Chapter 1: Background

1.1 Bladder Cancer Incidence and Etiology

Bladder cancer (BC) is the fifth most commonly diagnosed malignancy in the United States, with more than 70,000 new cases and more than 14,000 BC deaths reported in year 2009 [1]. The median age at diagnosis is 72 years for men and 73 years for women [2]. BC is three times more common among men than among women and is twice as common among whites as among African-Americans (AAs) [3]. The estimated lifetime risk of BC ranges from 0.5% for AA women to 3% for white men [4]. The causes of these disparities are not known, although animal studies suggest that the gender difference in incidence may be related to hormonal factors [5].

Despite lower incidence, women and AAs with BC are more likely to die from their disease when compared to white men. The origins of this phenomenon are not fully understood. Although women and AAs tend to present with more advanced stages and more aggressive tumor types, differences in age and tumor characteristics at presentation do not fully explain the excess hazard of death from BC in these demographic groups [2].

Established risk factors for development of BC include cigarette smoking, occupational exposure to certain chemical compounds, history of therapeutic radiation to the pelvis, exposure to a chemotherapeutic agent cyclophosphamide, and chronic bladder inflammation [4,6-18].
Cigarette smoking appears to be the most important risk factor. The risk of BC among smokers is two to four times greater than the risk among the non-smokers, with evidence of a dose-response relationship [6-9]. It has been estimated that at least 50% of BC cases among smokers might not have occurred if these individuals had not smoked [4]. Smoking cessation substantially reduces the risk, but the former smoker’s risk remains higher than the risk of a person who never smoked [9]. Among various chemical compounds present in cigarette smoke, polycyclic aromatic hydrocarbons, 4-amino-biphenyl, and unsaturated aldehydes have been identified as bladder carcinogens [10-12].

There are no known dietary factors with a clearly established causal link to BC, although there is some evidence that consumption of fruits may have a protective effect [7]. Artificial sweeteners are believed to confer little or no excess risk of BC, although initial studies suggested an association [4].

Among occupational risk factors, exposure to β-naphthylamine, benzidine, and 4-aminobiphenyl has been shown to be strongly associated with development of BC [13]. Occupational exposure to these chemicals occurred most frequently in the textile dye and rubber manufacturing industries. In the 1970s, Occupational Safety and Health Administration (OSHA) recognized β-naphthylamine as a human carcinogen and imposed strict regulations on the use of this compound. Similar regulations were applied to benzidin and 4-aminobiphenyl [13]. Although occupational exposure to these chemicals has been nearly completely eliminated in the United States, exposure to other
potential bladder carcinogens (e.g., \( o \)-toluidine) still occurs among workers involved in the manufacturing of dyes, rubber, pharmaceuticals, and pesticides [3,14]. It has been estimated that exposure to bladder carcinogens at workplace may be responsible for as many as 20% of all newly diagnosed bladder cancer cases [15].

Other factors associated with development of BC include history of therapeutic radiation to the pelvis [16], exposure to cyclophosphamide [17], and chronic bladder inflammation. In the Middle East, many cases of BC are attributed to bladder inflammation due to chronic infection with a urinary parasite Schistosoma haemotobium [18]. In the United States, chronic inflammation of the bladder often occurs in patients with spinal chord injury. These patients have an elevated risk of squamous cell carcinoma of the bladder [18].

Although the absolute number of BC cases diagnosed in the US increases each year, this trend is primarily attributed to aging of the population [8]. It is estimated that more than 500,000 men and women in the US currently live with a diagnosis of BC (either active disease or a past diagnosis with subsequent cure) [8].

### 1.2 Diagnosis of Bladder Cancer

#### 1.2.1 Signs and symptoms at presentation

The most common presenting sign of BC is painless hematuria. In a contemporary study of intravesical chemotherapy with over 1,000 participants with BC, 72% had gross hematuria (blood in the urine) as their presenting finding at the time of the initial
diagnosis [19]. Other conditions frequently associated with gross hematuria include kidney stones, trauma, and tumors of the upper urinary tract. All patients presenting with gross hematuria require a complete urologic evaluation [20].

Microscopic hematuria, either persistent or intermittent can be found in virtually all patients with BC if testing is performed on several occasions [21]. However, this finding may also be present in a wide variety of common non-neoplastic conditions affecting the urinary tract, including stones, infection, benign prostatic hyperplasia, and even increased physical activity (exercise hematuria) [20]. In a trial of hematuria home screening conducted in Wisconsin between 1988 and 1992, 1575 men ≥50 years of age tested their urine repetitively with a chemical reagent strip for hemoglobin. Either persistent or intermittent hematuria without obvious explanation (such as a urinary tract infection) was found in 258 (16%) of the study participants. These men underwent a complete urologic evaluation and 21 of them were diagnosed with BC. Hence the positive predictive value of hematuria for BC was 8% in this population [22]. Although most patients with intermittent microscopic hematuria do not have a urologic malignancy, a positive test for blood in the urine without a urinary tract infection or any other immediate explanation should raise a suspicion of BC, particularly for high risk patients such as older male smokers [20].

In addition to hematuria, signs and symptoms of BC may include urinary frequency, urgency, and dysuria (painful urination). Although these symptoms may also be caused by urinary tract infections, benign prostatic hyperplasia, and many other common
conditions affecting the lower urinary tract, in patients with BC they are often associated with locally advanced (muscle-invasive) disease or diffuse carcinoma in situ (flat high grade lesions confined to the bladder surface but with high potential for invasion) [3]. Dysuria may also occur due to physical obstruction of internal urethral orifice by a bladder tumor. Other, less common signs and symptoms of BC may include flank pain due to ureteral obstruction and symptoms of metastatic disease, such as weight loss, bone pain, or pain in any other metastatic site [3].

1.2.2 Cystoscopy

Cystoscopy plays a key role in the diagnosis of BC. During this procedure, a urologist inserts a flexible endoscope into the bladder through the urethra. This allows for visual examination of the lower urinary tract. Cystoscopy is a relatively well tolerated procedure which is typically performed under local anesthesia [23,24].

Most bladder tumors appear on cystoscopy as clearly visible papillary masses. Flat lesions such as carcinoma in situ may appear as erythematous velvety patches [25]. Unfortunately, a substantial proportion of flat lesions as well as some smaller papillary tumors can be missed on conventional white light cystoscopy [26]. This problem led to the development of a technique called fluorescence cystoscopy.

Fluorescence cystoscopy makes use of the fact that intravesical administration of 5-aminolevulenic acid (ALA) induces fluorescence in BC cells when the bladder surface is exposed to blue light. Recently, introduction of a hexyl ester of ALA
(hexylaminolevulinate or HAL) made this technique more practical because the use of HAL instead of ALA significantly shortens the amount of time needed for drug exposure prior to cystoscopy [26].

It is now clear that the use of fluorescence cystoscopy may often lead to detection of clinically important lesions which would be missed if only white light cystoscopy was used. In a recently published case series of 1713 cystoscopies performed in 875 patients, each patient was examined using the white light and the ALA-based procedure. An area of the bladder was biopsied if it appeared suspicious on cystoscopy (either ALA, white light, or both). Of all detected bladder tumors, 92% were located using the ALA and only 76% were located using conventional white light cystoscopy. It follows that 24% of all tumors would be missed if only the white light procedure was used. The benefit of ALA was most noticeable for detection of carcinoma in situ. Of all carcinoma in situ lesions detected in these series, 43% would be missed if patients were examined without fluorescence cystoscopy [27]. One undesirable feature of fluorescence cystoscopy which was observed in this study was a relatively low specificity, particularly in the presence of inflammation. Of all biopsies taken because of positive fluorescence, 29% were false positive (histologically benign). The most frequent histologic finding in these specimens was chronic cystitis [27]. Similar results were reported from other studies [28,29]. Although fluorescence cystoscopy (either ALA or HAL) improves detection of bladder tumors (particularly carcinoma in situ), it also results in some unnecessary biopsies [28,29].
The benefit of better detection seems to outweigh the inconvenience of additional biopsies. Several randomized controlled trials comparing white light cystoscopy to the ALA-based procedure showed that improved tumor detection led to superior patient outcomes, including lower rates of recurrence [26,29-34]. The studies of long-term patient outcomes with HAL are ongoing [34].

Once a tumor or a suspicious area is identified on cystoscopy, it is usually biopsied or excised (although smaller lesions may be destroyed without a biopsy). Biopsy and surgical excision provide pathological specimens which are used to make a definitive diagnosis of BC and to assess important tumor characteristics which determine prognosis and the choice of therapy. These tumor characteristics are reviewed in the next section.

1.3 Prognostic Factors for Bladder Cancer

1.3.1 Stage

Stage of bladder tumors is defined by the depth of invasion into surrounding tissues. Stage appears to be the most important prognostic factor for BC. It also affects the choice of therapy.

Histologically, normal bladder consists of three tissue layers: urothelium, lamina propria, and detrusor muscle (muscularis propria). Urothelium covers the inner surface of the bladder. It is composed of approximately six layers of transitional epithelial (urothelial) cells separated from lamina propria (the middle layer of connective tissue) by the
basement membrane. The outer layer (muscularis propria) is composed of smooth muscle fibers [35,36]. These tissue structures are shown as a diagram in Appendix A.

Staging of BC is based on the Tumor-Node-Metastasis (TNM) system developed by the American Joint Comission on Cancer (AJCC) [37]. The latest edition of this system was published in year 2009 (Appendix A). In BC, stage Ta is assigned to papillary tumors confined to the urothelium without invasion of lamina propria. Flat high grade lesions confined to the urothelium are referred to as carcinoma in situ (stage Tis). Tumors invading lamina propria (but not the detrusor muscle) are staged as T1. Muscle-invasive tumors which do not penetrate beyond the bladder wall are staged as T2. Stage T3 tumors invade perivesical fat, but not the neighboring organs. Bladder tumors involving other organs and/or abdominal or pelvic wall are staged as T4.

Nodal status (N) and distant metastases (M) are coded separately from the T stage (Appendix A). In BC, almost all node-positive and metastatic tumors have a T stage 2 or greater. For prognostic purposes, separate AJCC codes for tumor, nodes, and metastases are often combined in AJCC stage groups (Appendix A).

In clinical practice, the process of BC staging typically involves (1) bimanual examination following biopsy or transurethral resection, (2) microscopic examination of surgical specimens, and (3) imaging of regional and distant sites (to diagnose metastatic disease). Stages assigned based on microscopic examination of the tumor specimen removed by transurethral resection, bimanual examination of the bladder following
endoscopic surgery, and radiographic imaging are called “clinical” stages. Patients
treated with cystectomy (removal of the bladder) are also assigned a “pathological” stage,
which is determined based on microscopic examination of the cystectomy specimen
(including the bladder, the perivesical fat, the lymph nodes, and all other tissue removed
during surgery).

Distant metastases are usually detected by various imaging modalities, such as chest x-
ray, bone scans, and CT or MRI of various organs. The most common sites of distant
metastases include lungs, liver, and bone [37]. Elevated liver enzymes (AST, ALT) may
indicate the presence of metastatic disease in the liver.

The distribution of AJCC stage groups at the time of initial diagnosis by gender and race
is shown in Table 1. This table is based on the SEER data for years 2004 and 2005.
Similar results have been reported previously [2]. The relationship between AJCC stages
and TNM categories is summarized in the footnote to Table 1.
Table 1 Distribution of AJCC stage groups by gender and race, SEER 2004-2005

<table>
<thead>
<tr>
<th>AJCC stage group</th>
<th>White males</th>
<th>White females</th>
<th>Black males</th>
<th>Black females</th>
</tr>
</thead>
<tbody>
<tr>
<td>AJCC 0a/is</td>
<td>10,893</td>
<td>3,412</td>
<td>423</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td>(56%)</td>
<td>(53%)</td>
<td>(48%)</td>
<td>(38%)</td>
</tr>
<tr>
<td>AJCC I</td>
<td>4,530</td>
<td>1,358</td>
<td>216</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>(23%)</td>
<td>(21%)</td>
<td>(24%)</td>
<td>(24%)</td>
</tr>
<tr>
<td>AJCC II</td>
<td>2,078</td>
<td>798</td>
<td>112</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>(11%)</td>
<td>(13%)</td>
<td>(13%)</td>
<td>(15%)</td>
</tr>
<tr>
<td>AJCC III</td>
<td>886</td>
<td>337</td>
<td>46</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>(4%)</td>
<td>(5%)</td>
<td>(5%)</td>
<td>(8%)</td>
</tr>
<tr>
<td>AJCC IV</td>
<td>1,148</td>
<td>486</td>
<td>88</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>(6%)</td>
<td>(8%)</td>
<td>(10%)</td>
<td>(15%)</td>
</tr>
<tr>
<td>Total</td>
<td>19,535</td>
<td>6,391</td>
<td>885</td>
<td>527</td>
</tr>
<tr>
<td></td>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
</tr>
</tbody>
</table>

| Incidence rate (per 100,000 person-years) | 40.4 | 10.1 | 20.9 | 7.1 |

The AJCC stages are derived from the TNM categories as follows: 0a = Ta, N0, M0; 0is = Tis, N0, M0; I = T1, N0, M0; II = T2, N0, M0; III = T3, T4a, N0, M0; IV = T4b, and/or positive nodes and/or positive metastases.

A number of recent studies have suggested that for any given patient, accurate staging of BC may require a consensus opinion of two or more urological pathologists (consensus review). When stage is determined by a single pathologist, interobserver reproducibility may be low, particularly for differentiation between Ta and T1 tumors. For example, in one study, a total of 235 tumors which were originally classified as T1 were re-evaluated by a second urological pathologist. The second pathologist down-staged 35% of these tumors to Ta and upstaged 3% of the tumors to $T \geq 2$ [38]. In another study, 56% of 63 bladder tumors originally staged as T1 were down-staged to Ta and 13% were upstaged to $T \geq 2$ by central histology review [39].
Misclassification of muscle-invasive disease ($T < 2$ vs. $T \geq 2$), although less common than misclassification of non muscle-invasive tumors (Ta vs. T1) is of greater concern because of its impact on the choice of therapy. While muscle-invasive tumors ($T \geq 2$) are usually treated with cystectomy, non muscle-invasive tumors ($T < 2$) typically do not require this radical operation. It has therefore been recommended that muscle invasion be confirmed by at least two pathologists before radical forms of therapy are considered [38].

