove the dFdCTP and repair the growing DNA strand. With the addition of one more nucleoside to the growing DNA strand, there is complete inhibition of further DNA synthesis - “masked chain termination”

This appears to induce the programmed cellular death process known as apoptosis.

The exploration of Gemcitabine for intravesical treatment of non muscle-invasive tumours was begun in the early 2000’s via animal studies to a first clinical study in 2002 on patients with TCC refractory to BCG [14].

![Figure 4. 2'-deoxy-2', 2'-difluorocytidine monohydrochloride (β-isomer)](image)

The Tumour Marker Lesion Concept

How do we best assess new drugs for potential intravesical use without exposing an unnecessary number of patients to it?

The tumour marker lesion concept was introduced by the EORTC as a means to get an early indication whether or not a new candidate drug was active on TCC in the urinary bladder [15]. In the Phase II study setting patients with Ta or T1 tumours are recruited. The tumours are resected via TURBT and one well characterised tumour, the marker lesion, is left behind. The study drug (or drugs) is then instilled according to the study protocol and at a second operation the effect is evaluated. Any residual tumour is resected. This method is proposed as an alternative to the complete resection of tumours, instillation and subsequent follow up which has been used traditionally and which not only requires a larger number of patients but also takes longer time to evaluate. The latter approach is also considered less objective and reliable, resulting in a statistically less manageable situation.
As to the ethics of the marker lesion method the bioethicist McCollough [16] has stated that it is possible from an ethical point of view to leave a tumour behind at the first operation provided that the following three conditions have been met:

- Proper management of risk in the design and conduct of the study
- A proper informed consent process
- Proper monitoring of the study, preferably using a Data and Safety Monitoring Board (especially in Phase III trials)

Finding Targets in Metastasised Disease – the HER2 Receptor

The invasion of neoplastic cells into the muscular layers of the urinary bladder and beyond may be discovered at the first examination or diagnosed as an escalation despite efforts with intravesical treatment as described above. At present the five year survival for patients with muscle-invasive disease is a disappointing average of 50%. Subdivisions can be made to find survival differences but the fact remains that the prognosis for this group is poor. Recent work by Sherif et al has shown a survival benefit with neoadjuvant chemotherapy, particularly in the T3 subgroup where the absolute risk reduction was 11% [17]. The improvement is welcome and the therapy should be implemented but it does not alter the state of things in a major way.

Reviewer of the ASCO-meeting 2005 James Montie on the need for new therapies against advanced Bladder cancer (Editorial Comment):

"...There is a desperate need for new therapies to complement existing chemotherapy (which is highly effective at inducing a partial response in the tumor but poorly effective in providing a durable complete response). A better understanding of the mechanism of disruption of normal cellular mechanisms by malignant transformation has raised the possibility of interfering with cancer cell function by “targeting” these specific abnormal or altered pathways. There are fewer clinical trials with such new agents in bladder cancer than in many other malignancies, because industry priorities dictate investigations of more common malignancies. Unfortunately, the second line therapeutic options for relapsing bladder cancer are currently largely limited to alternative cytotoxic agents. We anxiously await the opportunity to study new targeted agents in a disease in which they may well be effective."

One of the possible targets for such therapy is the human epidermal growth factor receptor family, HER 1-4. This is a group of tyrosine kinase receptors (The HER1 receptor is also known as the EGFR).

Previous work in our group focused on the HER1-receptor and ways to use it for targeted therapy [18]. Due to possible problems with the distribution of receptors in normal tissue our focus turned to the next receptor, HER2. This receptor is a 185 kDa transmembrane protein with no known ligand. It acts via dimerization with the other receptors in the family or itself.
In breast cancer overexpression of the HER2 receptor is a well known alteration, and it is used as target for the anti-HER2 antibody trastuzumab (Herceptin®) treatment[19, 20].

The distribution of HER2 in normal tissue differs from that of HER1 [21, 22]. In the liver it is only weakly expressed on hepatocytes and in the bile ducts. It is also expressed to a lesser degree than HER1 in the digestive tract, skin, and reproductive organs. Thus, HER2 based targeting could be advantageous, since the uptake in most normal tissues should be limited.

In a recently published study [23] on a limited number of urinary bladder cancer patients (n=21) HER2 was overexpressed in 81% of the primary tumours and in 67% of the metastases. All HER2 positive metastases were from HER2 positive primary tumours. With the introduction of trastuzumab (Herceptin®) in the treatment of breast cancer, more standardised immunohistochemical procedures were developed; one of them being the FDA approved HercepTest® by DAKO. A possible risk of underdiagnosing receptor expression using this method on other cancer types was reported after tests of a modified protocol on prostate cancer tumours [24].
Aims of the Studies

I. To use the Swedish National Quality Registry for Bladder Cancer data base to analyse the following aspects both nationally and by health care region for Ta-T1 tumours: demography of the patients including age and gender, tumour characteristics (i.e. stage and grade), initial therapy and relative survival.

II. To evaluate at ten years a randomised prospective study comparing intravesical treatment of high risk non muscle-invasive bladder cancer with Bacillus Calmette-Guerin or Mitomycin-C regarding the need for additional and more aggressive treatment, progressive disease and survival (overall and cancer specific).

III. To use the tumour marker lesion concept in a randomised phase II study to assess the antitumoral effects on urothelial carcinoma and toxicity of gemcitabine administered intravesically using three different dose schedules.

IV. To assess HER2 expression for possible differences between primary tumours and metastases and between metastases at different locations in patients with transitional cell cancer of the urinary bladder. To compare two different immunohistochemical protocols; the FDA approved HercepTest® protocol versus a modified protocol and compare the standard evaluation criteria applied for breast cancer therapy with criteria proposed for radionuclide based targeting.
Patients and Methods

Paper I
Study Design
The Swedish National Quality Registry for Bladder Cancer database was used. During the period 1997-2001 9859 patients were registered with a diagnosis of urinary bladder cancer. The material consisted of the 6581 patients who were reported to have a Ta or T1-tumour (TNM-classification 1997). For survival the patients were followed from the date of diagnosis to the first event of death, emigration or the date 2003-09.

Statistics Paper I
Relationships between factors, i.e. health care region and treatment, were analysed in cross-tables by Pearson's chi-squared statistics under the hypothesis that the rows and columns in a two-way table are independent, or Fishers’ exact test when appropriate.

We used unconditional logistic regression analyses [25] to assess if treatment with endovesical therapy had increased during the studied period and if treatment with endovesical therapy was dependent on age at diagnosis or gender. Age at diagnosis was treated as a categorical variable divided in 5 groups: <65, 65-69, 70-75, 76-80 and ≥81 years. Year of diagnosis was evaluated both as a linear trend variable (on the log scale), and as a categorical variable where each year of diagnosis represented one category.

Since very few autopsies are performed in Sweden today the accuracy of the cause of death certificates can be questioned. Previous studies have also demonstrated the pitfalls of using death certificates for epidemiologic studies [26]. For this reason we chose to analyse survival by means of relative survival ratios (RSR). RSR are defined as the observed survival in the patient group divided by the expected survival of a comparable group of individuals. Observed survival was calculated by the actuarial method, and expected survival was calculated from Swedish population life tables stratified by gender, age and calendar time. All deaths are considered as events when calculating RSR and it therefore provides a measure of the total excess mortality associated with the diagnosis, i.e. it measures the excess mortality due to factors that are indirectly or directly associated with the diagnosis.
analyses were performed using the statistical software STATA (version 8.2, StataCorp LP).

Paper II

Study Design

**Inclusion and Exclusion Criteria:**
As described in the first publication from the study in 1996[27] patients with stage Ta, grades 1 to 3 or stage T1, grades 1 and 2 tumours were included provided there had been at least 3 tumour events during the prior 18 months. Patients with stage T1, grade 3 and those with primary or concomitant dysplasia or carcinoma in situ were included without having had prior tumour events. Additional prerequisites for inclusion were informed consent, normal liver and kidney function tests, and no chemotherapy during the prior 6 months. Exclusion criteria were previous or ongoing intravesical treatment with Mitomycin-C, BCG or radiotherapy, any secondary malignancy except treated carcinoma in situ of the uterine cervix or basal cell carcinoma of the skin, ongoing corticosteroid therapy, leukocytes less than 3,000/ mm., thrombocytes less than 100,000/mm., untreated urinary tract infection, urethral stricture preventing cystoscopy, active tuberculosis, pregnancy and expected difficulties during the follow-up (for example Karnofsky performance index less than 50, senility, psychotic disease or any other reason that might prevent follow-up)

**Patient Characteristics:**
Between 1987 and 1992, 12 hospitals in Sweden and Norway enrolled 218 men (84%) and 43 women (16%) and who had a mean age of 68 years (range 27 to 86). Fourteen patients (5%) had previously received intravesical chemotherapy. Of the patients 130 were randomly assigned to receive Mitomycin C and 131 to receive BCG after stratification was performed according to the different inclusion criteria listed below. BCG treatment was not yet considered standard therapy in Sweden or Norway at the time of designing the original study and so it was included as an option to cross over to the alternative treatment if one of the following occurred:

- in Ta-T1 GI-II if there were tumour recurrences on two consecutive follow-up visits,
- in T1 GIII on the first recurrence,
- in cases of dysplasia or CIS if cytology and biopsies were not benign after the first six months of treatment.
Treatment crossovers were performed in 60 cases: 39 patients from MMC to BCG and 21 patients from BCG to MMC.

Of the 261 recruited patients 11 were excluded from the efficacy analysis. These non evaluable patients were equally distributed between the two treatment groups. The distribution across the different stages for the two treatment groups is illustrated in table 3.

Table 3. Distribution across different stages for the treatment groups

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. Mitomycin C</th>
<th>No. BCG</th>
<th>Total No. Pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ta</td>
<td>51</td>
<td>53</td>
<td>104</td>
</tr>
<tr>
<td>T1</td>
<td>32</td>
<td>31</td>
<td>63</td>
</tr>
<tr>
<td>Dysplasia/Tis</td>
<td>42</td>
<td>41</td>
<td>83</td>
</tr>
<tr>
<td>Totals</td>
<td>125</td>
<td>125</td>
<td>250</td>
</tr>
</tbody>
</table>

Treatment Schedules

The patients were randomized via a centralized procedure to receive intravesical instillations of either 40 mgs of Mitomycin–C in 50 mls of saline with phosphate buffer or 120 mgs of Bacillus Calmette-Guerin (Danish strain 1331) according to the following: once weekly for the first six weeks then monthly up to one year and finally every third month for a further year.

Follow-up and Definition of Clinical Events

The clinics responsible for the initial treatment were contacted and given a questionnaire on progression, need for escalation to more aggressive treatment (such as cystectomy, radiation or systemic chemotherapy) and survival. Cause of death, bladder cancer or non-bladder cancer, was sought via the questionnaire and the National Cause of Death Register. Progression was defined as an increase in stage, e.g. Ta to T1 or T1 to T2.

Statistics Paper II

In the original study the recruitment goal was to have at least 250 randomized patients based on the probability of 50% with no evidence of disease in the Mitomycin C group and 70% in the BCG group after 2 years of treatment. A total of 96 evaluable patients were required in each treatment group, with a type 1 error rate of 5% and a power of 80%.

In the present 10 year follow-up analysis progression and survival were analyzed according to the Kaplan-Meier method. The log rank test was used for significance. Comparisons between the groups regarding later treatment were performed using the chi square test or Fischer’s exact test when appropriate, using a significance level of p<0.05. The statistical analyses were performed using the program Medlog™ version 2k (Information Analysis

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Paper III

Study Design

Inclusion and Exclusion Criteria:
Included patients were diagnosed with recurrent multiple Ta G1-2 urinary bladder cancer and all but one lesion, the marker lesion sized 0.5 to 1.0 cm (using the cutting loop of the resectoscope as reference), were resected. This procedure was monitored by a neutral physician. Cytology from the prestudy transurethral resection had to be without signs of high grade carcinoma. Previous treatments with intravesical agents had to have been terminated at least 3 months before inclusion for chemotherapy or 6 months for BCG. Malignancies of the upper urinary tract were excluded and a minimum of 150 mls of bladder capacity was required. Patients with serious infections or surgery within the urogenital sphere were not accepted if either occurred within one month prior to inclusion. No cases of chronic cystitis were accepted. Laboratory tests for liver, kidney, bone marrow and coagulation function had to be within the references set by the hospital laboratories. A performance status of ECOG 0-2 was required and informed consent was obtained. The study design was approved by all the ethical committees concerned.

Drug Schedule and Toxicity Monitoring:
The randomisation was done centrally. The patients were allotted to one of three dose schedules as presented in Figure 6. They were instructed to reduce their intake of fluids 12 hours prior to instillation. The optimal dose as described by previous authors[14] of 2000 mgs of gemcitabine was dissolved in 100 mls of an unbuffered solution of saline 9 mgs/ml and administered intravesically with a dwell time of one hour. Spasmolytic drugs were used in case of urgency.

Before each instillation, blood samples were taken and liver, kidney, bone marrow and coagulation function was assessed. After each installment a questionnaire was filled out regarding side effects or changes in concomitant medication by the study nurse and patient together.

In cases of side effects, the following actions were taken: If platelets decreased but were still over or equal to 100 000/mm3 or granulocytes equal to or over 1500/mm3 or a non haematological toxicity was 0 to 1 (NCI CTC) the instillation was carried out as planned. In cases of a platelet count of < 100 000 but still over or equal to 50 000/mm3 or poly was < 1500 but still over or equal to 1000/mm3 or the NCI CTC was 2 then the instillation was
postponed for one week. If any of the parameters described were worse than grade 2 the instillation was postponed until lab tests were normal. If this took more than two weeks the patient was taken out of the instillation programme but still underwent the follow-up cystoscopy.

At nine weeks after the first installation a follow-up cystoscopy was performed in the presence of a neutral physician and a transurethral resection was performed if any residual or new tumour was found.

**Figure 6. Study design and monitoring**

**Definition of Response:**
Based on the nine week cystoscopy:
- Complete response (CR) was defined as the complete disappearance of the marker lesion and no new tumours.
- No response (NR) was defined as the discovery of an unaffected marker lesion with no increase in size or other visible tumours.
- Increasing Tumour (IT) was defined as an increase in size of the marker lesion or the appearance of new tumours.

**Statistics Paper III:**
The study was set up as a feasibility study with no attempt to compare the three arms with each other. Any response rate less than 20% was to be considered ineffective. A response rate greater than 50% was to be considered effective and would motivate further trials. A sample size of 17 patients on
each arm was considered sufficient (two-sided alpha of 0.05 and an 80% power). Considering the risk of drop-outs and the risk of having not all patients evaluable, the inclusion of 20 patients in each arm was planned. The total number of patients in the study was thus planned to be 60.

Paper IV
Study Design
Tissue Samples
The study included 90 patients with metastatic transitional cell carcinoma of the urinary bladder. In all cases histological material from both primary tumours and metastases had to be available. The samples were consecutively collected between 1976 and 2003. Ten of the patients were also included in a sentinel node project [28]. All tissue specimens were formalin fixed and paraffin embedded according to standard procedures at our laboratory and the Department of Pathology, Uppsala, Sweden. For patient, tumour and metastasis characteristics see table 4.

Immunohistochemistry
Two different immunohistochemical staining protocols were applied, HercepTest® and modified HercepTest® (MH). We then evaluated HercepTest® stained sections using two scoring systems, the standard and a modified system, and MH using the modified scoring system.

For both methods, paraffin sections of 4-µm thickness were placed onto Superfrost/plus® slides (Mentzel, Germany), deparaffinised in xylen and rehydrated in graded alcohols.