Table 2 shows the 5-year survival proportions for patients diagnosed with different stages of BC in the geographic areas under SEER surveillance between 1990 and 2000. For the purpose of comparison, the 5-year survival proportion for the subset of the general population with the same age, gender, and racial distribution as the SEER subjects in Table 2 was approximately 80%.

**Table 2 Five-year survival proportions by stage, grade, and cystectomy; SEER 1990-2000 (N=74,707)**

<table>
<thead>
<tr>
<th></th>
<th>Low Grade</th>
<th>High Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>AJCC 0</td>
<td>76%</td>
<td>68%</td>
</tr>
<tr>
<td>AJCC I</td>
<td>67%</td>
<td>58%</td>
</tr>
<tr>
<td>AJCC II</td>
<td>59%</td>
<td>29%</td>
</tr>
<tr>
<td>AJCC III</td>
<td>44%</td>
<td>19%</td>
</tr>
<tr>
<td>AJCC IV M0</td>
<td>27%</td>
<td>5%</td>
</tr>
<tr>
<td>AJCC IV M1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Survival proportions for AJCC stages 0 and I are stratified on tumor grade because grade is a very important prognostic factor for non muscle-invasive (NMI) disease. Stratification of muscle-invasive (MI) tumors (AJCC stage II-IV) on grade is less relevant because all MI tumors are aggressive and the overwhelming majority of them are high grade. Tumor grade will be discussed in greater detail in section 1.3.3.

Note that AJCC stage II and III tumors in Table 2 are stratified on cystectomy. While cystectomy is rarely used as initial treatment of NMI BC, it is considered a standard of therapy for AJCC stage II and III tumors. Nevertheless, many patients with stage II and III BC do not undergo cystectomy either because of short life expectancy due to co-morbidities, or because they choose less aggressive (bladder-sparing) therapy. Cystectomy and other forms of BC therapy will be discussed in detail in section 1.4.

The AJCC stage IV tumors in Table 2 are stratified on distant metastases because survival of patients presenting with M1 disease is much worse than survival of patients with stage IV BC without distant metastases at presentation (M0/MX). Although prognosis of patients with stage IV M0/MX disease may be influenced to some degree by the T sub-stage (T2 vs. T3a vs. T3b), all patients with stage IV BC (even those without direct extravesical extension, that is, T2 N+) have a relatively poor prognosis (5-years survival <40%) [40,41]. In addition, presence of residual tumor at surgical margins is associated with a very poor prognosis independently of the T stage and the nodal status [41].
1.3.2 *Cell type*

In the United States, more than 95% of all bladder cancers are classified as urothelial carcinoma (UC). Of the remaining tumors, the majority are squamous cell carcinoma (<3%), or adenocarcinoma (<2%). Other cell types are very rare (<1%) [42]. A common feature of all non-urothelial bladder cancers is their tendency to present at very advanced stages. In a recent review of 1422 cases of squamous cell carcinoma (SCC) diagnosed in the United States and reported in SEER between 1988 and 2003, more than 85% of the tumors were muscle-invasive (AJCC stage $\geq 2$) at the time of the initial diagnosis. For comparison, only 22% of urothelial carcinomas diagnosed in the area under SEER surveillance during the same years were muscle-invasive at presentation [43]. Furthermore, of all non-MI SCCs, three quarters invade lamina propria. In contrast, invasion of lamina propria is present in less than one third of non-MI UCs (Table 1).

This highly unfavorable stage distribution of SCCs has been reported from other case-series and similar stage distribution has been reported for adenocarcinoma of the bladder [44-46]. It also appears that even with stage adjustment, SCC may be more aggressive than urothelial carcinoma, particularly among patients treated with bladder-sparing therapy [43]. The biological reasons for such aggressive behavior of non-urothelial tumors are not known.

In some parts of the world, particularly in the Middle East, SCC of the bladder represents a substantial proportion (more than 50%) of all bladder cancers [47]. The high incidence
of SCC in these regions is explained by the high prevalence of chronic infection with a urinary parasite Schistosoma haematobium which has been strongly associated with the development of SCC [18,47].

Although pure squamous tumors of the bladder are rare in the rest of the world, squamous differentiation co-existing with malignant urothelial histology is a fairly common condition observed in a substantial proportion of muscle-invasive BCs, although the prevalence may vary by geographic location [48-52]. In a recent study from the US, 16% of 243 muscle-invasive BCs had squamous differentiation in addition to malignant urothelial component [52]. Squamous differentiation is much less common in tumors invading lamina propria (T1) and is very rare in superficial (Ta) BC [52]. Urothelial carcinoma may also include a glandular component, although this is slightly less common than squamous differentiation. At this time, the prognostic role of non-urothelial histology in urothelial carcinoma remains uncertain. Existing evidence will be reviewed in detail in section 3.1. In the current urological practice, urothelial tumors with non-urothelial component are treated as pure UC [34].

1.3.3 Grade
Grading is a method by which pathologists evaluate cytologic features and growth pattern of tumor cells in an attempt to predict the clinical course of disease. The current grading system for urothelial tumors was developed by the International Society of Urological Pathologists (ISUP). This system was first proposed in 1998 and validated in subsequent studies. In 2004, the ISUP system was formally adopted as the WHO classification of
bladder tumors (Table 3) [35]. The rest of this section will be focused on description of individual diagnostic categories in Table 3.

Table 3 WHO/ISUP classification of tumor grade

- Normal
- Hyperplasia
  - Flat hyperplasia
  - Papillary hyperplasia
- Flat lesions with atypia
  - Reactive (inflammatory) atypia
  - Dysplasia (low grade intraurothelial neoplasia)
  - Carcinoma in situ (high grade intraurothelial neoplasia)
- Papillary neoplasms
  - Papilloma
  - Papillary urothelial neoplasm of low malignant potential
  - Papillary carcinoma, low grade
  - Papillary carcinoma, high grade

The first two categories, normal urothelium and hyperplasia, both imply lack of cytologic atypia (that is, only normal-looking cells are present in the specimen). The difference between normal urothelium and hyperplasia is in the number of cell layers. While normal urothelium typically consists of less than 7 cell layers, hyperplastic urothelium consists of numerous (much greater than 7) layers of cells which are cytologically normal [53]. It has been suggested however that papillary urothelial hyperplasia may be a precursor of malignant papillary tumors [54].

The category labeled “flat lesions with atypia” includes reactive changes, flat low grade lesions (dysplasia), and flat high grade lesions (carcinoma in situ). Reactive changes result from acute or chronic inflammation due to bacterial infections, calculi, trauma, or
no apparent cause. In bacterial infections, neutrophils and bacterial colonies are often present. Patients with reactive changes do not have an increased risk of bladder cancer [25]. The cellular abnormalities in dysplasia include cell crowding with nuclear enlargement and variation of nuclear shape. Nucleoli are usually small and inconspicuous. Mitotic figures are usually absent. Approximately 15%-20% of patients with urothelial dysplasia develop carcinoma in situ (CIS) or more invasive lesions [55-57].

Urothelial CIS is a flat high grade lesion with marked cytologic abnormalities such as loss of polarity, clumped chromatin with prominent nucleoli, and frequent mitotic figures [54]. The cells of CIS may form a single layer or multiple layers. Most cases of urothelial CIS are diagnosed in patients who also have papillary tumors. In one recent study of fluorescence cystoscopy, 30% (58/196) of patients with primary or recurring bladder cancer had CIS. Of all patients with CIS in this study, 60% (35/58) also had papillary tumors [28]. By definition, CIS is confined to urothelium (without invasion of lamina propria), however, the risk of stage progression is high. Without treatment, 50%-80% of urothelial CIS will progress to muscle-invasion. The probability of stage progression can be reduced by intravesical immunotherapy (which will be discussed in detail in section 1.4.1) [58].

The category labeled “papillary neoplasms” in Table 3 includes urothelial papilloma, papillary urothelial neoplasm of low malignant potential, low grade papillary urothelial carcinoma, and high grade papillary urothelial carcinoma. In the WHO/ISUP system, urothelial papilloma is a benign tumor composed of a fibro-vascular core covered by
normal urothelium. Urothelial papilloma is uncommon, representing less than 1% of all newly diagnosed bladder tumors [53].

Papillary urothelial neoplasm of low malignant potential is a papillary tumor consisting of > 7 layers of cells with minimal cytologic atypia. The nuclei are slightly enlarged but polarity is preserved. Mitoses are rare and when present, have basal location [53]. The probability of recurrence following resection of this tumor may be as high as 60% but stage progression is uncommon (< 10%) and tumor-related death occurs in less than 4% of all cases [59].

Low grade papillary urothelial carcinoma is characterized by variation in nuclear size and shape with some loss of polarity. Nucleoli may be visible. Mitoses may occur in the basal, intermediate, or superficial layers of the urothelium [53]. The characteristic features of high grade papillary urothelial carcinoma include marked loss of polarity, prominent nucleoli, and frequent mitoses [53].

Without intravesical chemo- or immunotherapy, as many as 80% of NMI papillary urothelial carcinomas recur after initial resection [60]. The probability of recurrence can be considerably reduced by intravesical therapy with Bacillus-Calmette-Guerin or mitomycin C (this will be discussed in greater detail in section 1.4) [58]. The probability of any stage progression for low grade papillary urothelial carcinoma is approximately 10% and tumor-related mortality occurs in ≤ 5% of all cases [59]. Grade progression
occurs in 10%-25% of low grade tumors, although grade regression from high grade non-
MI cancers is also common and may occur in as many as 45% of all cases [61].

The probability of stage progression in high grade papillary urothelial carcinoma is high
even with intravesical chemotherapy. In a series of papillary tumors treated with
Bacillus-Calmette-Guerin with 15 years of follow-up, any stage progression occurred in
39% (49/125) of high grade Ta’s and in 56% (41/73) of high grade T1’s. Among patients
with high grade Ta tumors, progression to muscle-invasion was reported in 13% (16/125)
of all cases [62]. Of all patients presenting with non muscle-invasive (Ta/T1) high grade
papillary urothelial carcinoma of the bladder, as many as 17%-21% may eventually die
from bladder cancer [22,63].

The prevalence of high grade tumors at the time of initial diagnosis increases with
increasing AJCC stage. For example, among SEER cases diagnosed in 2004-2005, the
proportions of high grade (poorly differentiated and undifferentiated) tumors were 23%,
68%, and 91% for AJCC stages 0, I, and II-IV respectively. It is important to recognize
that a single urothelial neoplasm may contain areas of well differentiated and poorly
differentiated cells. In such cases, the tumor should be graded according to the highest
grade [64].
1.3.4 *Other prognostic factors*

In addition to grade, stage, and cell type, established prognostic factors for BC include multiplicity (presence of more than one tumor in the bladder). Although multiplicity does not seem to affect survival, it has a very strong impact on the probability of intravesical recurrence. In one recent study of low grade non muscle-invasive UC, approximately 30% of all tumors were multifocal. The two-year recurrence probabilities were 38% and 72% for unifocal and multifocal tumors, respectively (p < 0.001) [65].

Another factor which may affect the probability of intravesical recurrence of bladder tumors is the tumor size [66,67]. This factor appears to be independent of multiplicity in the sense that larger solitary tumors are more likely to recur than smaller solitary tumors. A recently published update on treatment guidelines for non muscle-invasive bladder cancer (from the American Urological Association) recognized both, multiplicity and tumor size as prognostic factors for recurrence [67].

Many other tumor characteristics, such as lymphovascular invasion and specific molecular markers (described below) have been investigated as predictors of intravesical recurrence, response to therapy, and survival. Except for the lymphovascular invasion, none of these tumor characteristics can be viewed as established prognostic factors for BC at this time because of limited and/or conflicting evidence.
Lymphovascular invasion is now recognized as an independent prognostic factor with significant effect on survival [68]. Presence of tumor cells in the blood and lymphatic vessels significantly increases the risk of regional and distant metastases [68].

In recent years, a number of molecular markers have been investigated as prognostic factors for BC. Two of these markers, the fibroblast growth factor receptor 3 (FGFR3) gene and the p53 gene received much attention in the literature.

Mutations in the FGFR3 gene (located on chromosome 4) appear to be associated with low tumor grade and stage and may be associated with lower risk of recurrence and/or progression [69]. Between 70% and 90% of low grade tumors have mutations in the FGFR3 gene. The most frequent of these mutations are S249C (70%), Y375C (15%), and R248C (7%). The prevalence of FGFR3 mutations in high grade tumors is between 15% and 30% [69,70].

According to one recent report, the FGFR3 status may help identify high grade tumors with very high potential for stage progression (wild type FGFR3) and distinguish them from less aggressive high grade tumors which are unlikely to progress in stage (mutated FGFR3) [70]. However, in other studies, the FGFR3 status was not an independent predictor of progression after grade was taken into account [71]. Likewise, while some studies reported the association of FGFR3 mutations with lower probability of recurrence, this was not confirmed in other studies [68-71]. At this time, the prognostic role of FGFR3 mutations remains uncertain because existing evidence is limited and conflicting.
Same applies to other molecular markers investigated as prognostic factors for BC. In addition to FGFR3, much attention was focused on abnormal expression of the p53 gene (a tumor suppressor gene located on chromosome 17). Accumulation of dysfunctional p53 protein in tumor cells seems to be associated with higher grade and stage and more aggressive tumor behavior. According to some reports, FGFR3 mutations and abnormal expression of p53 are almost mutually exclusive tumor characteristics [72,73]. It is thought that these mutations may represent two distinct pathways of carcinogenesis. Mutations in FGFR3 seem to result in less aggressive tumors with low grade and stage, while tumors with abnormal expression of p53 are characterized by high grade and more advanced stage [72,73]. However, it is not clear whether p53 status adds independent prognostic information once known prognostic factors are taken into account. Existing evidence appears to be inconclusive [74].

Other molecular markers investigated as prognostic factors for BC include the Rb protein (a tumor suppressor), the p27Kip1 protein (a cell cycle inhibitor), the Ki-67 protein (a marker of cell proliferation detected by monoclonal antibody MIB-1) and other gene products [69,70,74]. As was the case with FGFR3 and p53, because of limited and/or conflicting evidence, none of these markers can be viewed as established prognostic factors for BC at this time [74].

One tumor characteristic which has not been thoroughly investigated as a prognostic factor for BC is the presence of non-urothelial differentiation in UC. Theoretically,
urothelial tumors with non-urothelial components may not respond to therapy in the same way as pure UC. Such mixed tumors may also be more (or less) likely to produce micrometastases before the initiation of definitive therapy. This may have an impact on survival which is independent of the apparent TNM stage at presentation.

The next section (section 1.4) will focus on bladder cancer therapy. Available evidence on the effect of mixed histology on response to therapy and survival will be reviewed in section 1.5.

1.4 Management of Bladder Cancer

1.4.1 Non muscle-invasive disease

In 2007, the American Urological Association published an update on treatment guidelines for non muscle-invasive bladder cancer (Ta, T1, and Tis) [67]. The guidelines were prepared by the American Urological Association expert panel based on available evidence in published literature. When available evidence was inconclusive, guideline statements were based on expert opinion.

The American Urological Association guideline statements are divided in three groups according to the degree of flexibility in their application: (1) standards, (2) recommendations, and (3) options. A guideline statement is a standard if the health outcomes of the alternative interventions are sufficiently well known to permit meaningful decisions, and there is virtual unanimity about which intervention is preferred. A guideline statement is a recommendation if the health outcomes of the alternative
interventions are sufficiently well known to permit meaningful decisions, and an appreciable but not unanimous majority agrees on which intervention is preferred. A guideline statement is an option if the health outcomes of the interventions are not sufficiently well known to permit meaningful decisions, or preferences are unknown or equivocal. The American Urological Association guidelines for treatment of non muscle-invasive BC are included in Appendix B and are summarized in the following paragraph.

Most patients with non muscle-invasive BC can be successfully treated with transurethral resection of bladder tumors (TURBT) which does not require removal of the bladder. During this procedure, surgical instruments are inserted in the bladder through the urethra and the tumors are removed from the bladder wall. For all patients, surgeons should attempt to resect all visible tumors by TURBT, although the presence of obvious deep muscle invasion may prevent complete resection. For cases without obvious muscle-invasion, a single post-operative dose of intravesical mitomycin C is an option. For patients with multifocal histologically confirmed low grade Ta or recurrent low grade Ta tumors, an induction course of intravesical therapy with Bacillus Calmette-Guerin or mitomycin C is recommended and a maintenance therapy with these same agents is an option. For patients with initial histologically confirmed high grade Ta, T1, and/or carcinoma in situ, induction and maintenance therapy with Bacillus Calmette-Guerin is recommended and cystectomy is an option. For patients with high grade Ta, T1, and/or carcinoma in situ which has recurred promptly (i.e. within a year) after prior intravesical chemotherapy, cystectomy is recommended and further intravesical therapy is an option. For all patients with T1 BC, re-staging TURBT must be performed to minimize the
probability of under-staged muscle-invasive disease [67]. These guidelines are summarized in Table 4.

Table 4 Summary of the treatment guidelines for non-muscle invasive BC

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>First solitary low grade Ta</td>
<td>TURBT+</td>
</tr>
<tr>
<td>Recurrent or multifocal low grade Ta</td>
<td>Single post-operative dose of IVT (option)</td>
</tr>
<tr>
<td></td>
<td>TURBT+</td>
</tr>
<tr>
<td></td>
<td>Induction IVT (recommendation)</td>
</tr>
<tr>
<td></td>
<td>Maintenance IVT (option)</td>
</tr>
<tr>
<td>First high grade Ta or T1 or Tis</td>
<td>TURBT+</td>
</tr>
<tr>
<td></td>
<td>Induction+maintenance IVT (recommendation)</td>
</tr>
<tr>
<td></td>
<td>Cystectomy (option)</td>
</tr>
<tr>
<td>Recurrent high grade Ta or T1 or Tis</td>
<td>TURBT+</td>
</tr>
<tr>
<td></td>
<td>Cystectomy (recommendation)</td>
</tr>
<tr>
<td></td>
<td>Further IVT (option)</td>
</tr>
</tbody>
</table>

TURBT = transurethral resection of bladder tumor; IVT = intravesical therapy

Additionally, for all patients with non muscle-invasive BC who do not undergo cystectomy, periodic surveillance cystoscopy is a standard of care according to the American Urological Association guidelines. However, neither the ideal interval nor the duration of follow-up has been defined. Given the variable risk of recurrence and progression, a risk-adapted approach is recommended. Patients with high risk disease should receive more intensive surveillance [67]. According to the recommendations of the National Comprehensive Cancer Network, cystoscopy should be performed every 3 months during the first year, every 3 to 6 months (depending on risk factors) during the next 3 years and then annually. Other professional organizations, such as European Association of Urology, British Medical Research Council, and French Urological
Association published their own surveillance guidelines [75]. None are based on prospective data however. Rather, they are based on expert opinion [75].

In addition to cystoscopy, post-operative surveillance of non muscle-invasive BC may include urinary cytology and measurement of certain molecular markers in voided urine. Urinary cytology is highly sensitive (90%-95%) and specific (>97%) for high grade tumors, including carcinoma in situ [75]. Because high grade tumors are associated with substantial risk of stage progression, their early detection is critical. Unfortunately, the sensitivity of urinary cytology for low grade tumors is very low (approximately 35%) [76]. This technique is also highly dependent on expertise of the cytopathologist.

Because cystoscopy is an invasive procedure and urinary cytology lacks sensitivity for low grade tumors, a number of molecular markers of intravesical recurrence have been investigated in recent years with the hope to reduce the need for surveillance cystoscopy. Performance of these markers has been examined in a recent systematic review [76] and is summarized in Table 5.
Table 5 Molecular markers for bladder cancer surveillance

<table>
<thead>
<tr>
<th>Test</th>
<th>Studies</th>
<th>Patients</th>
<th>Median Sensitivity</th>
<th>Range</th>
<th>Median Specificity</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTAstat</td>
<td>17</td>
<td>1377</td>
<td>58</td>
<td>29-74</td>
<td>2084</td>
<td>73</td>
</tr>
<tr>
<td>BTAtrack</td>
<td>4</td>
<td>360</td>
<td>71</td>
<td>60-83</td>
<td>195</td>
<td>66</td>
</tr>
<tr>
<td>NMP22</td>
<td>15</td>
<td>838</td>
<td>71</td>
<td>47-100</td>
<td>1203</td>
<td>73</td>
</tr>
<tr>
<td>FDP</td>
<td>4</td>
<td>168</td>
<td>54</td>
<td>47-68</td>
<td>173</td>
<td>61</td>
</tr>
<tr>
<td>ImmunoCyt</td>
<td>6</td>
<td>276</td>
<td>67</td>
<td>52-100</td>
<td>683</td>
<td>75</td>
</tr>
<tr>
<td>Cytometry</td>
<td>5</td>
<td>364</td>
<td>60</td>
<td>45-85</td>
<td>89</td>
<td>82</td>
</tr>
<tr>
<td>Quanticyt</td>
<td>5</td>
<td>129</td>
<td>58</td>
<td>45-65</td>
<td>227</td>
<td>76</td>
</tr>
<tr>
<td>Hb-dipstick</td>
<td>2</td>
<td>117</td>
<td>40</td>
<td>37-41</td>
<td>113</td>
<td>87</td>
</tr>
<tr>
<td>LewisX</td>
<td>3</td>
<td>95</td>
<td>75</td>
<td>68-79</td>
<td>215</td>
<td>85</td>
</tr>
<tr>
<td>FISH</td>
<td>4</td>
<td>165</td>
<td>79</td>
<td>70-86</td>
<td>147</td>
<td>70</td>
</tr>
<tr>
<td>Telomerase</td>
<td>3</td>
<td>146</td>
<td>39</td>
<td>29-66</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Microsatellite</td>
<td>6</td>
<td>108</td>
<td>82</td>
<td>75-92</td>
<td>153</td>
<td>89</td>
</tr>
<tr>
<td>CYFRA21-1</td>
<td>3</td>
<td>156</td>
<td>85</td>
<td>75-8</td>
<td>323</td>
<td>82</td>
</tr>
<tr>
<td>UBC</td>
<td>5</td>
<td>267</td>
<td>60</td>
<td>21-80</td>
<td>480</td>
<td>87</td>
</tr>
<tr>
<td>Cytokeratin20</td>
<td>2</td>
<td>117</td>
<td>85</td>
<td>79-87</td>
<td>61</td>
<td>76</td>
</tr>
<tr>
<td>BTA</td>
<td>5</td>
<td>436</td>
<td>48</td>
<td>32-58</td>
<td>216</td>
<td>92</td>
</tr>
<tr>
<td>TPS</td>
<td>3</td>
<td>179</td>
<td>65</td>
<td>50-80</td>
<td>246</td>
<td>83</td>
</tr>
<tr>
<td>Cytology</td>
<td>26</td>
<td>2213</td>
<td>35</td>
<td>13-75</td>
<td>3322</td>
<td>94</td>
</tr>
</tbody>
</table>

Note that even the most sensitive markers in Table 5 (such as Microsatellite, CYFRA21-1 and Cytokeratin20) had a sensitivity of less than 80% in some studies. This means that at least 20% of patients with recurrent bladder cancer were considered negative (disease-free) according to these tests. After publication of the systematic review summarized in Table 5, a promising new marker called bladder cancer antigen 4 was proposed for BC surveillance. This marker had a sensitivity of 89% and a specificity of 95% in pilot studies [77]. Although urinary markers may reduce the need for surveillance cystoscopy in the future, at the present time most patients with history of non muscle-invasive BC treated by TUR undergo frequent cystoscopic examinations. In the 2007 update on treatment guidelines for non muscle-invasive BC, the American Urological Association
expert panel made no recommendations on the use of molecular markers for post-operative surveillance [67].

1.4.2 Muscle-invasive disease

As was discussed in the previous section, most patients with non muscle-invasive BC can be successfully treated with TURBT, and do not require removal of the bladder (cystectomy). Although the probability of intravesical recurrence is high (60%-80%), most recurrences can be controlled with repeat procedures. Among patients with non muscle-invasive BC, removal of the bladder (cystectomy) is only recommended for tumors with high risk of stage progression, such as high grade T1’s [67].

In muscle invasive (MI) disease without distant metastases, cystectomy is currently the standard of care because complete resection of the tumor with TURBT alone is usually not feasible and any residual MI disease in the bladder will almost certainly result in metastases and death from BC. Additionally, exorable local symptoms ensue which are very difficult to prevent or treat, including pelvic pain, massive hematuria, and urinary retention. In MI BC without distant metastases or involvement of abdominal or pelvic wall (stages T2-T4a, M0), cystectomy is a potentially curative therapy. In cases of unresectable disease (T4b and/or M1), cystectomy may be performed for palliation [78].

In most cases, cystectomy implies removal of the anterior pelvic organs, including bladder with its peritoneum, prostate and seminal vesicle in men, or bladder, uterus, cervix, fallopian tubes, ovaries, anterior wall of the vagina, and anterior pelvic
peritoneum in women [78]. Extensive pelvic lymphadenectomy is also performed as part of this procedure. It has been shown that removal of fewer than 10 nodes is associated with increased probability of local recurrence and decreased survival [79]. Thirty-day surgical mortality with cystectomy is approximately 2% [78,80].

After the bladder is removed, a urinary diversion procedure is performed. The two main types of urinary diversion procedures are urostomy and continent diversion.

In urostomy, an opening (stoma) is created on the abdomen and the ureters are connected to the stoma. Although the ureters can be connected to the stoma directly (ureterostomy), this is rarely done because of high rate of complications. More frequently, the surgeon removes a short segment of intestine and reconnects the remaining intestine so that it functions normally. The distal end of the removed intestinal segment is attached to the stoma and the proximal end is connected to the ureters. The urine is collected in a pouch which is attached to the skin around the stoma. The most commonly used intestinal segment in this procedure is ileum (and the procedure is called an ileal conduit) [81].

In continent diversion, the surgeon creates a urine reservoir inside the body from a section of the stomach or intestine. The ureters carry urine to the reservoir, where it is stored. There are two main sub-types of continent diversion: (1) continent cutaneous diversion, and (2) orthotopic neo-bladder. In continent cutaneous diversion, the urine is emptied through a stoma by intermittent self-catheterization. In contrast, patients with
orthotopic neo-bladder can urinate through the urethra as they did with their original bladders (although different pelvic muscles must be used with neo-bladders) [81].

All urinary diversion procedures can result in a wide variety of long-term complications including stenosis, infection, stones, and metabolic acidosis. Additional concern with orthotopic neo-bladders is the possibility of tumor recurrence in the urethra (because urethra must be preserved for this form of urinary diversion). The probability of urethral recurrence may be as high as 37% in patients presenting with tumors in the bladder neck or prostatic urethra. Without these risk factors, the probability of urethral recurrence is between 3% and 7% [82].

Despite the fact that cystectomy is a very aggressive surgical procedure, it does not cure all patients with T2-T4a N0 M0 BC. Among patients with T2 N0 M0 and T3-T4a N0 M0 BC treated with cystectomy as the only form of definitive therapy, the 5-year survival probabilities are approximately 60% and 35%, respectively [83]. BC deaths occurring among these patients after removal of the bladder are caused by initially undiagnosed micro-metastatic disease which may be present in as many as 50% of all patients with MI BC at the time of cystectomy [84]. These micro-metastases become detectable within weeks or months after definitive surgery (most frequently in extra-pelvic sites, such as lung, liver and bone) and usually cause death within two years from diagnosis [84].
1.4.2.1 Adjuvant and neo-adjuvant therapies for bladder cancer

Because cystectomy is associated with considerable morbidity and often does not result in cure of MI BC (due to occult micro-metastatic disease at the time of definitive surgery), additional forms of therapy for MI BC, such as radiation and systemic chemotherapy have been investigated. In most studies, radiation or chemotherapy administered before surgery (neo-adjuvant therapy) or after surgery (adjuvant therapy) were compared to cystectomy alone. The neo-adjuvant approach received more attention because it provided an opportunity to treat micro-metastatic disease as early as possible. At this time, there are no randomized controlled trials (RCT) comparing cystectomy to bladder sparing therapy (TURBT plus pelvic radiation and systemic chemotherapy without cystectomy) because cystectomy is believed to provide a better chance of cure and better control of local symptoms for most patients [78].