HercepTest® staining was done according to the instructions from the manufacturer of the HercepTest kit® (DAKO, Glostrup, Denmark) using an automated immunostaining instrument, Autostainer® (DAKO). Four cases were not stained due to technical reasons.

The MH staining method was applied on all 90 patients, using the Autostainer® (DAKO) as for HercepTest® staining. Prior to the MH staining method, slides were immersed in Target retrieval solution® (DAKO), pH 9 and boiled for 7 minutes in a Decloacing chamber® (Biocare Medical, Walnut Creek, CA, USA). Endogenous peroxidase was blocked in H2O2 (DAKO) for 20 minutes. Sections were incubated with a rabbit polyclonal anti-HER-2 antisera (DAKO), diluted 1:500, for 30 minutes. Incubation with anti-rabbit peroxidase-conjugated Envision® (DAKO) was done for 30 minutes followed by Diaminobenzidine (DAKO) for 10 minutes. Finally sections were counterstained in Harris hematoxylin (Sigma, St. Louis, MO, USA).
For both immunostaining methods, parallel sections where the primary antibody was replaced with PBS, served as negative controls and commercially available control slides (DAKO), containing cultured cells (SK-BR-3, MDA 175 and MDA231) with unique expression levels of HER-2 were used as positive controls.

Table 4. Patient and tumour characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>66 (73)</td>
</tr>
<tr>
<td>Female</td>
<td>24 (27)</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>66 (range 35-87)</td>
</tr>
<tr>
<td>Source of primary tumour</td>
<td></td>
</tr>
<tr>
<td>TUR</td>
<td>76 (84)</td>
</tr>
<tr>
<td>Cystectomy</td>
<td>14 (16)</td>
</tr>
<tr>
<td>Histological grades</td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Grade II</td>
<td>10 (11)</td>
</tr>
<tr>
<td>Grade III</td>
<td>79 (88)</td>
</tr>
<tr>
<td>Time between diagnosis of primary tumour and metastases (months)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>10 (range 0-82)</td>
</tr>
<tr>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>&lt;1</td>
<td>25</td>
</tr>
<tr>
<td>&lt;12</td>
<td>66</td>
</tr>
<tr>
<td>Metastatic locations</td>
<td></td>
</tr>
<tr>
<td>Regional lymph glands</td>
<td>39 (43)</td>
</tr>
<tr>
<td>Distant lymph glands</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Liver</td>
<td>7 (8)</td>
</tr>
<tr>
<td>Lung</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Skeleton</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Intestinal</td>
<td>14 (16)</td>
</tr>
<tr>
<td>Prostate</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Vagina</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Other</td>
<td>11 (12)</td>
</tr>
</tbody>
</table>
**Evaluation of Immunohistochemistry**

Evaluation of immunohistochemical staining was performed by three of the authors (TG, MT and KW). At the time of evaluation, the association between the primary tumours and their corresponding metastases was blinded to avoid bias.

**HercepTest score (the breast cancer criteria); ≥10% stained tumour cells**

The HER2 expression was scored according to the HercepTest score using the 0 to 3+ scale, 0 was completely negative, 1+ faint perceptible staining of the membrane, 2+ moderate staining of the entire membrane observed in more than 10% of the tumour cells and 3+ was strong circumferential staining of the entire membrane creating a fishnet pattern. Cytoplasmic staining was considered non-specific and was not included in the scoring.

**Target score; ≥2/3 stained tumour cells**

The HER2 expression was also analysed according to the Target score criteria [23]. The intensity and staining patterns were judged in a similar way as for the HercepTest score criteria. Positive staining was, for HER2:

1) More than 2/3 of the tumour cells should be stained
2) The staining intensity should be moderate to intense (2+ or 3+)

The staining pattern should be membranous, with or without concomitant cytoplasmic staining

Using the above criteria, a tumour classified as positive should fulfil all three, i.e. express HER2 at an adequate level with a distribution that might allow for successful radionuclide targeted therapy. Examples of positive and negative staining classifications are given in figure 1.

**Statistics Paper IV**

The metastatic sites were collected into groups according to distance from the primary tumour and type of tissue and comparisons between the groups regarding HER2 overexpression were performed using the chi square test or Fischer’s exact test when appropriate, using a significance level of $p<0.05$. 

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Results

Paper I

**Demography and Tumour Characteristics**

Table 5 reveals that the majority of the 6581 included patients with Ta-T1 tumours were Ta; regional variation was 61 to 72%. The majority of both Ta and T1 patients were male (76%).

Figure 7 shows that the age distribution was very similar for stages Ta and T1. The disease was rare in younger ages, the median age at diagnosis being 72 for Ta and 73 years for T1. A slight shift towards more T1 tumours was found in the age groups older than 70 years. The age and sex distributions in the different health care regions, not depicted in the tables, were similar.

Distribution of tumour grade in Ta-tumours differed significantly between the six health care regions (p<0.001). Likewise there was also a significant difference in T1 tumours between the health care regions (p<0.001). The most deviating distribution was observed in the Stockholm–Gotland Region, using a different grading system, for both Ta and T1 tumours (figures 8 and 9).

<table>
<thead>
<tr>
<th>Region</th>
<th>Ta</th>
<th>T1</th>
<th>Ta+T1</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-G</td>
<td>813</td>
<td>395</td>
<td>1 208</td>
</tr>
<tr>
<td>U-Ö</td>
<td>934</td>
<td>434</td>
<td>1 368</td>
</tr>
<tr>
<td>S-E</td>
<td>509</td>
<td>230</td>
<td>739</td>
</tr>
<tr>
<td>S</td>
<td>820</td>
<td>523</td>
<td>1 343</td>
</tr>
<tr>
<td>W</td>
<td>910</td>
<td>388</td>
<td>1 298</td>
</tr>
<tr>
<td>N</td>
<td>456</td>
<td>169</td>
<td>625</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>4 442</strong></td>
<td><strong>2 139</strong></td>
<td><strong>6 581</strong></td>
</tr>
</tbody>
</table>
Figure 7. Age distribution for stages Ta and T1
Figure 8. Distribution of grade for Ta tumours per region

Figure 9. Distribution of grade for T1 tumours per region
**Treatment**

The most common treatment both among Ta and T1 patients by far was resection only: 90%, and 67%, respectively. Patients with G3 tumours received immunotherapy more often than patients with lower tumour grades, in both Ta- and T1-tumour patients (p<0.001) (figure 10). Moreover, there was a variation in treatment activity in different health care regions, illustrated by the fact that the Northern region reported immunotherapy in 38% of the T1G3 patient cases in contrast to the South-East region where only 10% reported immunotherapy usage (Table 6).