Unfortunately, the studies of radiation therapy did not demonstrate any advantage of radiation therapy plus cystectomy over cystectomy alone [85]. The studies of adjuvant (post-cystectomy) chemotherapy (AC) were interpreted as inconclusive by most investigators because only 2 of 5 randomized trials showed a significant improvement in overall survival following AC (compared to observation) [86]. It must be recognized however that the two trials that did show survival advantage from AC used multi-drug combinations (cisplatin plus two or three other drugs) and included primarily high risk patients with extravesical (T3/T4) tumors and/or positive nodes [85]. In contrast, out of 3 trials with no difference in overall survival, one trial used cisplatin as a single agent and another trial enrolled primarily lower risk patients (no one had positive nodes) [85]. Thus
existing evidence from randomized trials suggests that multi-drug AC may potentially improve survival of high risk patients (Table 6); however, this question is still very controversial and clear evidence-based guidelines on this matter are lacking.

Table 6 Meta-analysis of adjuvant chemotherapy trials for locally advanced BC

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>N</th>
<th>HR</th>
<th>95% LCL</th>
<th>95% UCL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skinner et al.</td>
<td>1991</td>
<td>91</td>
<td>0.71</td>
<td>0.52</td>
<td>0.97</td>
<td>0.027</td>
</tr>
<tr>
<td>Stockle et al.</td>
<td>1992</td>
<td>49</td>
<td>0.62</td>
<td>0.39</td>
<td>1.01</td>
<td>0.048</td>
</tr>
<tr>
<td>Studer et al.</td>
<td>1994</td>
<td>77</td>
<td>0.96</td>
<td>0.58</td>
<td>1.59</td>
<td>0.877</td>
</tr>
<tr>
<td>Freheia et al.</td>
<td>1996</td>
<td>60</td>
<td>0.71</td>
<td>0.43</td>
<td>1.22</td>
<td>0.152</td>
</tr>
<tr>
<td>Bono et al.</td>
<td>1997</td>
<td>83</td>
<td>0.82</td>
<td>0.56</td>
<td>1.15</td>
<td>0.313</td>
</tr>
<tr>
<td><strong>Combined</strong></td>
<td></td>
<td>350</td>
<td>0.75</td>
<td>0.62</td>
<td>0.90</td>
<td>0.002</td>
</tr>
</tbody>
</table>


Neo-adjuvant (pre-cystectomy) platinum-based combination chemotherapy has been investigated in 7 RCTs. In a meta-analysis of these trials, addition of neo-adjuvant platinum-based combination chemotherapy to definitive treatment was associated with an increase in 5-year survival from 45% to 50% (HR = 0.86, p = 0.003, Figure 1) [87].
Figure 1 Meta-analysis of neo-adjuvant chemotherapy trials for locally advanced BC

The hazard ratio is for all-cause mortality; ends of horizontal bars denote the 99% confidence intervals and inner bar marks denote the 95% confidence intervals. Modified from: Advanced Bladder Cancer (ABC) Meta-analysis Collaboration. Neoadjuvant chemotherapy in invasive bladder cancer: update of a systematic review and meta-analysis of individual patient data. Eur Urol 2005;48(2):202-205

One very interesting effect of neo-adjuvant platinum-based combination chemotherapy is its ability to down-stage a substantial proportion of muscle-invasive tumors to pT0 (no evidence of BC in cystectomy specimen). In the most recent trial of neo-adjuvant MVAC (methotrexate, vinblastine, adriamycin, cisplatin), 50% (26/52) of patients with initial T2 disease and 30% (22/74) of patients with initial T3-T4a disease had no evidence of BC in cystectomy specimens after neo-adjuvant MVAC. In contrast, only 15% (18/121) of patients in the “cystectomy only” arm had no evidence of BC in the cystectomy specimen (this means that the entire tumor was removed by TURBT in these patients) [83]. In this study, 85% of patients with no residual disease in the cystectomy specimen were alive at five years from diagnosis. This is comparable to the 5-year survival of healthy individuals of the same age (the median age of these patients was 63 years; expected 5-year survival of a 63 y.o. white male is 90%). In contrast, the 5-year survival of patients with residual disease in the cystectomy specimen following MVAC was only 45% [83].
These results suggested that for a sizable proportion of patients with MI BC, MVAC therapy can completely eliminate all evidence of disease in the bladder. Because the 5-year survival of patients with complete response (pT0) to MVAC appeared to be similar to the 5-year survival of the general population of the same age, most of the patients with complete response were probably cured from BC by combination of chemotherapy and cystectomy. This raises an interesting question. Given the fact that no tumor was found in the cystectomy specimens of these patients, could they be cured with a combination of transurethral resection and systemic chemotherapy (a bladder sparing treatment)?

Unfortunately, this question is difficult to answer at this time because there are currently no reliable predictors of complete pathological response to the MVAC regimen. In other words, it is not possible to determine whether any given patient responded completely to MVAC without removing the bladder. It is now clear that the absence of visible tumor on cystoscopy and even the absence of BC in the TURBT specimen following MVAC therapy can not guarantee complete pathological response (pT0). In one recently published report, 63 patients with complete clinical response to MVAC (based on cystoscopy and post-MVAC TURBTs) who refused cystectomy were followed for 5 years. During this time, 23 (37%) of these patients died from BC and 83% (19/23) of the BC deaths resulted from tumors recurring first in the bladder (as opposed to distant sites), indicating that the majority of these BC deaths could potentially be prevented by cystectomy following chemotherapy [93].
In a recent trial conducted by the Southwest Oncology Group, complete clinical response to a combination of paclitaxel, carboplatin and gemcitabine was observed in 34 of 74 (46%) of patients with the initial diagnosis of cT2-T4a BC. Of the 34 patients with complete clinical response, 10 elected immediate cystectomy. Residual tumor in the cystectomy specimen was found in 6 of these 10 patients, indicating that complete clinical response to neo-adjuvant chemotherapy has a low positive predictive value for complete pathological response [123].

One promising approach to identification of prognostic markers is the study of genome-wide gene expression by micro-array analysis. Recently, a group from Japan developed a prognostic system for prediction of response to MVAC based on expression of 14 genes. Unfortunately this system does not separate complete responders (pT0) from partial responders (patients who regress in stage but not to pT0), although it may help identify patients who are unlikely to experience any stage regression in the bladder (non-responders). So far, the system has been tested in a small number of patients. In a pilot study, among 14 patients identified by the system as responders (complete or partial), 11 patients actually showed complete or partial response (PPV = 0.79); among 8 patients identified by the system as non-responders, no one responded to MVAC (NPV = 1) [94]. These results require confirmation in larger studies. Also, because this system cannot separate complete responders from partial responders, it cannot be used as a decision tool for bladder preservation following apparent clinical response to MVAC.

Other molecular markers investigated as predictors of response to neo-adjuvant chemotherapy included p53 (a tumor suppressor protein), mdm-2 (a negative regulator of
p53), and bcl-2 (a regulator of apoptotic pathway). In a recently published report, none of these markers, either alone or in combination showed association with down-staging following neo-adjuvant MVAC [95].

Because there are no reliable predictors of complete pathological response (pT0) to neo-adjuvant chemotherapy at this time, cystectomy remains the standard of care for resectable (T2-T4a, M0) muscle-invasive BC, even in the presence of complete clinical response to neo-adjuvant chemotherapy. The most recent treatment guidelines for muscle-invasive bladder cancer were published by the European Association of Urology [84]. A summary of these guidelines is included in Appendix E.

1.4.2.2 Some controversial issues surrounding the use of neo-adjuvant chemotherapy for bladder cancer

Currently, the use of neo-adjuvant chemotherapy for management of MI BC is associated with two major controversies

(1) It is not clear what patients are most likely to benefit from the chemotherapy. The average survival benefit from the use of neo-adjuvant chemotherapy is relatively small in magnitude (an improvement in 5-year survival form 45% to 50%, or a hazard ratio of 0.86) [87]. It is possible that for some patients (particularly those who show no evidence of any pathological response), chemotherapy may actually result in decreased survival because it delays cystectomy by more than 3 months. This delay has been associated with decreased survival in some patient populations [96]. Although several variables (such as
age, sex, clinical T stage, clinical N stage, and grade) have been investigated as potential modifiers of the effect of chemotherapy on survival, none were found to result in substantial modification of the treatment effect [87]. One characteristic that has not been studied in this context is the histologic composition of the tumor specimen (the presence of squamous, glandular, or other non-urothelial differentiation in urothelial carcinoma). Hence, it is currently unknown whether the effect of neo-adjuvant chemotherapy on survival of patients with resectable MI urothelial carcinoma of the bladder treated with radical cystectomy is influenced by the presence of non-urothelial component in the tumor. This question is important because mixed histology is a fairly common finding, observed in as many as 40% of MI BCs in the US and pure non-urothelial BCs tend to be less sensitive to chemotherapy than urothelial carcinoma [52].

(2) Platinum-based combination chemotherapy regimens used in the trials of neo-adjuvant chemotherapy for locally advanced bladder cancer are relatively toxic. Although these regimens rarely cause death by direct toxicity (< 1% of all patients treated), severe myelosupression, nephrotoxicity, and other side effects are common [83,89]. For example, MVAC, which appears to be the most effective regimen in terms of survival benefit, causes severe granulocytopenia (<500 cells/mm$^3$) in one third of all patients treated [83]. In clinical practice, a less toxic neo-adjuvant chemotherapy regimen composed of gemcitabine and cisplatin (GC) is now commonly substituted for neo-adjuvant MVAC [97,98]. In the setting of metastatic disease, GC seems to result in the same survival as MVAC based on report from one randomized trial [99]. However, it has been argued in the literature that extrapolation of these results to resectable (non-metastatic) BC is
potentially invalid, because the biology of metastatic and locally advanced BC may be different, and survival of patients with metastatic disease is usually very poor despite the use of combination chemotherapy (MVAC or other regimens) [97]. Hence, the ability of GC to induce complete pathological response and improve survival of patients with resectable MI BC remains unknown.

1.4.2.3 Summary

In summary, systemic platinum-based combination chemotherapy is used for treatment of locally advanced bladder cancer with the hope to reduce the risk of disease recurrence. However, it is currently unknown whether platinum-based combination chemotherapy is effective for all most commonly encountered histologic sub- types of BC (pure UC and UC with squamous and/or glandular differentiation). It is also uncertain whether GC chemotherapy, which is the regimen most frequently used in practice, can induce complete pathological response and improve survival of patients with locally advanced BC. These questions were examined in the current research investigation.
Chapter 2: Study Purpose and Specific Aims

The following specific aims (SA) and corresponding hypotheses (H) have been addressed in the study.

**SA1:** To determine whether the effect of neo-adjuvant chemotherapy with MVAC on pathological down-staging and survival of patients with locally advanced UC of the bladder treated with radical cystectomy is influenced by the presence of squamous and/or glandular component in the tumor.

H.1.1. The magnitude of the additive effect of neo-adjuvant MVAC on the probability of pathological stage zero at cystectomy differs between patients with mixed tumors and those with pure UC, after adjusting for clinical stage at presentation.

H.1.2. The magnitude of the multiplicative effect of neo-adjuvant MVAC on the hazard of death from all causes differs between patients with mixed tumors and those with pure UC, after adjusting for covariates associated with mortality.

**SA2:** To investigate the effect of neo-adjuvant chemotherapy with GC on pathological down-staging and survival of patients with locally advanced urothelial carcinoma of the bladder treated with cystectomy.

H.2.1 Patients treated with neo-adjuvant GC are more likely to have pathological stage zero at cystectomy compared to patients who do not receive neo-adjuvant chemotherapy, after adjusting for clinical stage at presentation.
H.2.2. Patients treated with neo-adjuvant GC have decreased hazard of death from all
causes compared to patients who do not receive neo-adjuvant chemotherapy, after
adjusting for covariates associated with mortality.

H.2.3. Patients treated with neo-adjuvant GC have decreased hazard of death from BC
compared to patients who do not receive neo-adjuvant chemotherapy, after adjusting for
covariates associated with mortality.
Chapter 3: Specific Aim 1

3.1 Introduction

Bladder cancer (BC) is the fifth most commonly diagnosed malignancy in the United States, with more than 70,000 new cases and more than 14,000 BC deaths reported in year 2009 [1]. The overwhelming majority of deaths from BC occur among patients with muscle-invasive disease (stages T2-T4). Standard therapy for resectable (T2-T4a) muscle-invasive BC without known metastases includes radical cystectomy with pelvic lymphadenectomy [84]. Unfortunately, many patients with apparently resectable muscle-invasive BC have undiagnosed micrometastatic disease at the time of definitive surgery. In a series of 1,054 patients treated with radical cystectomy and pelvic lymphadenectomy between 1977 and 1997, with a median follow-up of 10.2 years, BC recurred in 311 patients (30%) with a median time to recurrence of 12 months. Three quarters of all patients with disease recurrence had distant metastases [102].

Early treatment of micrometastatic disease with neo-adjuvant platinum-based combination chemotherapy (PBCC) administered before definitive local treatment (cystectomy and/or radiotherapy) has been compared to local treatment alone in several randomized trials. A meta-analysis of these trials demonstrated that addition of a neo-adjuvant PBCC regimen to local treatment improves the average 5-year survival by 5% on the additive scale (from 45% to 50%) [87]. Several trials also reported that the use of neo-adjuvant PBCC may increase the probability of pathological stage zero (pT0) at cystectomy from approximately 12%-15% in the “cystectomy only” arm to 33%-38% in the “PBCC plus cystectomy” arm [83,89].
Although most patients who are treated with radical cystectomy for muscle-invasive BC have pure urothelial carcinoma (UC), tumors with mixed histologic features (UC co-existing with non-urothelial histology) are also common. For example, in a series of 243 patients with clinical stage $\geq T2$, 96 patients (40%) had mixed histologic features, most frequently UC with squamous and/or glandular differentiation [52].

It is currently unknown whether the effect of neo-adjuvant PBCC on pathological downstaging and survival of patients with apparently resectable muscle-invasive UC treated with radical cystectomy depends on the presence of non-urothelial component in the tumor. Observational studies suggested that among patients with metastatic BC, patients with pure UC as well as patients with mixed tumors can achieve complete clinical response to PBCC (disappearance of all clinical and radiographic evidence of disease) [103,104]. In one series of metastatic BC, complete clinical response to PBCC was reported in 39% of 74 patients with pure UC and in 25% of 20 patients with mixed histology (UC with squamous, glandular, or spindle cell components) [103]. In another series of metastatic UC, any clinical response to PBCC (complete or partial) was observed in 44% of 389 patients with pure UC and in 34% of 42 patients with mixed urothelial and squamous histology [104]. In both series, survival did not differ significantly by histologic type [103,104].

These findings reported for metastatic UC may not be directly applicable to patients with apparently resectable BC because biology of metastatic and locally advanced tumors may be different. In particular, complete clinical response of metastatic lesions (which is
determined primarily by imaging studies) may not be equivalent to complete pathological response of the primary tumor in the bladder (stage pT0 at cystectomy). In addition, survival of patients with metastatic BC is usually very poor. While histologic type may not have a strong impact on survival of patients with this very advanced form of disease, it may influence both response to chemotherapy and survival of patients with less advanced tumors treated with cystectomy and neo-adjuvant PBCC with curative intent, because patients with lower tumor burden are more likely to be cured. Some authors hypothesized that the presence of mixed histologic features in locally advanced BC may confer resistance to PBCC [112]. This hypothesis has never been formally tested in epidemiological studies.

The purpose of the current study was to determine whether the effect of neo-adjuvant PBCC on pathological downstaging and survival of patients with locally advanced UC of the bladder treated with radical cystectomy is influenced by the presence of non-urothelial component in the tumor. The following hypotheses have been addressed in this study:

H.1.1. The magnitude of the additive effect of neo-adjuvant MVAC on the probability of pathological stage zero at cystectomy differs between patients with mixed tumors and those with pure UC, after adjusting for clinical stage at presentation.
H.1.2. The magnitude of the multiplicative effect of neo-adjuvant MVAC on the hazard of death from all causes differs between patients with mixed tumors and those with pure UC, after adjusting for covariates associated with mortality.

3.2 Materials and Methods

3.2.1 Study Design

This is a secondary analysis of the Southwest Oncology Group (SWOG) trial of neo-adjuvant MVAC (methotrexate, vinblastine, doxorubicin, cisplatin) followed with cystectomy versus cystectomy alone (SWOG 8710) [83]. Eligibility criteria for the trial included clinical stage T2-T4a N0 M0 UC of the bladder, no prior pelvic radiation, adequate renal, hepatic, and hematologic function (creatinine clearance > 50 ml/min, WBCs > 3.5 × 10^9/L, platelets > 100× 10^9/L) and a performance status of 0 or 1 (0 = asymptomatic, normal activity; 1 = symptomatic, ambulatory, able to carry out activities of daily life) [83,87]. A total of 307 eligible patients were enrolled between 1987 and 1998 and were randomized to either MVAC plus cystectomy (n=153) or cystectomy alone (n=154) (Figure 2).
Randomization was carried out using the method of Pocock and Simon with stratification on binary age (younger than 65 years vs. 65 years or older) and clinical stage (T2 vs. T3-T4a) [124]. The choice of the cut-off for binary age was determined by expected median age in the study sample. The purpose of this method of randomization is to reduce the probability of unequal allocation of important prognostic factors between the treatment arms [124]. This method can be particularly useful in trials with a large number of stratification variables (where adjustment for unbalanced covariates in the analysis can be highly inefficient) but it is also applicable to trials with a small number of strata, as in the current study. Balanced allocation of prognostic factors is achieved by adjusting the probability of treatment assignment for any given patient based on observed imbalance of prognostic factors at that point in the trial. Details of this process are described elsewhere [124].
Patients randomized to neo-adjuvant chemotherapy in the current study were to be given three 28-day cycles of MVAC, as follows: methotrexate (30 mg per square meter of body surface area) on days 1, 15, and 22; vinblastine (3 mg per square meter) on days 2, 15, and 22; and doxorubicin (30 mg per square meter) and cisplatin (70 mg per square meter) on day 2.

According to the study protocol, two pathological reviews were planned for each patient: (1) a central pathological review of the pre-registration biopsy (transurethral resection) specimen to confirm eligibility, and (2) a review of the cystectomy specimen to determine the pathological stage. As was discussed in the original publication, the first review was not performed for 46 patients because slides were not submitted or were lost in shipment [6]. These patients were enrolled in the trial and underwent randomization.

Of the remaining patients, 4 had missing information on histologic type from the first review (due to inadequate specimen submission). For these 50 patients (46 + 4), histologic type was determined from the institutional pathology reports. For all other patients, histologic type was determined by the central pathological review.

The original purpose of this trial was to determine whether administration of neo-adjuvant MVAC can improve survival of patients with locally advanced BC treated with radical cystectomy. The primary analyses showed an estimated 25% reduction in the hazard of death from all causes among those treated with MVAC, based on the Cox model with stratification on clinical stage and binary age (HR = 0.75, p = 0.06). Neither clinical stage (p = 0.45) nor age (p=0.74) had a significant interaction with treatment [83].
For the purpose of analyses reported in this paper, tumors were classified based on the presence of non-urothelial components as either pure UC (n=236) or mixed tumors (n=61). For 10 patients, tumors could not be definitively classified as either pure or mixed based on available information. These 10 patients were excluded from our analysis. Non-urothelial components included squamous histology (n=37), adenocarcinoma (n=20), squamous histology with adenocarcinoma (n=2) and other histologic types (n=2). The two patients with other histologic types (1 small cell and 1 signet ring) were also excluded to make the mixed histology group a more homogenous pathological entity (UC with squamous and/or glandular differentiation). Therefore, secondary analyses were based on 236 patients with pure UC (115 randomized to MVAC-plus-cystectomy and 121 randomized to cystectomy-only) and 59 patients with mixed tumors (32 randomized to MVAC-plus-cystectomy and 27 randomized to cystectomy only) (Figure 3).

Figure 3 Secondary Analyses of the SWOG study
The primary exposure variables of interest in the current analyses were “treatment” (MVAC + cystectomy vs. cystectomy alone) and “histologic type” (pure UC vs. mixed tumors). Other covariates included age at randomization (in years), clinical stage (T2 vs. T3-T4a), sex (male or female), and race (white or other race). The clinical stages were defined according to the fourth edition of the American Joint Committee on Cancer staging manual [83,105]. Two outcome measures were examined in the current study: (1) the probability of no residual tumor in the cystectomy specimen (stage pT0 at cystectomy), and (2) the hazard of death from all causes (all-cause mortality). All-cause mortality was the primary end-point of the trial according to the original study protocol. Survival time was measured from randomization until death from any cause. Patients were censored at their last contact date. All patients provided written informed consent, and the study was approved by the ethics committees of participating institutions.

3.2.2 Methods of Data Analysis

3.2.2.1 Tumor down-staging

Proportions of patients with stage pT0 at cystectomy were compared between the two treatment arms separately for patients with pure UC and for patients with mixed tumors using the Fisher’s exact test. The same method was used to compare the proportions of pT0s at cystectomy between patients with pure UC and patients with mixed tumors within each treatment arm. The additive effects of treatment and histologic type on the probability of tumor down-staging to pT0 with adjustment for clinical stage were estimated using the modified least squares model with identity link function and robust variance estimator (described in detail in section 3.2.2.3.1) [106]. This model was also
used to test for the interaction of treatment with histologic type with adjustment for clinical stage (Hypothesis 1.1). The statistical interaction between treatment and histologic type would indicate that the effect of treatment in patients with mixed tumors is different in magnitude from the effect of treatment in patients with pure UC. For sensitivity analysis, the estimated effects of treatment and histologic type on tumor downstaging were also controlled for clinical stage by direct standardization [107]. This procedure is explained in section 3.2.2.3.2

3.2.2.2 Survival analysis

The effect of MVAC on all-cause mortality was estimated separately for patients with mixed tumors and for patients with pure UC using the Cox model. This model was also used to estimate the effect of histologic type (mixed tumors vs. pure UC) on all-cause mortality within each treatment arm and to test for treatment-by-histologic type interaction (Hypothesis 1.2) [108]. All models were stratified on clinical stage and included age as a continuous covariate. Hence, the estimated effects of treatment and histologic type on the hazard of death from all causes were controlled for age and clinical stage at randomization in all comparisons reported in this section.

To express the effect of treatment and histologic type on all-cause mortality in terms of the absolute risk with adjustment for age and clinical stage, model-based five-year survival probabilities were estimated for each combination of treatment with histologic type and clinical stage, holding the value of age fixed at the average age of all patients in the study. Details of the estimation process are described in section 3.2.2.3.3
The assumption of proportional hazards was tested for each covariate in the Cox model by inclusion of covariate-by-time interaction terms. The assumption of linearity of age (the only continuous covariate) with respect to the log-hazard was tested by inclusion of quadratic terms in the model. Product terms were used to test for treatment-by-histologic type interaction [109,110]. All analyses were performed in SAS version 9.2. All reported p-values are two-sided.

3.2.2.3 Statistical models

3.2.2.3.1 Additive probability model

In the additive probability model the probability of a binary outcome is modeled as a linear function of covariates and regression parameters [106]

\[ E(y \mid X) = X\beta \]  

(1)

The regression parameters \( \beta \) can be estimated by maximizing the Bernoulli likelihood function

\[ L(\beta) = \prod_{i=1}^{n} \pi_{i}^{y_{i}} (1 - \pi_{i})^{1-y_{i}} \]

\[ \ln L(\beta) = \sum_{i=1}^{n} [y_{i} \ln \pi_{i} + (1 - y_{i}) \ln(1 - \pi_{i})] = \sum_{i=1}^{n} [y_{i} \ln \beta' x_{i} + (1 - y_{i}) \ln(1 - \beta' x_{i})] \]

Solution of the corresponding estimating equations can be obtained with the Fisher scoring algorithm. At convergence, the estimated coefficients are consistent, asymptotically normal and fully efficient. Their covariance matrix is estimated by the inverse of the information matrix evaluated at \( \hat{\beta} \). When the maximum-likelihood
algorithm fails to converge, as may occur in the presence of empty cells for some combinations of categorical covariates, model parameters can be estimated by the method of least squares [106].

Note that the ordinary least squares (OLS) estimators \( \hat{\beta} = (X'X)^{-1}X'y \) are unbiased for regression parameters in equation (1) if the model for conditional expectation of the outcome was specified correctly

\[
E(\hat{\beta} \mid X) = E[(X'X)^{-1}X'(y \mid X)] = (X'X)^{-1}X'E(y \mid X) = (X'X)^{-1}X'X\beta = \beta
\]  

(2)

The variance of \( \hat{\beta} \) can be estimated with the robust covariance matrix estimator

\[
V(\hat{\beta} \mid X) = V[(X'X)^{-1}X'(y \mid X)] = (X'X)^{-1}X'V(y \mid X)X(X'X)^{-1},
\]  

(3)

where the diagonal elements of \( V(y \mid X) \) are estimated by \( e_i^2 = (y_i - \hat{\beta}'x_i)^2 \).

3.2.2.3.2 Direct standardization

Let \( y \) denote a binary outcome variable (such as stage pT0 at cystectomy), let \( x_1 \) denote a binary exposure variable (such as the use of neo-adjuvant chemotherapy), let \( x_2 \) denote a potential confounder (such as clinical stage), and let \( W_i \) denote the proportion of subjects in the \( i \)th stratum of \( x_2 \) in some standard population. The additive effect of \( x_1 \) on the probability of \( y = 1 \) standardized to the distribution of \( x_2 \) is defined as

[107,116,117]

\[
\delta = \sum_{i=1}^{k} W_i [\Pr(y = 1 \mid x_1 = 1, x_2 = i) - \Pr(y = 1 \mid x_1 = 0, x_2 = i)] = \sum_{i=1}^{k} W_i (\pi_{1i} - \pi_{0i})
\]
If we replace $\pi_{1i}$ and $\pi_{0i}$ by their maximum likelihood estimators (assuming Bernoulli distribution for $y$), we obtain an unbiased estimator of $\delta$

$$\hat{\delta} = \sum_{i=1}^{k} W_i (\hat{\pi}_{1i} - \hat{\pi}_{0i})$$

The estimated variance of $\hat{\delta}$ is

$$\hat{V}(\hat{\delta}) = \sum_{i=1}^{k} W_i^2 \hat{V}(\hat{\pi}_{1i} - \hat{\pi}_{0i}) = \sum_{i=1}^{k} W_i^2 \left[ \frac{\hat{\pi}_{1i} (1 - \hat{\pi}_{1i})}{n_{1i}} + \frac{\hat{\pi}_{0i} (1 - \hat{\pi}_{0i})}{n_{0i}} \right],$$

where $n_{1i}$ and $n_{0i}$ are the numbers of exposed and unexposed subjects (respectively) in stratum $i$. This expression for the variance can be used for interval estimation. For the purpose of hypothesis testing, the variance of $\hat{\delta}$ under the null hypothesis ($H_0 : \delta = 0$) can be estimated by

$$\hat{V}(\hat{\delta}) = \sum_{i=1}^{k} W_i^2 \left[ \frac{\hat{\pi}_i (1 - \hat{\pi}_i)}{n_{1i}} + \frac{\hat{\pi}_i (1 - \hat{\pi}_i)}{n_{0i}} \right],$$

where $\hat{\pi}_i = \frac{n_{1i}}{n_{1i} + n_{0i}} \hat{\pi}_{1i} + \frac{n_{0i}}{n_{1i} + n_{0i}} \hat{\pi}_{0i}$ is an estimator of the common stratum-specific probability which is assumed to be the same for both levels of exposure under $H_0$ [107].

The test statistic for hypothesis testing is given by

$$\chi^2_{df/1} = \frac{\hat{\delta}^2}{\hat{V}(\hat{\delta})}$$

It is clear that unless $(\pi_{1i} - \pi_{0i})$ are equal to the same value for all $i$ (that is, there is no interaction of $x_1$ with $x_2$ on the additive probability scale), the value of $\delta$ will depend
on the choice of the standard distribution for \( x_2 \). This choice should be determined by the target of causal inference. In randomized controlled trials, it is often meaningful to choose the distribution of \( x_2 \) in the combined sample of exposed and unexposed subjects as the standard [116]. The standardized measure of effect in this case would be approximating the causal effect of exposure in the entire study cohort when the following two assumptions hold: (1) the distribution of \( x_2 \) is not causally affected by the exposure, and therefore would be the same in the counterfactual exposed and in the counterfactual unexposed cohort and (2) there is no residual confounding within the levels of \( x_2 \). The first assumption is guaranteed to hold in the current study because the clinical stage (\( x_2 \)) cannot be a consequence of chemotherapy (\( x_1 \)), which is administered after the clinical stages are documented. The second assumption is also very reasonable since the distribution of unmeasured and unknown determinants of the outcome is the same in expectation within each treatment arm due to randomization.