The fact that the Stockholm region in contrast to the other regions reported management during the first six months did not increase the number of additional treatments compared to the other regions.
Table 6. The four most common initial treatments in high risk Ta and T1 bladder cancer patients in different health care regions (%).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>S-G</th>
<th>U-Ö</th>
<th>S-E</th>
<th>S</th>
<th>W</th>
<th>N</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TURBT only</td>
<td>23</td>
<td>28</td>
<td>39</td>
<td>33</td>
<td>35</td>
<td>22</td>
<td>180</td>
</tr>
<tr>
<td>Chemo</td>
<td>3 (8)</td>
<td>5 (9)</td>
<td>0 (0)</td>
<td>3 (6)</td>
<td>3 (5)</td>
<td>4 (10)</td>
<td>18</td>
</tr>
<tr>
<td>Immuno</td>
<td>8 (22)</td>
<td>16 (30)</td>
<td>5 (11)</td>
<td>12 (24)</td>
<td>18 (31)</td>
<td>14 (35)</td>
<td>73</td>
</tr>
<tr>
<td>Cystectomy</td>
<td>1 (3)</td>
<td>3 (6)</td>
<td>0 (0)</td>
<td>0 (2)</td>
<td>1 (2)</td>
<td>1 (3)</td>
<td>6</td>
</tr>
<tr>
<td>TURBT only</td>
<td>184 (78)</td>
<td>144 (77)</td>
<td>88 (89)</td>
<td>199 (81)</td>
<td>136 (75)</td>
<td>53 (77)</td>
<td>804</td>
</tr>
<tr>
<td>Chemo</td>
<td>0 (0)</td>
<td>9 (5)</td>
<td>0 (0)</td>
<td>6 (2)</td>
<td>5 (3)</td>
<td>2 (3)</td>
<td>22</td>
</tr>
<tr>
<td>Immuno</td>
<td>29 (12)</td>
<td>20 (11)</td>
<td>2 (2)</td>
<td>30 (12)</td>
<td>26 (14)</td>
<td>7 (10)</td>
<td>114</td>
</tr>
<tr>
<td>Cystectomy</td>
<td>6 (3)</td>
<td>10 (5)</td>
<td>2 (2)</td>
<td>3 (1)</td>
<td>6 (3)</td>
<td>3 (4)</td>
<td>30</td>
</tr>
<tr>
<td>TURBT only</td>
<td>79 (52)</td>
<td>112 (53)</td>
<td>81 (70)</td>
<td>116 (49)</td>
<td>105 (61)</td>
<td>34 (36)</td>
<td>527</td>
</tr>
<tr>
<td>Chemo</td>
<td>7 (5)</td>
<td>14 (7)</td>
<td>7 (6)</td>
<td>14 (6)</td>
<td>8 (5)</td>
<td>14 (15)</td>
<td>64</td>
</tr>
<tr>
<td>Immuno</td>
<td>37 (24)</td>
<td>60 (28)</td>
<td>12 (10)</td>
<td>77 (32)</td>
<td>37 (21)</td>
<td>36 (38)</td>
<td>259</td>
</tr>
<tr>
<td>Cystectomy</td>
<td>15 (10)</td>
<td>25 (12)</td>
<td>5 (4)</td>
<td>14 (6)</td>
<td>16 (9)</td>
<td>2 (2)</td>
<td>77</td>
</tr>
</tbody>
</table>

Nationally, quite a low proportion of the high risk patients (TaG3, T1G2, and T1G3) received immuno- or chemotherapy. However, treatment activity was found to increase during the study period (Table 7). There was a tendency, though not statistically significant, that women received less immuno- or chemotherapy. Older patients received less intravesical treatment compared to younger patients.
Table 7. Odds ratios$^1$ for endovesical therapy in high risk non muscle-invasive bladder carcinoma patients (TaG3, T1G2 and T1G3)

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Year of diagnosis</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1997</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1998</td>
<td>0.8</td>
<td>0.6-1.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1999</td>
<td>1.1</td>
<td>0.8-1.5</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>1.4</td>
<td>1.0-1.9</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>1.6</td>
<td>1.2-2.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Trend</td>
<td></td>
<td>1.2</td>
<td>1.1-1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age group</td>
<td>&lt;65</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>65-69</td>
<td>0.9</td>
<td>0.6-1.2</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>70-75</td>
<td>0.8</td>
<td>0.6-1.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>76-80</td>
<td>0.6</td>
<td>0.4-0.8</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>$\geq$81</td>
<td>0.4</td>
<td>0.3-0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.8</td>
<td>0.6-1.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

$^1$ Odds ratio is for instance the ratio between the odds of intravesical therapy in the age group 65-69 compared to the age group <65.

Outcome

The five year relative survival for patients with all stages of bladder cancer (Ta-T4) during the observation period was 70%. The corresponding survival was found to be 93% for patients with Ta tumours and 75% for the T1 tumour patients (table 8).

Table 8. Relative survival for all patients (Tis, Ta, T1-T4, and Tx) registered in the Swedish bladder cancer registry (95% Confidence Intervals).

<table>
<thead>
<tr>
<th>Year of follow-up</th>
<th>All stages</th>
<th>Ta only</th>
<th>T1 only</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.85 (0.84-0.86)</td>
<td>0.99 (0.98-0.99)</td>
<td>0.95 (0.93-0.96)</td>
</tr>
<tr>
<td>2</td>
<td>0.78 (0.77-0.79)</td>
<td>0.98 (0.97-0.99)</td>
<td>0.88 (0.86-0.90)</td>
</tr>
<tr>
<td>3</td>
<td>0.74 (0.73-0.75)</td>
<td>0.97 (0.95-0.98)</td>
<td>0.82 (0.80-0.84)</td>
</tr>
<tr>
<td>4</td>
<td>0.72 (0.70-0.73)</td>
<td>0.95 (0.93-0.96)</td>
<td>0.78 (0.76-0.81)</td>
</tr>
<tr>
<td>5</td>
<td>0.70 (0.68-0.71)</td>
<td>0.93 (0.91-0.95)</td>
<td>0.75 (0.72-0.78)</td>
</tr>
</tbody>
</table>

Five year relative survival curves are presented in Figs 11 and 12. Subgroup analysis by grade revealed that the T1G3 group had the lowest relative survival, 69%. T1G3 patients had a significantly higher excess mortality compared to both T1G2, and TaG3 patients (p-values <0.001 and 0.009, respectively).
Even though there was a trend in favour of more intensive therapy, there were no statistically significant differences in relative survival between different health care regions. Five year relative survival rates for patients with TaG3, T1G2 or T1G3 tumours by region were:
- Stockholm-Gotland 0.71 (95% CI=0.63-0.79)
- Uppsala-Örebro 0.78 (95% CI=0.70-0.85)
- South Eastern 0.74 (95% CI=0.64-0.83)
- Southern 0.78 (95% CI=0.71-0.84)
- Western 0.80 (95% CI=0.72-0.87)
- Northern 0.76 (95% CI=0.64-0.86)

**Paper II**

Median observation time for the survivors was 123 months (range 46-176). Progression, defined as a shift to a higher stage, occurred in 23% (58) of the cases, in the MMC group 27% (34/125) and in the BCG group 19% (24/125). Among patients with progression median time to progression was 27 months (range 2-171) and is illustrated in fig 13.

![Figure 13](image)

Death from cancer occurred among 76% (26/34) in the MMC group and 79% (19/24) in the BCG group, fig 14.
As is illustrated in fig. 15 later treatment was given to a total of 19% (47/250) of the patients, 26 in the MMC group and 21 in the BCG group (p-value for difference = 0.42). This later treatment was given to eight patients, five in the MMC group and three in the BCG group who had been recorded as not progressing. The most common later treatment was cystectomy in the MMC group, 13 cases, and radiation therapy in the BCG group, 10 cases. At the time of the present evaluation the majority of patients, 56% (140), were dead: 32% (45/140) from urinary bladder cancer and 68% (95/140) due to other causes. Death from cancer in patients undergoing crossover treatment was 22% (13/60) while it was 17% (32/190) in those who did not cross over, fig 16. The curves for overall and cancer specific survivals including log rank analysis are illustrated in figs 17 and 18. As is shown in the two figures the survival curves cross and no differences in impact on survival between the agents can be seen.
Figure 15.

Figure 16.
Figure 17.

Figure 18.
Paper III

Inclusion:
A total of thirty-two patients were included at five centres from January 2002 to March 2004 and none were lost to follow-up. Due to recruitment problems, the study was terminated prematurely. Two patients had to be excluded due to protocol violation. Among the remaining 30 patients the mean age was 67 years (45-85) and the gender distribution was 23 male and 7 female. Fourteen patients were G1 and sixteen were G2.

Response:
All but one patient were evaluable regarding efficacy. The complete response rate over all was 31% (9/29). The corresponding rate per arm was in the single dose arm 10% (1/10), twice weekly 40% (4/10) and once weekly 44% (4/9). Increased tumour was found in 38% (11/29). All these patients had new tumour occurrences and in two cases there was also marker tumour enlargement.
The distribution of response is illustrated in table 9 and figure 19.