To compare two standardized additive effects in independent samples, the following test statistic can be used [118]

\[
\chi^2_{df} = \frac{(\hat{\delta}_1 - \hat{\delta}_2)^2}{\hat{V}(\hat{\delta}_1) + \hat{V}(\hat{\delta}_2)}
\]

Alternatives to direct standardization include regression-based methods discussed in the previous section. Although model-based covariate-adjusted risk differences do not depend on the choice of the standard (which is their advantage from a certain point of
view), they do depend on the assumption of effect homogeneity, which may be difficult to test in small to moderate size samples. In contrast, directly standardized risk differences remain well defined as a weighted average of the stratum-specific effects even in the absence of effect homogeneity [116]. When homogeneity is present, model-based estimates are more efficient than the standardized estimates.

3.2.2.3.3 Cox model

The Cox model assumes multiplicative effect of covariates on the hazard function but does not assume any particular distribution of event times

\[ h(t \mid x_1, \ldots, x_k) = h_0(t) \exp(\beta_1 x_1 + \ldots + \beta_k x_k) \]

Regression coefficients are estimated by maximizing the partial likelihood function [109,110]

\[ L(\beta) = \prod_{j=1}^{D} \frac{\exp(\beta'x_j)}{\sum_{i=1}^{R(j)} \exp(\beta'x_i)} \]

where the product is taken over the set of distinct ordered event times, \( x_j \) is the vector of covariates for the subject who failed at the \( j \) th ordered event time, and \( R(j) \) is the number of persons in the \( j \) th risk set. In the presence of ties, a modified form of partial likelihood is maximized [109,110].
The covariance matrix of $\hat{\beta}$ is estimated by inverting the matrix of second partial derivatives of the log-partial likelihood function taken with respect to $\beta$ and evaluated at $\hat{\beta}$.

In the analysis of the effect of covariates on the hazard of death from a given cause, deaths from other causes are regarded as censoring events. A theoretical justification for this practice is based on the fact that the likelihood function in the presence of multiple failure types is completely specified by the cause-specific hazard functions and can be factored into a component for each failure type. This is reviewed in references [109,111].

The Cox model can also be used to obtain an estimate of the cumulative survival function for a given covariate pattern $\mathbf{x}_i$ at some time $\tau$. For example, $\tau$ can be equal to 5 years from time zero, as in the analysis of five-year survival [109,110]

For estimation of the cumulative survival function, the model can be written as

$$S(\tau \mid x_1, x_2, ..., x_k) = S_0(\tau)^{\exp(\beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k)}$$

Several estimators can be used for estimation of the baseline cumulative survival function in the last expression. For example, one estimator (proposed by Breslow) which is available in SAS has the form [109]

$$\hat{S}_0(\tau) = \prod_{j=1}^{\hat{R}(j)} \left[ \exp \left( \frac{-d_j}{\sum_{i=1}^{R(j)} \exp(\hat{\beta}'x_i)} \right) \right]$$
where $d_j$ is the total number of deaths at the $j$th ordered event time.

The variance of this estimator has a very complicated form and will not be reproduced here. The expression for the variance is given for example in [109], p220-221. An alternative estimator of $S_\theta(\tau)$ (also available in SAS) was proposed by Kalbfleisch and Prentice [121]. In practice, the two estimators produce very similar results [109,110].

### 3.2.3 Power and Sample Size Considerations

According to the primary hypothesis of Specific Aim 1, the effect of treatment on the hazard of death from all causes may depend on the histologic composition of the tumor.

To approximate the power for the test of this interaction effect, we will consider a Cox model stratified on histologic type (mixed tumors vs. pure UC) with a regression coefficient for treatment (MVAC-plus-cystectomy vs. cystectomy-only) and a regression coefficient for the interaction of treatment with histologic type [125]. We will assume that the proportion of patients with mixed tumors in the study cohort is 20%-40% (based on previously reported case-series) [52], the hazard ratio for the treatment effect among patients with mixed tumors is near or above unity, and the exponentiated regression coefficient for the interaction effect is equal to 2, that is, the ratio of the treatment effects is equal to 2, as shown in Table 7.

The hazard ratio for the treatment effect among the patients with mixed tumors would be equal to unity if the presence of mixed histologic features confers resistance to MVAC but does not decrease survival (relative to the cystectomy-only arm) by delaying
cystectomy. Because there is evidence that delaying cystectomy beyond three months from diagnosis of muscle-invasion (which is the time needed to complete chemotherapy) may decrease survival [96], it is reasonable to assume that the hazard ratio for the treatment effect among patients with mixed tumors may take values above unity. A range of possible hazard ratios with a two-fold interaction effect is shown in Table 7.

Table 7 Treatment effect as a function of histologic type with a two-fold interaction

<table>
<thead>
<tr>
<th>HR(MVAC \ mixed)</th>
<th>HR(MVAC \ pure UC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>0.50</td>
</tr>
<tr>
<td>1.10</td>
<td>0.55</td>
</tr>
<tr>
<td>1.20</td>
<td>0.60</td>
</tr>
<tr>
<td>1.30</td>
<td>0.65</td>
</tr>
</tbody>
</table>

The power of the interaction test is related to the total number of deaths within each stratum (mixed tumors vs. pure UC) and the magnitude of the interaction effect as shown in the following equation [125]

\[
\frac{d_1 d_2}{d_1 + d_2} = \frac{(z_{1-\alpha/2} + z_{power})^2}{pq(ln 2)^2},
\]

where \(d_1\) and \(d_2\) are the stratum-specific death counts, \(p = 0.5\) is the proportion of patients randomized to MVAC-plus-cystectomy within each stratum, and \(q = 1 - p\).

To approximate power, we will assume that \(d_1 = d_2 = 190/2 = 95\), where 190 is the total number of deaths in the study (reported in the original publication). The assumption of \(d_1 = d_2\) is reasonable since the proportion of patients with mixed tumors in the study
cohort is expected to be <50% but these patients are thought to experience higher mortality. Now, to compute the power, we re-arrange the last equation as follows

\[
 z_{\text{power}} = \frac{d_1 d_2}{d_1 + d_2} \times pq \times (\ln 2)^2 - z_1-\alpha/2
\]

At \( \alpha = 0.05 \), the power of the two-sided test can be approximated as

\[
 z_{\text{power}} = \frac{95^2}{190} \times 0.5^2 \times (\ln 2)^2 - 1.96 = 0.428 , \text{ and power} = \Phi(0.428) = 0.67 \text{ or 67%}
\]

At \( \alpha = 0.10 \), the power of the two-sided test can be approximated as

\[
 z_{\text{power}} = \frac{95^2}{190} \times 0.5^2 \times (\ln 2)^2 - 1.645 = 0.743 , \text{ and power} = \Phi(0.743) = 0.77 \text{ or 77%}
\]

To achieve a reasonable balance between the type I and the type II error probabilities, a p-value of < 0.1 from the test of the interaction effect will be interpreted as evidence of statistical interaction. Although these calculations are based on a stratified Cox model (with histologic type as a stratification variable) they also provide a reasonable approximation to the power of the test for interaction in a model with a separate coefficient for treatment, histologic type, and their interaction effect [125].

It may also be of interest to examine the power for the test of the secondary hypothesis of Specific Aim 1. According to this hypothesis, the magnitude of the additive effect of treatment on the probability of tumor down-staging to pT0 may depend on the histologic composition of the tumor. As was stated in the section on data analysis, this hypothesis
will be tested using the additive risk model estimated by maximizing the Bernoulli
likelihood function. If the Fisher scoring algorithm fails to converge, model parameters
will be estimated by the method of least squares with robust covariance matrix estimator.

If the presence of mixed histologic features confers resistance to MVAC, then the
additive down-staging effect of chemotherapy will be present among the patients with
pure UC, but not among the patients with mixed tumors. According to the original
publication, among the patients who received cystectomy, the proportions of patients
with stage pT0 were 38% in the MVAC-plus-cystectomy arm and 15% and in the
cystectomy-only arm [83].

For the purpose of power analysis, we will assume that tumor down-staging only occurs
among patients with pure UC and that the probabilities of stage pT0 at cystectomy for
each combination of treatment with histologic type are

\[
\Pr(pT0 = 1 \mid MVAC = 1, MIXED = 0) = 0.45 \\
\Pr(pT0 = 1 \mid MVAC = 0, MIXED = 0) = 0.15 \\
\Pr(pT0 = 1 \mid MVAC = 1, MIXED = 1) = 0.15 \\
\Pr(pT0 = 1 \mid MVAC = 0, MIXED = 1) = 0.15
\]

The corresponding model parameters are

\[
\Pr(pT0 = 1 \mid MVAC, MIXED) = 0.15 + 0.30MVAC + 0MIXED - 0.30MVAC \times MIXED
\]
If the proportion of patients with mixed tumors is equal to 20%, then the marginal (with respect to histologic type) probabilities of \( pT0 \) within each treatment group are

\[
\Pr(pT0 = 1 | MVAC = 1) = 0.45 \times 0.8 + 0.15 \times 0.2 = 0.39
\]

\[
\Pr(pT0 = 1 | MVAC = 0) = 0.15 \times 0.8 + 0.15 \times 0.2 = 0.15
\]

Simulated power, based on 1,000 realizations of the Bernoulli process is shown in Table 8.

Table 8 Power analysis: additive risk model:

\[
\beta_0 = 0.15, \quad \beta_1 = 0.30, \quad \beta_2 = 0, \quad \beta_3 = -0.30, \quad n_1 = 153, \quad n_2 = 154.
\]

\[
\Pr(MIXED = 1 | MVAC = 1) = \Pr(MIXED = 1 | MVAC = 0) = 0.2
\]

<table>
<thead>
<tr>
<th>Estimation method</th>
<th>Convergence</th>
<th>Mean</th>
<th>Coverage</th>
<th>Power ((\alpha = 0.05))</th>
<th>Power ((\alpha = 0.10))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum likelihood</td>
<td>0.99</td>
<td>-0.30</td>
<td>0.96</td>
<td>0.83</td>
<td>0.89</td>
</tr>
<tr>
<td>Least squares</td>
<td>1.00</td>
<td>-0.30</td>
<td>0.96</td>
<td>0.81</td>
<td>0.88</td>
</tr>
</tbody>
</table>

\( n_1 \) = number of patients randomized to MVAC-plus-cystectomy; \( n_2 \) = number of patients randomized to cystectomy-only; Convergence = estimated probability of model convergence; Mean = mean of the estimated regression coefficient for the interaction effect; Coverage = estimated coverage probability of the 95% confidence intervals given model convergence; Power = estimated power given model convergence.

Hence, the test of interaction at alpha = 0.05 will provide a power of approximately 80%.

Power would be lower for smaller effect size.

3.3 Results

The distribution of patient characteristics for each combination of treatment and histologic type is shown in Table 9. In the mixed histology group, patients randomized to the cystectomy-only arm were on average older, had a higher proportion of deeply invasive tumors, and included fewer females and fewer white patients, compared to
patients randomized to the MVAC-plus-cystectomy arm. Among patients with pure UC, the distribution of patient characteristics did not differ substantially between the two treatment arms.

### Table 9 Patient characteristics for each combination of treatment with histologic type

<table>
<thead>
<tr>
<th></th>
<th>Mixed UC</th>
<th>Pure UC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MVAC + RC</td>
<td>RC alone</td>
</tr>
<tr>
<td>N</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>Mean age</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>% cT3-4a*</td>
<td>59%</td>
<td>70%</td>
</tr>
<tr>
<td>% Females</td>
<td>31%</td>
<td>15%</td>
</tr>
<tr>
<td>% Whites</td>
<td>91%</td>
<td>78%</td>
</tr>
</tbody>
</table>

*cT3-T4a = clinical stage T3 or T4a

#### 3.3.1 Tumor down-staging

Table 10 shows numbers and percentages of patients who had known pT0 status and numbers and percentages of patients who had stage pT0 based on pathological examination of the cystectomy specimen. The pT0 status was known for 266 patients who either received cystectomy (n=243) or had surgery cancelled or aborted due to overt disease progression / unresectable disease. The pT0 status was not known for 29 patients who did not undergo cystectomy for reasons other than overt disease progression (e.g., refused cystectomy for personal reasons).
Table 10 Numbers and percentages of patients with known pT0 status and numbers and percentages of patients with stage pT0, by treatment-histology combinations

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>Treatment arm</th>
<th>N</th>
<th>No. (%) with known pT0 status</th>
<th>No. with pT0</th>
<th>%¹ with pT0</th>
<th>%² with pT0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed tumors</td>
<td>MVAC + RC</td>
<td>32</td>
<td>28 (88%)</td>
<td>11</td>
<td>39%</td>
<td>34%</td>
</tr>
<tr>
<td></td>
<td>RC alone</td>
<td>27</td>
<td>24 (89%)</td>
<td>1</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Pure UC</td>
<td>MVAC + RC</td>
<td>115</td>
<td>100 (87%)</td>
<td>33</td>
<td>33%</td>
<td>29%</td>
</tr>
<tr>
<td>Pure UC</td>
<td>RC alone</td>
<td>121</td>
<td>114 (94%)</td>
<td>17</td>
<td>15%</td>
<td>14%</td>
</tr>
</tbody>
</table>

%¹ with pT0 = (No. with pT0 / No. with known pT0 status)*100%
%² with pT0 = (No. with pT0 / N)*100%

Table 10 contains two sets of percentages for stage pT0. The first set (denoted by %¹) was computed by dividing the number of patients with pT0 by the number of patients whose pT0 status was known. The second set (denoted by %²) was computed by dividing the number of patients with pT0 by the total number of patients in the corresponding combination of treatment with histologic type, with the conservative assumption that all patients whose pT0 status was not known had residual disease (did not have stage pT0).

When only patients with known pT0 status were included in the analysis, the crude estimated effect of chemotherapy on tumor down-staging was equal to 39% - 4% = 35% (p = 0.003) for patients with mixed tumors, and 33% - 15% = 18% (p = 0.002) for patients with pure UC (Table 11). The estimated effect of histologic type on tumor down-staging in the crude analysis was equal to 39% - 33% = 6% (p = 0.65) in the MVAC-plus-cystectomy arm, and 4% - 15% = -11% (p = 0.20) in the cystectomy-only arm.