<table>
<thead>
<tr>
<th>Distribution of response per dose schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>response</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>CR</td>
</tr>
<tr>
<td>NR</td>
</tr>
<tr>
<td>IT</td>
</tr>
<tr>
<td>NE</td>
</tr>
<tr>
<td>sum</td>
</tr>
</tbody>
</table>
Toxicity:

All patients were evaluable regarding toxicity. A total of eight patients reported toxicity, see Table 11; all but one in the multiple dose groups. The latter had pruritus and asthenia. The most common side effect was nausea (n=5). One patient in the 2 doses per week group had to stop after 4 instillations due to nausea and fever (>39°C). She was evaluated as NR at follow-up. One patient in the once weekly group had to delay instillation one week due to mild thrombocytopenia. Mild reversible anaemia developed during treatment of one patient in the twice weekly group. No patients were excluded due to pathological changes in haematological or other laboratory parameters. Females were overrepresented with regards to toxicity, 4 out of 7 included. Ten patients were unable to retain the drug intravesically for the whole hour. Spasmolytic medication was used in 8 cases.
Table 10. Patients with side effects arranged according to gender, age, grade of side effect (NCI-CTC) and response

<table>
<thead>
<tr>
<th>gender</th>
<th>age</th>
<th>fever</th>
<th>nausea</th>
<th>diarrhoea</th>
<th>headache</th>
<th>asthenia</th>
<th>pruritus</th>
<th>thrombocytopenia</th>
<th>anaemia</th>
<th>response</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>47</td>
<td>(2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CR</td>
</tr>
<tr>
<td>male</td>
<td>67</td>
<td>(1)</td>
<td>(2)</td>
<td></td>
<td>(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CR</td>
</tr>
<tr>
<td>male</td>
<td>73</td>
<td>(2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NR</td>
</tr>
<tr>
<td>male</td>
<td>79</td>
<td></td>
<td>(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PD</td>
</tr>
<tr>
<td>female</td>
<td>62</td>
<td>(2)</td>
<td>(2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NR</td>
</tr>
<tr>
<td>female</td>
<td>80</td>
<td>(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CR</td>
</tr>
<tr>
<td>female</td>
<td>80</td>
<td></td>
<td>(1)</td>
<td>(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CR</td>
</tr>
<tr>
<td>female</td>
<td>81</td>
<td>(1)</td>
<td>(2)</td>
<td>(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PD</td>
</tr>
</tbody>
</table>

Paper IV

HercepTest® Staining and HercepTest Score (≥10% stained tumour cells)

HER2 staining was positive in 55% of the primary tumours and in 40% of the metastases when the HercepTest® staining and score criteria were applied. Furthermore, 34% of the patients with positive primary tumours had negative metastases. In 8% the metastases were positive even though the primary tumours were negative. In 78% the primary tumours and the metastases were similar (0/1+ or 2+/3+ in both). The results are summarised in table 11.

Table 11. HercepTest scores for the analysed primary tumours and the corresponding metastases (n=86). Standardised HercepTest staining and analysis

<table>
<thead>
<tr>
<th>Primary tumour HercepTest scores</th>
<th>Metastases HercepTest scores</th>
<th></th>
<th></th>
<th></th>
<th>Σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
<td>0</td>
</tr>
<tr>
<td>1+</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>2+</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>3+</td>
<td>3</td>
<td>3</td>
<td>10</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>Σ</td>
<td>45</td>
<td>7</td>
<td>20</td>
<td>14</td>
<td>86</td>
</tr>
</tbody>
</table>
HercepTest® Staining and Target Score (≥2/3 stained tumour cells)
HER2 staining was positive in 45% of the primary tumours and in 37% of the metastases when the HercepTest® staining and the Target score criteria were applied. Thus, the positive cases were somewhat lower than when the HercepTest score was applied. About 36% of the patients had negative metastases despite positive primary tumours and in 5% the metastases were positive even though the primary tumours were negative. In 76% the primary tumours and the metastases were similar (0/1+ or 2+/3+ in both). The results are summarised in table 3.

Table 12. Target scores for the analysed primary tumours and the corresponding metastases (n=86). Target score analysis based on HercepTest staining.

<table>
<thead>
<tr>
<th>Primary tumour Target scores</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>∑</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>38</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>45</td>
</tr>
<tr>
<td>1+</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2+</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>3+</td>
<td>4</td>
<td>2</td>
<td>9</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>∑</td>
<td>50</td>
<td>4</td>
<td>18</td>
<td>14</td>
<td>86</td>
</tr>
</tbody>
</table>

MH Staining and Target Score (≥2/3 stained tumour cells)
HER2 staining was positive in 79% of the primary tumours and in 62% of the metastases when the MH staining and the Target score criteria were applied. Thus, the positive scores were higher when MH instead of HercepTest® staining was applied. About 27% of the metastases were negative despite the fact that the primary tumours were positive and in 21% the metastases were positive even though the primary tumours were negative. In 74% both the primary tumours and the metastases were similar (0/1+ or 2+/3+ in both). The results are summarised in tables 13 and 14.

Table 13. Target scores for the analysed primary tumours and the corresponding metastases (n=90). Target score analysis based on MH staining.

<table>
<thead>
<tr>
<th>Primary tumour Target scores</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>∑</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>1+</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2+</td>
<td>6</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>3+</td>
<td>9</td>
<td>2</td>
<td>6</td>
<td>36</td>
<td>53</td>
</tr>
<tr>
<td>∑</td>
<td>28</td>
<td>6</td>
<td>14</td>
<td>42</td>
<td>90</td>
</tr>
</tbody>
</table>
Table 14.

<table>
<thead>
<tr>
<th>Description</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tumours with 2+ or 3+</td>
<td>72/90</td>
<td>≈ 80%</td>
</tr>
<tr>
<td>Metastases with 2+ or 3+</td>
<td>56/90</td>
<td>≈ 62%</td>
</tr>
<tr>
<td>Patients which had 0 or 1+ in their primary tumours and remained so in their metastases</td>
<td>14/18</td>
<td>≈ 78%</td>
</tr>
<tr>
<td>Patients which had 2+ or 3+ in their primary tumours and remained so in their metastases</td>
<td>52/72</td>
<td>≈ 72%</td>
</tr>
</tbody>
</table>

**Sentinel and Regional Lymph Nodes versus Distant Metastases, MH Staining and Target Score (≥2/3 stained tumour cells)**

In regard to the location of metastases, the frequency of cases with HER2 positivity decreased with distance from the primary tumour. Thus sentinel nodes were positive in 90% while regional nodes as a group were positive in 74%. The corresponding fraction for distant metastases was only 47%. See Table 15.

Table 15. Target scores for the analysed primary tumours and the corresponding metastases as a function of the type of metastases. The target score analysis was based on MH staining. 0 and 1+ scores were pooled as well as 2+ and 3+ scores.

**Sentinel nodes vs distant metastases and regional vs distant metastases, p <0.05: chi-square.**

<table>
<thead>
<tr>
<th></th>
<th>Primary tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0/1+</td>
</tr>
<tr>
<td>Sentinel lymph node metastases</td>
<td></td>
</tr>
<tr>
<td>0/1+</td>
<td>0</td>
</tr>
<tr>
<td>2+/3+</td>
<td>0</td>
</tr>
<tr>
<td>Regional lymph node metastases (including sentinel nodes)</td>
<td></td>
</tr>
<tr>
<td>0/1+</td>
<td>5</td>
</tr>
<tr>
<td>2+/3+</td>
<td>1</td>
</tr>
<tr>
<td>Distant metastases (except genital tract) including distant lymph nodes</td>
<td></td>
</tr>
<tr>
<td>0/1+</td>
<td>9</td>
</tr>
<tr>
<td>2+/3+</td>
<td>2</td>
</tr>
</tbody>
</table>
General Discussion

The conducted studies included in this thesis are concerned with the epidemiology, current and possible future treatment of urothelial cancer of the urinary bladder. We explore how the clinicians use the current therapies and the outcome on a national and regional level. Since the registry has only been running since 1997 a long term analysis on the efficacy of the two most common therapies for non muscle-invasive tumours is performed in the second paper. In search of further improvements, a new drug is used in the established treatment tradition of repeated intravesical instillations. Finally we leave the field of traditional concepts altogether and turn towards an entirely new treatment modality, the radionuclide target therapy.

Non muscle-invasive bladder cancer is much more common in men. In the studied Swedish population about 75% of the Ta and T1 patients were men. However; this proportion might change in the future, since cigarette smoking, one established risk factor for bladder cancer, has decreased among men in Sweden and is now equally prevalent between men and women [29].

The incidence of both Ta and T1 tumours peaks in the age range 70-79 years, and the age distribution is similar to previous reports [30]. We observed a tendency towards an increasing number of T1 tumours with increasing age. This might be due to a delay in diagnosis among older individuals [31].

Our study revealed considerable differences between health care regions with respect to the reported initial additional treatment given to high risk patients (TaG3, T1G2 and T1G3 patients). However, one limitation of these data and analysis could be that registration of some of the treatments might be missed, since only treatments during the first three months are reported. This implies that, if due to a late second look resection a patient receives BCG treatment after four months then the intravesical therapy will not be registered. The restricted three month time span for reporting initial treatment was originally introduced to detect any possible delay with the intention to elucidate the quality of the care of bladder cancer patients in the Swedish health care system [32]. The Stockholm-Gotland region has deviated from this time limit, reporting initial treatments within six months after diagnosis. This means that this region is not fully comparable with the other regions. Still, despite the three additional months, the Stockholm-Gotland region is at the lower end of the range of reported additional treatments.
among the six regions. The restriction on reporting treatment within three months has been debated, and as of the year 2002 all regions will use the six month time limit. Moreover, the increased use of computerised patient data management in Swedish hospitals will hopefully make it possible to simplify the recording process for the individual urologist, allowing the introduction of registering a one year treatment follow-up period.

Transurethral resection as single treatment of Ta-T1 tumours was the major therapy, especially in the South-East region. Many Swedish urologists seem to consider that tumours reported as TaG3 are more serious than T1G2 tumours, as observed in the determination of additional treatment. This could possibly be from fear of undertreating some T1G3 tumours mistakenly understaged as TaG3 tumours.

Adherence to national and international guidelines for patients with TaG3, T1G2, and T1G3-tumours seems to be increasing as the number of patients treated with a more aggressive approach increased during the study period. Elderly patients were treated less actively with intravesical therapy. The reason might be that they were considered unfit to endure the side effects of such treatment.

Moreover, there have been improvements in treatment procedures, such as early post operative instillations of chemotherapy [33], improved instruments for resection with video support and fluorescence cystoscopy, and maintenance BCG. All of these measures should hopefully be helpful to improve the prognosis of bladder cancer. Since non muscle-invasive bladder cancer patients constitute more than 2/3 of all bladder cancer, an improvement in survival for that group should affect the overall survival in bladder cancer patients per se. The 5-year relative survival of all 9589 patients in the register 1997-2001, 70%, is unfortunately not better than the 5-year relative survival of bladder cancer patients in the Swedish Cancer Registry between 1980 and 1984[34], also 70%. One reason for this might be that with a follow-up period for the patients in our study of only 5 years the observation time may be too short to identify a possible survival benefit. There may also have been changes in the patient and reference populations that invalidate a comparison between the different time periods.

One of the major known risk factors for bladder cancer, tobacco smoking, is also associated with other severe diseases that have a relatively high mortality rate. This might lead to an underestimation of the relative survival rates for bladder cancer when using the general Swedish population as the comparison group. Patients with low grade Ta tumours (TaG1 and TaG2) are known to have very low disease specific mortality, and these patients might be more comparable to other bladder cancer patients regarding unmeasured confounding factors than the general Swedish population. In a forthcoming study we plan to calculate expected survival of TaG3 and T1 patients using individuals with low grade Ta tumours as comparison population.
There was a tendency to a lower relative survival in the region with the lowest reported frequency of intravesical therapy. However, the inherent heterogeneity in tumour classification makes it difficult to compare survival across the health care regions.

Patients with Ta tumours have, as expected, a better prognosis than patients with T1 tumours. Relative five year survival was very similar for patients with TaG3-and T1G2-tumours (82%). Since a higher proportion of TaG3 patients received additional treatment compared to T1G2 patients, one might possibly expect a larger improvement in survival in TaG3 patients in the future.

In conclusion, this first study provides an insight into the management of Ta T1 tumours in Sweden. The outcome of the slight but promising increase in the use of additional treatment, possibly reflecting the impact of published guidelines, will be evaluable in the future using this unique continuously updated register.

Meta-analyses of studies comparing BCG with other types of treatment have concluded an advantage for BCG regarding time to recurrence. The impact on progression and survival has been under discussion as one recent metaanalysis comparing BCG to other treatments (including no treatment) found an advantage for BCG [12] while other analyses did not. The selection of studies included in the analyses differs and the follow-up times are short. One meta-analysis discusses uncertainties regarding patients who had previous treatment with chemotherapeutic drugs, possibly rendering them refractory to further chemotherapy and hence affecting the outcome negatively for the chemotherapy arm of a study [10]. A comparison of patients previously treated with neither BCG nor chemotherapy would be preferable. In the second paper in this thesis, our long term follow-up could not prove any advantages for BCG. The number of events for progression was lower than would have been desired from a statistical point of view. This was also partly true for disease specific survival. The guidelines published reflect the conclusions drawn by the respective key researchers involved based on the publications available at the time of writing. The latest guideline, by the SIU group, does recommend BCG in T1 tumours but sets the level of evidence at a relatively low C.

Conservative treatment with instillations or up front cystectomy in high grade T1 is still a matter of debate and as long as we do not have more accurate prognostic tools we will have to accept that concomitant CIS, multiplicity of tumours and failure to respond to primary intravesical treatment are cause to discuss cystectomy with the patient. This issue was addressed in a recent paper by Sylvester where outcome data from 2596 patients from previous EORTC studies were pooled and a scoring system was set up separating four different risk groups for recurrence and four for progression [35].
For Carcinoma in situ there seems to be less controversy with maintenance
BCG as the recommended treatment.

Both the treatments with BCG and MMC have undergone modifications
since our study was designed. For BCG the importance of maintenance
treatment has been shown and dose reductions have been tried to reduce side
effects. An optimization of the concentration and procedure for instillation of
MMC has been proven efficacious [36]. Further possible improvements for
current drugs include thermo-chemotherapy and electromotive drug admini-
stration [37].

If the described measures bring out the best in the currently available
drugs and the cure rate remains less than 100% a move to new drugs seems
necessary. The third paper reflects such a step involving gemcitabine which
has already been proven in advanced disease. The once weekly dose sched-
ule did not seem to be less efficient compared to the twice weekly one and
has so far been the one used in published studies (see the next section on
methodological considerations). So far no studies have reported on serious
adverse events or side effects but one has to bear in mind that this category
of patients may not tolerate what would be considered modest problems in
patients with advanced disease. The patient who was withdrawn in our study
in accordance with the study protocol did not reach over Grade II on the
NCI-CTC scale. Whether or not gemcitabine is better than current drugs is
not possible to conclude without Phase III studies with long follow-up times.

Our previous finding of a tendency towards lower HER2 expression with
increasing distance from the primary tumour [23] was confirmed in the pre-
sent study with a shift from 89% down to 47% when comparing sentinel
nodes and distant metastases. The differences were statistically significant.
The reason for this is not clear. HER2 overexpression can be regarded as
overexpression of an oncogene product. Loss or a decrease in HER2 expres-


We will be further discussed in the future prospects section.
Methodological Considerations

Paper I

The purpose of The Swedish National Quality Registry for Bladder Cancer was to study the epidemiology and management of patients with bladder cancer in different health care regions with the aim of improving the care of the patients. Due to the unique personal identification numbers that everyone has in Sweden, information about deaths can be identified by the Swedish census. This makes it possible also to study outcome. The coverage of the registry averages at 94% and analyses of patients who were not registered has not revealed any systematic deviations regarding age, sex, or outcome. The registry provides an excellent database to study different factors in a large number of patients.