The estimated effects of treatment and histologic type on tumor down-staging to pT0 with model-based adjustment and direct standardization for clinical stage are also shown.
in Table 11. The adjusted and the standardized effects were very similar to the effects estimated in the crude analysis in terms of their magnitude and statistical significance.

The interaction of treatment with histologic type on the additive probability scale was not statistically significant in either the crude (p = 0.14) or the adjusted (p = 0.15) analysis (Hypothesis 1.1).

Table 11 Estimated effects of treatment and histologic type on tumor downstaging to pT0 based on patients with known pT0 status

<table>
<thead>
<tr>
<th>Patients included</th>
<th>N</th>
<th>Variable</th>
<th>Crude Effect</th>
<th>P-value</th>
<th>Standardized Effect</th>
<th>P-value</th>
<th>Adjusted Effect</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed UC</td>
<td>52</td>
<td>MVAC</td>
<td>35%</td>
<td>0.003</td>
<td>33%</td>
<td>0.001</td>
<td>32%</td>
<td>0.003</td>
</tr>
<tr>
<td>Pure UC</td>
<td>214</td>
<td>MVAC</td>
<td>18%</td>
<td>0.002</td>
<td>18%</td>
<td>0.002</td>
<td>18%</td>
<td>0.001</td>
</tr>
<tr>
<td>MVAC+RC</td>
<td>128</td>
<td>Mixed</td>
<td>6%</td>
<td>0.65</td>
<td>9%</td>
<td>0.37</td>
<td>7%</td>
<td>0.43</td>
</tr>
<tr>
<td>RC alone</td>
<td>138</td>
<td>Mixed</td>
<td>-11%</td>
<td>0.20</td>
<td>-8%</td>
<td>0.32</td>
<td>-8%</td>
<td>0.11</td>
</tr>
<tr>
<td>All patients</td>
<td>266</td>
<td>Interaction</td>
<td>17%</td>
<td>0.14</td>
<td>16%</td>
<td>0.18</td>
<td>15%</td>
<td>0.15</td>
</tr>
</tbody>
</table>

1Additive effect (risk difference) directly standardized to the distribution of clinical stage among all patients included in the analysis; 2Additive effect (risk difference) adjusted for clinical stage using the additive risk model estimated by least-squares (Fisher scoring algorithm did not converge because there were no success among patients with cT3-T4a mixed tumors treated with cystectomy alone)

We also estimated the effects of chemotherapy and histologic type on tumor downstaging under a conservative assumption that all patients with unknown pT0 status had residual disease. The corresponding estimates (Table 12) were very similar to the estimates reported in Table 11.
Table 12 Estimated effects of treatment and histologic type on tumor down-staging to pT0 under the assumption that all patients with unknown pT0 status had residual disease

<table>
<thead>
<tr>
<th>Patients included</th>
<th>N</th>
<th>Variable</th>
<th>Crude Effect</th>
<th>P-value</th>
<th>Standardized Effect</th>
<th>P-value</th>
<th>Adjusted Effect</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed UC</td>
<td>59</td>
<td>MVAC</td>
<td>30%</td>
<td>0.004</td>
<td>28%</td>
<td>0.004</td>
<td>27%</td>
<td>0.004</td>
</tr>
<tr>
<td>Pure UC</td>
<td>236</td>
<td>MVAC</td>
<td>15%</td>
<td>0.007</td>
<td>15%</td>
<td>0.004</td>
<td>15%</td>
<td>0.004</td>
</tr>
<tr>
<td>MVAC+RC</td>
<td>147</td>
<td>Mixed</td>
<td>5%</td>
<td>0.52</td>
<td>6%</td>
<td>0.51</td>
<td>6%</td>
<td>0.52</td>
</tr>
<tr>
<td>RC alone</td>
<td>148</td>
<td>Mixed</td>
<td>-10%</td>
<td>0.20</td>
<td>-9%</td>
<td>0.22</td>
<td>-8%</td>
<td>0.10</td>
</tr>
<tr>
<td>All patients</td>
<td>395</td>
<td>Interaction</td>
<td>15%</td>
<td>0.15</td>
<td>14%</td>
<td>0.19</td>
<td>13%</td>
<td>0.17</td>
</tr>
</tbody>
</table>

1 Additive effect (risk difference) directly standardized to the distribution of clinical stage among all patients included in the analysis; 2 Additive effect (risk difference) adjusted for clinical stage using the additive risk model estimated by least-squares

The estimated down-staging effects adjusted for clinical stage using the robust least-squares model (last two columns of Table 12) are also reported in Table 13 along with the 95% confidence intervals.

Table 13 Down-staging effects of treatment and histologic type estimated under the assumption that all patients with unknown pT0 status had residual disease – stage-adjusted point estimates with 95% CIs and p-values

<table>
<thead>
<tr>
<th>Model</th>
<th>Patients included</th>
<th>N</th>
<th>Contrast</th>
<th>Adjusted Effect</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mixed UC</td>
<td>59</td>
<td>MVAC+RC vs. RC-only</td>
<td>27%</td>
<td>(9%, 45%)</td>
<td>0.004</td>
</tr>
<tr>
<td>2</td>
<td>Pure UC</td>
<td>236</td>
<td>MVAC+RC vs. RC-only</td>
<td>15%</td>
<td>(5%, 25%)</td>
<td>0.004</td>
</tr>
<tr>
<td>3</td>
<td>MVAC+RC</td>
<td>147</td>
<td>Mixed UC vs. pure UC</td>
<td>6%</td>
<td>(-12%, 24%)</td>
<td>0.52</td>
</tr>
<tr>
<td>4</td>
<td>RC-only</td>
<td>148</td>
<td>Mixed UC vs. pure UC</td>
<td>-8%</td>
<td>(-17%, 2%)</td>
<td>0.10</td>
</tr>
<tr>
<td>5</td>
<td>All patients</td>
<td>295</td>
<td>MVAC*mixed Interaction</td>
<td>14%</td>
<td>(-6%, 33%)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Adjusted effect = additive down-staging effect, adjusted for clinical stage using the least-squares model with identity link function and robust covariance matrix estimator; CI = confidence intervals
The effect of MVAC on tumor down-staging had the same direction within each clinical stage for both pure UC and for mixed tumors, without sufficient evidence of treatment-by-stage interaction ($p = 0.91$).

Among patients with mixed tumors randomized to MVAC-plus-cystectomy, stage pT0 at the time of definitive surgery was observed in 6 of 20 patients with urothelial and squamous differentiation and in 5 of 10 patients with urothelial and glandular differentiation. Hence, pathological down-staging following chemotherapy was observed in both sub-types of mixed tumors, and clearly was not limited to only one subtype (squamous or glandular).

### 3.3.2 Survival analysis

Table 14 shows the estimated hazard ratios for the effect of MVAC on all-cause mortality among patients with mixed tumors (model 1) and among patients with pure UC (model 2). Also included in Table 13 are the estimated hazard ratios for the effect of mixed histology on all-cause mortality among patients randomized to MVAC-plus-cystectomy (model 3) and among patients randomized to cystectomy alone (model 4). An estimate of the treatment-by-histologic type interaction effect was obtained from model 5. For all models in Table 14, model assumptions were tested as described in the methods section and could not be rejected at the conventional level of significance (all $p$-values $> 0.05$).
### Table 14 Estimated hazard ratios

<table>
<thead>
<tr>
<th>Model</th>
<th>Patients included in the model</th>
<th>N</th>
<th>Contrast</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mixed UC</td>
<td>59</td>
<td>MVAC+RC vs. RC-only</td>
<td>0.46</td>
<td>(0.25, 0.87)</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>Pure UC</td>
<td>236</td>
<td>MVAC+RC vs. RC-only</td>
<td>0.90</td>
<td>(0.67, 1.21)</td>
<td>0.48</td>
</tr>
<tr>
<td>3</td>
<td>MVAC+RC</td>
<td>147</td>
<td>Mixed UC vs. pure UC</td>
<td>0.69</td>
<td>(0.42, 1.13)</td>
<td>0.14</td>
</tr>
<tr>
<td>4</td>
<td>RC-only</td>
<td>148</td>
<td>Mixed UC vs. pure UC</td>
<td>1.28</td>
<td>(0.80, 2.06)</td>
<td>0.30</td>
</tr>
<tr>
<td>5</td>
<td>All patients</td>
<td>295</td>
<td>MVAC*mixed Interaction</td>
<td>0.52</td>
<td>(0.28, 1.09)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

HR = hazard ratio, adjusted for age (as a continuous covariate) and clinical stage (as a stratification variable); CI = confidence intervals

There was evidence of a survival benefit from chemotherapy in patients with mixed tumors (HR=0.46; 95% CI: 0.25, 0.87; p=0.02). Patients with pure UC had improved survival on the chemotherapy arm however the survival benefit was not statistically significant (HR=0.90; 95% CI: 0.67, 1.21; p=0.48). There was marginal evidence that the survival benefit of chemotherapy in patients with mixed tumors was greater than it was for patients with pure UC (statistical interaction, p=0.09) (Hypothesis 1.2). These analyses also suggested that compared to pure UC, mixed tumors may be associated with increased mortality when treated with cystectomy alone and with decreased mortality when treated with MVAC-plus-cystectomy, although the estimated hazard ratios were not statistically significant in these two comparisons (rows 3 and 4 of Table 14). Table 15 shows the age-standardized five-year survival estimates by treatment, histologic type, and clinical stage obtained from the Cox model. The estimated improvement in five-year survival associated with MVAC was greater in magnitude among patients with mixed tumors than among patients with pure UC.
Table 15 Estimated five-year survival probabilities

<table>
<thead>
<tr>
<th>Stage</th>
<th>Treatment</th>
<th>Pure UC</th>
<th></th>
<th>Mixed UC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5-yr survival</td>
<td>95% CI</td>
<td>5-yr survival</td>
<td>95% CI</td>
</tr>
<tr>
<td>cT2</td>
<td>RC-only</td>
<td>0.61 (0.52,0.72)</td>
<td></td>
<td>0.54 (0.39,0.74)</td>
<td></td>
</tr>
<tr>
<td>cT2</td>
<td>MVAC+RC</td>
<td>0.64 (0.55,0.74)</td>
<td></td>
<td>0.73 (0.62,0.86)</td>
<td></td>
</tr>
<tr>
<td>cT3-T4a</td>
<td>RC-only</td>
<td>0.42 (0.34,0.53)</td>
<td></td>
<td>0.34 (0.21,0.55)</td>
<td></td>
</tr>
<tr>
<td>cT3-T4a</td>
<td>MVAC+RC</td>
<td>0.46 (0.37,0.56)</td>
<td></td>
<td>0.58 (0.45,0.75)</td>
<td></td>
</tr>
</tbody>
</table>

†adjusted for age by conditional standardization (conditioned on the average age of all patients in the study); cT2 = clinical stage T2, cT3-T4a = clinical stage T3-T4a

All estimates in Tables 14 and 15 were controlled for age and clinical stage. These covariates were pre-specified in the original study protocol as stratification factors for survival analysis. Because the covariates gender and race also appeared to be somewhat unbalanced between the comparison subgroups (Table 9), we performed sensitivity analyses by including these covariates in the model. Gender and race were not independently associated with all-cause mortality in these analyses (after adjusting for age, clinical stage and histologic type) and had no substantial impact on reported findings.

3.4 Discussion

The purpose of this study was to determine whether the effect of neo-adjuvant MVAC on pathological down-staging and survival of patients with locally advanced UC of the bladder treated with radical cystectomy depends on the presence of squamous and/or glandular components in the tumor. This question is important because squamous and/or glandular differentiation co-existing with malignant urothelial histology is a fairly common finding in muscle-invasive BC. To our knowledge, it has never been clearly demonstrated that neo-adjuvant PBCC can induce complete pathological response (stage pT0) and improve survival of patients with these mixed tumors. Before results of the
current analyses became available, we considered it possible that the presence of non-urothelial components could render UC resistant to MVAC because pure non-urothelial cancers of the bladder tend to be resistant to chemotherapy [112]. If this was confirmed, then patients with mixed tumors could potentially benefit from immediate cystectomy (without neo-adjuvant chemotherapy), especially since delaying cystectomy for more than 3 months from diagnosis of muscle invasion has been associated with decreased survival in some patient populations [96].

Our current analyses have provided no evidence that the presence of squamous and/or glandular differentiation in locally advanced UC of the bladder confers resistance to MVAC. Evidence of tumor down-staging to pT0 following chemotherapy was clearly present among patients with pure UC (ADE = 15%, $p = 0.004$) and among patients with mixed tumors (ADE = 27%, $p = 0.004$) (Table 13). These down-staging effects may be underestimated because we included in the analyses patients whose pT0 status was unknown (Table 10), making a conservative assumption that all of these patients would have residual disease in the cystectomy specimen if their definitive surgery was performed (i.e., they were all considered not to be pT0). However, it is possible that some patients who refused cystectomy did so because of complete clinical response to chemotherapy. If some of these patients in fact had no residual disease, then the down-staging effect of chemotherapy reported in Table 8 could be underestimated. This down-staging effect (estimated under the most conservative assumptions) was nevertheless relatively large in magnitude, especially for patients with mixed tumors (27% on the additive scale or 27 extra pT0s per 100 patients treated, $p = 0.004$).
The estimated survival benefit of chemotherapy among patients with mixed tumors was also relatively large (HR = 0.46, p = 0.02). For patients with pure UC, the estimated improvement in survival following chemotherapy was not as substantial (HR = 0.90, p = 0.48). We used the hazard of death from all causes as opposed to the hazard of death from BC as the outcome measure in survival analysis for the following reasons. First, unlike the BC-specific hazard, all-cause mortality is not affected by potential misclassification of the causes of death. For example, patients who die from comorbid conditions exacerbated by BC progression or BC therapy (such as MVAC) may potentially be counted as “non-BC” fatalities in the analysis of BC-specific hazard. This may result in misleading estimates of survival benefit associated with a given therapeutic intervention. Another limitation of BC-specific hazard as the outcome measure (relative to all-cause mortality) is decreased precision of estimation. The variance of the estimated regression coefficients in the Cox model depends very strongly on the total number of terminal events in the study sample. In the analysis of BC-specific hazard, deaths from causes other than BC are regarded as censoring events [111]. Hence, the estimated effect of covariates on disease-specific hazard tends to be less precise than the estimated effect of covariates on the hazard of death from all causes. All-cause mortality was also the primary end-point of the trial according to the original study protocol.
3.4.1 *Strengths and limitations*

The strengths of this study include randomized treatment allocation, central pathological review (performed for 85% of the patients), the use of the standard treatment protocol (MVAC as the only form of neo-adjuvant chemotherapy, no prior pelvic irradiation, etc.), and rigorous follow-up (median 8.5 years). However, some limitations of this study exist. First, the results of the current analyses may be difficult or impossible to generalize to mixed urothelial tumors with non-urothelial components other than squamous cell or adenocarcinoma. For example, there is evidence to suggest that small cell tumors of the bladder may respond better to neuroendocrine regimens than to urothelial regimens such as MVAC [112]. Second, even for squamous and glandular components, survival could not be analyzed separately in the current study because the estimated hazard ratios would be highly imprecise due to small sample size for individual histologic sub-types (for adenocarcinoma, this comparison would be based on only 10 patients in each treatment arm). However, our analyses suggested that pathological down-staging following chemotherapy occurs in both sub-types of mixed tumors (squamous and glandular), and clearly is not limited to only one subtype. Hence, it is unlikely that improvement in survival following chemotherapy is limited to only one of the two sub-types.