Any undertaking of epidemiologic studies in non muscle-invasive urinary bladder cancer is fraught with the risk of validity problems. Not only the urologists but also the pathologists must agree on which classification system to use and how to interpret the individual cases. The data must then be reported in an orderly manner and registered at the respective Regional Oncological Centre before they can be analysed at the national level. Studies on interobserver variation between pathologists have shown reviewer agreement with the original report in only 68.5 and 62.3% for Ta and T1 specimens [38]. Histological examination after cystectomy in T1 cases has also revealed considerable clinical understaging of up to 40% [39].

The variation in reported tumour grade between health care regions can be attributed to the use of different grading systems between pathology laboratories (see introduction) and also to variation between individual pathologists. This issue was addressed by migrating to a common grading system [4, 38] in 2003 within Sweden. A recent study looked at the observation that a small number (108 cases; 1.6%) of patients were reported to have T1G1-tumours. That study showed that all of these cases were misclassified and could be placed in other groups, mainly Ta (69%). The remaining tumours were either upstaged or upgraded or both [40]. The quality register working group has performed a number of other validity studies and concluded that urologists and pathologists should intensify their cooperation and that the reports in patients with aggressive tumours should always be reviewed [41]. Moreover, familiarity with the TNM-system and a correct staging of the TUR specimen are necessary components of the classification process of bladder tumours.

The data from the national data base do not differ extensively in comparison with smaller but more in depth analyses performed in Sweden (table 17). A subdivision of the T1 category for the presence of concomitant CIS would have been an advantage and may be considered in the future registration. All this taken into account the database represents a unique opportunity to fol-
low the treated and untreated natural history of the disease without the risk of the selection bias adherent to the clinical study setting.

Table 15. *Three recent epidemiological studies from Sweden[42, 43]*

**Recent Studies on Non Muscle-invasive Bladder Cancer in Sweden**

<table>
<thead>
<tr>
<th></th>
<th>Holmäng et al(ref)</th>
<th>Larson et al(ref)</th>
<th>Sw Blca Reg</th>
</tr>
</thead>
<tbody>
<tr>
<td>nr of years</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>nr pat included</td>
<td>701</td>
<td>538</td>
<td>9859</td>
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Paper II
The evaluation of long term outcome in the treatment of non muscle-invasive disease in a study such as is presented by our group can be complicated by several factors.

A clinically useful choice of endpoints must be made with minimal confounding. The standard set including overall survival and progression remain of interest but may not capture events influencing the therapeutic choices made. A persistent T1 tumour may lead to cystectomy even though no progression as defined has occurred. Lamm et al took this into account when they evaluated the endpoint “worsening free survival” [44]. We chose to address this by reporting later treatments. Overall survival is an important measure of treatment efficacy but poses a problem in the bladder cancer patient group. Due to the old age of the patients at diagnosis, frequent deaths occur due to intercurrent disease. Thus natural censoring will limit the number of possible events for both progression and death due to the cancer. Bearing in mind the potential pitfalls of the uncertain validity of death certificates the evaluation of cancer specific survival still seems justified. We chose to report both overall and cancer related death for reasons of clarity.

Potentially small differences in outcome mean that large numbers of patients need to be recruited in order to attain statistical significance. In the future a meta-analysis of more studies with long term follow-up, including more of the endpoint events, would be desirable. In the present study we did not find a difference between the two original treatment groups regarding survival or progression; the Kaplan Meier curves overlap.

The problem with failure to control for previously administered chemotherapy has been addressed by Huncharek et al [10]. The potential risk of comparing patients with resistance to chemotherapy with BCG-naïve ones was discussed. This kind of “early contamination”, previous chemotherapy, was present in six per cent (14/250) of the patients in our study, a proportion that we consider too low to affect outcome and too few for a subgroup analysis. A meta-analysis of several trials would be necessary to address this problem. In our study an option to cross over to the alternative treatment was available. This could be described as “late contamination”, diluting the results. The reason for the potential for cross-over was the ethical dilemma of introducing a new treatment. At the time of designing the original study treatment with BCG was considered promising but it was not established in Sweden or Norway. There was concern that this might affect the recruitment for the study. MMC patients might feel that they did not receive a new and potentially more effective treatment and BCG patients might feel that they were subjected to an experiment with a useless drug instead of the proven one.
Paper III

The choice of the marker lesion concept as previously described[15] seemed natural for this phase II study since we wanted to gain information on the chemoablative properties of gemcitabine in a short time in few patients rather than making a complete resection in order to assess the prophylactic effect. We chose the Ta category, omitting the T1, for safety reasons in that it conferred less risk of systemic progression if the drug proved ineffective.

The pattern of toxicity in our study indicates that some systemic uptake must have taken place but apart from nausea that led to one drop out, the frequencies are obviously lower than when administered intravenously and less severe. Unfortunately the occurrence of toxicity was not accompanied by an increase in the chemoablative effect. The higher incidence of side effects in women (four out of seven included) warrants further study of gender specific uptake and metabolism. The generally thinner bladder walls in females compared to males could be the main reason for this finding.

Of the previously published studies on intravesical gemcitabine[14, 45-49] one has reported on the efficacy of 6 weekly doses using the marker lesion concept. The study which included thirty-nine patients reached a CR rate of 56% and no progression was seen among the non-responders [46]. We were not able to reproduce these results. One difference was that we only included Ta patients. To find out if the sensibility to gemcitabine increases with the de-differentiation of the tumour more patients would be needed, including cases with grade 3 tumours.

Another difference that may be of importance was that we used a lower concentration of 20 mg/ml (100 mls), as recommended at the planning of our study, rather than 40 mg/ml (50 mls) [46]. The fact that we experienced more toxicity than reported previously is however puzzling. Could the greater volume affect the uptake systemically without a benefit in antitumoral efficacy? The relatively large volume may also explain why 10 patients had shorter dwell times than planned.

Since the publication of our study several others have been published confirming a solid foundation for phase III studies [50-55].
Only one other group has reported results comparing primary urinary bladder tumours with its corresponding metastases [56]. Of 60 metastatic cases, 39 patients had evaluable histological material from both locations. The conclusion was that overexpression in the primary tumour consistently predicts overexpression in distant or regional metastasis but also that HER2 negative primary tumours may show overexpression in their corresponding metastasis (45% of lymphoglandulae and 67% of distant metastases). A direct comparison between that and the present study is difficult. The immunohistochemical retrieval and staining protocols were different in that the Ventana immunostainer, a steamer, antibody dilution 1:200 and Aminoethylcarbazole were used rather than the DAKO Autostainer, a decloacing chamber, dilution 1:300 and Diaminobenzidine, as in the present study. It is our opinion that this combination has a higher sensitivity. Furthermore, the scoring methodology in the study by Jimenez et al[56] was clear regarding intensity but less clear regarding the quantity of stained cancer cells.

It is hence worth noting that reported variations in HER2 expression may not only be a result of true biological variations but also reflect differences in the immunohistochemical staining protocols [23, 24, 57]. We applied two immunohistochemical staining procedures and found that there were more positive cases for both primary tumours and metastases using the modified HercepTest® MH, than when applying the standard procedure. The HercepTest® kit is usually employed to decide whether a breast cancer patient should be treated with trastuzumab or not. This is the procedure approved by the FDA and accepted as a standard method. Our results indicate that transitional cell carcinoma from the urinary bladder may need a modified protocol.

Variations in reported HER2 expression might also be due to differences in scoring criteria. The HER2 evaluation criteria applied for breast cancer (≥10% stained tumour cells) were compared to the Target score criteria (≥2/3 stained tumour cells). The breast cancer criteria are sometimes also referred to as "HercepTest score" since they are part of the FDA approved test kit previously mentioned. The Target score criteria were developed in relation to targeted radionuclide therapy since it is assumed that most tumour cells must bind the radiolabelled targeting agent to give a good treatment effect. The Target score criteria gave somewhat fewer positive primary tumours and metastases when applied to the HercepTest® stained slides while the combination of MH staining and Target score resulted in an increased number of HER2 positive cases. There were over 50% more HER2 positive cases for both primary tumours and metastases when the combination of the MH staining and Target score was compared to the combination HercepTest® staining and HercepTest score. The fact that not all antibodies are able
to identify all protein variants from a gene product was minimised as much as possible by our use of polyclonal antibodies.

Regarding FISH analysis, which is frequently used in the diagnosis of breast cancer, a poor correlation between gene amplification and receptor overexpression has already been reported for urinary bladder transitional cell carcinoma [58]. Since our primary goal was to analyse overexpressed receptors for possible treatment with HER2 targeting agents we used the immunohistochemical analysis.
Future Prospects

Will urologists in Sweden respond to recommended changes in management and therapy of urinary bladder cancer? Will these changes have an impact on survival? The registry is now active and as time goes by the possibility to draw conclusions regarding these questions will increase. The importance of continuous feedback to the registering clinicians cannot be overemphasised as they are the very foundation of a working quality assurance system. The working group in charge of the registry will continue to publish annual reports for feedback and to update the registry procedures.

For the choice of therapy in non muscle-invasive disease the established drugs have their respective advantages and disadvantages. The superiority of BCG in recurrence and progression will have to be reconfirmed in new meta-analyses of studies with long follow-up times of high risk patients.

More accurate prognostic tools must be made available so that the decision on therapy can be made based on firmer evidence by the patients and urologists. A classification by microarray expression profiling can possibly be a step in that direction [59].

Any major breakthrough for new drugs must come via properly performed Phase III studies including an adequate number of patients. For immediate postoperative instillation studies have already been started [60]. A study comparing gemcitabine with Mitomycin-C for repeated instillations would be of great interest, but due to the new rules and regulations for the performance of clinical studies it would be very costly and may not be attainable.

Targeting therapies in advanced urinary bladder cancer have so far followed the path of what has been done in breast cancer. Combination therapy with paclitaxel, carboplatin and gemcitabine has been tried in a multi-centre phase II NCI trial by Hussain et al and the results were presented at the ASCO meeting in 2005. An overall response rate of 70% was reported including complete response in 11%. A small study with six patients by Peyromaure et al concluded that Trastuzumab may be safe and effective in the treatment of advanced urinary bladder cancer [61].

The concept of targeting the HER2 overexpressing cancer cells with radionuclide carrying antibodies or affibodies, which are smaller, is currently under exploration at the department of Biomedical Radiation Sciences at Uppsala University. A recent study has shown that astatinated trastuzumab is a promising candidate [62].
Another antibody that is being considered as a carrier of radionuclides is pertuzumab, Omnitarg, which acts as a receptor dimerization inhibitor. In preliminary trials it has been successfully coupled with $^{177}$Lu as shown in figure 21[63].

*Figure 20. Targeting cancer cells with radionuclides, the concept*

*Figure 21. An example of HER2-targeting: Gamma camera images of mice injected with $^{177}$Lu pertuzumab 3 days post injection. Note the extensive uptake in the tumours. The mice contours were obtained from digital camera images collected at the time of gamma camera analysis[63]. Published with kind permission of Springer Science and Business Media.*
In the future treatment of muscle-invasive disease the patients may be examined for overexpression of HER2 in the primary tumour and if so systemic treatment with targeted radionuclides may be used in conjunction with a cystectomy and removal of local lymph glands to eradicate the disease in one swift strike. If metastases are found at initial diagnosis the current data suggest that the chances for HER2 expression are good if the primary tumour overexpressed the receptor. Since it may be assumed that the treatment will be expensive an examination of biopsies from the metastasis will probably be performed just to make sure.

While waiting for this possibility the concept may be used at the diagnostic stage. The sentinel node concept concerns finding the lymph glands draining the tumour and hence first in line for invasion. So far the methods to visualise these particular glands have not been able to answer whether or not they are invaded by cancer cells, just their location. A tumour specific antibody carrying detectable radiation could add this information.
Sammanfattning på svenska

Avhandlingen berör fyra aspekter av urotelial cancer i urinblåsan och går från ett makro- till ett mikroskopiskt perspektiv innefattande en utvärdering av såväl etablerade terapier som möjliga nya behandlingsmetoder och principer.

Arbete 1


Arbete 2

Bacillus Calmette Guerin (immunstimulerare) och Mitomycin-C (cytostatikum) används vid intravesikal behandling av icke muskelinvasiva tumörer av högrisktyp i enlighet med publicerade rekommendationer. Arbetet utvärderar 10årsresultaten av behandling med dessa medel hos 250 patienter (125 behandlade med endera läkemedlet efter randomisering) avseende överlevnad, övergång till mer framskriden sjukdom och behov av senare behandlingar. Majoriteten av patienterna var avlidna, de flesta av andra orsaker än bläscancer. Inga skillnader avseende överlevnad, utveckling till mer framskriden sjukdom eller senare behandlingsbehov kunde påvisas mellan de båda medlen.
Arbete 3

Behovet av effektivare medel för behandling av urinblåsecancer bekräftades i de tidigare arbetena och i detta tredje arbete prövades ett nytt cytostatikum, gemcitabine för intravesikalt bruk. Studien var upplagd som en randomiserad Fas II studie med användande av markörtumörkonceptet. Detta innebär att en väldefinierad tumör lämnas kvar i samband med den första resektionen och att effekten av den nya drogen givet intravesikalt på denna markörtumör utvärderas vid en andra operation. Totalt inkluderades 32 patienter i studien och randomiserades till ett av tre behandlingsschemata: endast en dos, två doser per vecka till sex doser eller en dos per vecka till sex doser. En patient kunde ej utvärderas avseende effekt och två ströks pg av protokollavvikelser. Fullständig remission uppnåddes i 31 % (9/29) av fallen. Endosregimen tycktes inte effektiv. Biverkningarna var överlag milda; vanligast var illamående. Slutsatsen blev att gemcitabine upphävsade en antitumoral effekt givet vid upprepade instillationer och att biverkningsgrader var låg.

Arbete 4

Spridning av cancer från urinblåsan, metastasering, är den huvudsakliga orsaken till dödlig utgång och detta avhandlas i det fjärde arbetet. Med syftet att utvärdera möjligheten att målsöka mot en spridd tumörsjukdom undersöks uttrycket av den epidermala tillväxtfaktorn HER2s receptor på cellytan av såväl primärtumör som metastaser av urinblåsecancer. Både regionala lymfkörtelmetastaser och mer perifera metastaser undersöktes. Graden av receptoruttryck jämfördes mellan de olika lokalerna dels med två olika vävnadsbehandlingsmetoder men också med två olika utvärderingsprotokoll. En statistiskt signifikant nedgång i receptoruttryck sågs med avstånd till primärtumören. Sett till alla regionala metastaser befanns uttryck i 74 %; för fjärrmetastaserna var siffran 47 %. Av dem som uttryckte HER2 i primärtumören uttrycktes receptorn också i metastasen i 72 %. Slutsatsen blev att en förbättrad metod för karaktärisering av vävnadsuttryck i kombination med ett protokoll anpassat för framtida målsökande strålbehandling mot urinblåsecancer tycktes bättre än de metoder som idag används vid behandling av bröstcancer.
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References


11. Shelley, M.D., T.J. Wilt, J. Court, B. Coles, H. Kynaston, and M.D. Mason, *Intravesical bacillus Calmette-Guerin is superior to mitomycin C in reducing tumour recurrence in high-risk superficial*


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