It must also be recognized that definition and documentation of mixed histology may vary between pathologists. In our study, histologic type of 50 patients was determined by institutional pathology report because slides were not available for the central review (these patients contributed 12 of the 59 mixed histology cases). Hence some potential misclassification of histologic types in our study could occur, and this must be recognized
as a limitation. Similarly, proportions of non-urothelial components in mixed histology
tumors could not be obtained from this analysis (it was rarely reported) and thus the
impact of this proportion on response to MVAC or outcome in the cystectomy-only arm
could not be ascertained. The biological reasons for apparent sensitivity of mixed tumors
to MVAC are not clear at this point. It is possible that non-urothelial differentiation in
UC of the bladder is a manifestation of high grade disease with rapid cell proliferation.
Because chemotherapy such as MVAC affects primarily actively dividing cells, mixed
tumors may be more sensitive to it than pure UC. This explanation however is only
hypothetical at this time since no data are currently available to support or refute it.

Another important question which needs to be considered is whether results reported in
this paper are fully applicable to PBCC regimens other than MVAC. In the setting of
metastatic disease, MVAC seems to result in the same survival as a less toxic regimen
composed of gemcitabine and cisplatin (GC) [99]. However, the two regimens have never
been directly compared in the setting of apparently resectable BC. Hence, the ability of
GC to induce complete pathological response and improve survival of patients with
locally advanced UC of the bladder with non-urothelial components remains uncertain.

3.4.2 Future studies

Some of the questions that could not be fully answered in this study may potentially be
examined in secondary analyses of other trials of neo-adjuvant PBCC for locally
advanced BC, and possibly in pooled analyses of two or more trials. For example, a
European trial of CMV (cisplatin, methotrexate, and vinblastine) enrolled more than 900
patients with stage T2-T4a N0/NX M0 BC [89]. To our knowledge, the effect of CMV in that trial has not been examined separately for patients with pure UC and for patients with mixed tumors. A pooled analysis of two or more trials would improve statistical power and increase the precision of estimation. This would be particularly beneficial for the formal test of statistical interaction between treatment and histologic type. Unfortunately, interaction effects are difficult to detect in studies powered for the analysis of the main effects [126]. Even large interactions often produce p-values above $\alpha = 0.05$ (the conventional level of significance for the main effects) in moderate sized studies. Secondary analyses of larger trials and pooled analyses of two or more trials may be particularly beneficial in these situations.

### 3.5 Conclusion

In summary, presence of squamous or glandular differentiation in locally advanced UC of the bladder does not confer resistance to MVAC and in fact may be an indication for the use of neo-adjuvant chemotherapy prior to radical cystectomy.
Chapter 4: Specific Aim 2

4.1 Introduction

Analyses reported in the “Results” section of Specific Aim 1 demonstrated that the use of neo-adjuvant MVAC in patients with locally advanced UC of the bladder treated with radical cystectomy may substantially increase the probability of pathological stage zero at the time of definitive surgery and improve survival. When patients with pure UC and patients with mixed tumors were considered separately, the down-staging effect of MVAC was statistically significant in both groups (pure UC = 15%, p = 0.004; mixed tumors = 27%, p = 0.004; interaction = 0.17). There was evidence of a survival benefit from chemotherapy in patients with mixed tumors (HR=0.46; 95%CI: 0.25, 0.87; p=0.02). Patients with pure UC had improved survival on the chemotherapy arm however the survival benefit was not statistically significant (HR=0.90; 95%CI: 0.67, 1.21; p=0.48). There was marginal evidence that the survival benefit of chemotherapy in patients with mixed tumors was greater than it was for patients with pure UC (statistical interaction, p=0.09)

Although in the trials of neo-adjuvant chemotherapy, MVAC (administered in 3 cycles) appeared to be the most effective regimen in terms of pathological down-staging and survival, it had a relatively unfavorable toxicity profile, producing severe granulocytopenia (grade 4 or < 500 cells / microL) in one third of all patients treated [83]. In clinical practice, a less toxic neo-adjuvant chemotherapy regimen composed of gemcitabine and cisplatin (GC) is now commonly substituted for neo-adjuvant MVAC [97]. In the setting of metastatic disease, GC seems to result in the same survival as
MVAC but with better toxicity profile, based on one report from a randomized trial [99]. In this study, severe (grade 4) granulocytopenia was observed in 65% of patients after a median of 4 cycles of MVAC and in 30% of patients after a median of 6 cycles of GC. Overall survival did not differ between the two chemotherapy regimens in this study (p = 0.75). However, it has been argued in the literature that extrapolation of these results to resectable (non-metastatic) BC is potentially invalid, because the biology of metastatic and locally advanced BC may be different, and survival of patients with metastatic disease is usually very poor despite the use of combination chemotherapy (MVAC or other regimens) [97]. The effect of GC on pathological down-staging and survival of patients with locally advanced BC has been investigated in two small single-institution series with somewhat conflicting results [97,98].

In the series published by Dash et al, 42 patients received neo-adjuvant GC and 54 patients received neo-adjuvant MVAC prior to cystectomy for locally advanced UC of the bladder. Chemotherapy allocation was primarily determined by calendar time (patients treated in more recent years received GC). The distribution of clinical stages (which is known to affect the probability of pT0) favored MVAC: 45% of patients in the GC group and 59% of patients in the MVAC group had clinical stage cT2 (organ-confined disease) (Table 16). Pathological stage zero at the time of definitive surgery was documented in 26% (11/42) of patients treated with GC and in 28% (15/54) of patients treated with MVAC (Table 16). Survival of patients in the two chemotherapy groups was not formally compared in this study [98].
In the second series published by Weight et al, 20 patients treated with neo-adjuvant GC followed with cystectomy were compared to 88 patients treated with cystectomy alone. The decision to administer neo-adjuvant chemotherapy in this cohort was determined by patients’ ability to tolerate chemotherapy as well as patients’/physicians’ preferences. Among patients treated with chemotherapy, 79% had clinical stage T2. The corresponding proportion of cT2s for patients treated with cystectomy alone was not reported. Pathological stage zero at the time of definitive surgery was documented in 10% (2/20) of patients treated with neo-adjuvant GC followed with cystectomy, and in 9% (8/88) of patients treated with cystectomy alone (Table 16). Overall survival did not differ significantly between the two treatment groups in this series (log-rank p = 0.91) [97].

The two observational studies of neo-adjuvant GC discussed in this section seem to provide somewhat conflicting evidence regarding the effect of GC on tumor down-staging in locally advanced BC (Table 16). The first series (Dash et al 2008) suggested that GC may be as effective as MVAC in terms of pathological response, although the effect of chemotherapy relative to cystectomy alone was not reported in that study. The second series (Weight et al 2009) documented a lower proportion of pT0s following neo-adjuvant GC compared to Dash et al’ series (10% vs. 26%). The proportion of pT0s reported by Weight et al was nearly identical to the proportion observed among patients treated with cystectomy alone in their study cohort, indicating lack of tumor down-staging following chemotherapy.
Table 16 Case-series of neo-adjuvant GC for locally advanced BC

<table>
<thead>
<tr>
<th>Comparison groups</th>
<th>Dash et al [98]</th>
<th>Weight et al [97]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GC+ cystectomy</td>
<td>MVAC+ cystectomy</td>
</tr>
<tr>
<td>N</td>
<td>42</td>
<td>54</td>
</tr>
<tr>
<td>%cT2</td>
<td>45%</td>
<td>59%</td>
</tr>
<tr>
<td>No. of cycles (median)</td>
<td>4</td>
<td>Not reported</td>
</tr>
<tr>
<td>%pT0</td>
<td>26%</td>
<td>28%</td>
</tr>
</tbody>
</table>

cT2 = clinical stage T2, pT0 = pathological stage 0

The differences between the two studies could potentially be attributed to certain systematic factors, although the effect of random error (sampling variation) alone could also be very substantial due to small sample size. In fact, a formal hypothesis test for equality of two binomial proportions: 0.26 (Dash 2008) and 0.10 (Weight 2009) would result in a two-sided p-value of 0.19 (Fisher’s exact test). Hence, the null hypothesis of equality would not be rejected at the conventional level of significance.

Among the systematic factors that could contribute to the observed difference in the proportions of pT0s following neo-adjuvant GC reported from the two studies one could consider differences in the distribution of clinical stages and the number of chemotherapy cycles completed. Among patients treated with neo-adjuvant chemotherapy, the proportion of patients with organ-confined disease based on clinical staging (cT2) was much higher in Weight et al’s cohort (79%) than in Dash et al’s series (45% for GC and 59% for MVAC, Table 16). Hence, Dash et al observed a higher proportion of pT0s despite less favorable distribution of clinical stages [97,98]
The average number of chemotherapy cycles completed appeared to be greater in Dash et al’s series, where 93% of all patients completed $\geq 4$ cycles of GC, while Weight et al reported a median of 3 cycles (which means that less than half of all patients received $\geq 4$ cycles, Table 16). Most patients in Dash et al’s study received chemotherapy in 21-day cycles with one of two schedules: (1) 70 mg/m$^2$ of cisplatin on day 1 and 1000 mg/m$^2$ of gemcitabine on days 1 and 8, or (2) 35 mg/m$^2$ of cisplatin and 1000 mg/m$^2$ of gemcitabine on days 1 and 8. The chemotherapy schedule (per cycle) in Weight et al’s cohort was not reported [97,98]. Due to small sample size, it is difficult to determine whether the difference in the average number of chemotherapy cycles completed could explain the observed difference in the outcome in these two series, although this hypothesis is biologically plausible and deserves further investigation.

In summary, it is currently not clear whether neo-adjuvant chemotherapy with GC can induce tumor down-staging and improve survival of patients with locally advanced BC treated with radical cystectomy. It is also not known whether the effect of GC on these measures of outcome depends on the presence of mixed histologic features in the tumor. The impact of the number of chemotherapy cycles completed on tumor down-staging also remains uncertain.

The purpose of the current study is to investigate the effect of neo-adjuvant chemotherapy with GC on pathological down-staging and survival of patients with locally advanced urothelial carcinoma of the bladder treated with cystectomy at Strong
Memorial Hospital (Rochester NY) during the years of 1999-2009. This study addressed the following hypotheses:

H.2.1 Patients treated with neo-adjuvant GC are more likely to have pathological stage zero at cystectomy compared to patients who do not receive neo-adjuvant chemotherapy, after adjusting for clinical stage at presentation.

H.2.2. Patients treated with neo-adjuvant GC have decreased hazard of death from all causes compared to patients who do not receive neo-adjuvant chemotherapy, after adjusting for covariates associated with mortality.

H.2.3. Patients treated with neo-adjuvant GC have decreased hazard of death from BC compared to patients who do not receive neo-adjuvant chemotherapy, after adjusting for covariates associated with mortality.

Additional (exploratory) analyses included investigation of the effect of chemotherapy on alternative end-points of tumor down-staging such as (1) residual muscle-invasive disease at cystectomy (present or absent), (2) direct extravesical extension at cystectomy (present or absent), (3) nodal status at cystectomy (nodal metastases present or absent), and (4) surgical margins at cystectomy (positive or negative). We also examined the effect of mixed histology and the number of chemotherapy cycles completed on tumor down-staging by neo-adjuvant GC.
4.2 Materials and Methods

4.2.1. Study Design

This retrospective cohort study included patients with locally advanced UC of the bladder (clinical stages T2-T4a, N any, M0/MX) treated with radical cystectomy with or without neo-adjuvant chemotherapy at Strong Memorial Hospital (Rochester NY) during the years of 1999-2009. Patients who received neo-adjuvant chemotherapy other than GC and/or pelvic irradiation prior to cystectomy were excluded.

The primary exposure variable of interest was defined as the use of neo-adjuvant chemotherapy with GC. This variable took the value of 1 if neo-adjuvant chemotherapy was administered and the value of 0 otherwise.

The primary outcome variable was defined as pathological stage at cystectomy. This variable took the value of 1 if no tumor was found in the cystectomy specimen (pT0 based on the pathology report) and the value of 0 otherwise. This outcome variable was used in the test of the primary hypothesis of the proposed study (H.2.1). Secondary measures of outcome were defined as the hazard of death from all causes (H.2.2) and the hazard of death from BC (H.2.3).
Alternative end-points of tumor down-staging in the exploratory analyses were defined as
(1) residual invasion of lamina propria at cystectomy (present or absent), (2) residual
muscle invasive at cystectomy (present or absent), (3) direct extravesical extension at
cystectomy (present or absent), (4) nodal status at cystectomy (nodal metastases present
or absent), and (5) surgical margins at cystectomy (positive or negative).

Other covariates selected for inclusion in the study database are listed in Table 17. These
covariates were used for description of the study cohort and/or for modeling purposes as
described in the next section.
Table 17 Variables selected for inclusion in the study database

<table>
<thead>
<tr>
<th>Demographics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (in years)</td>
</tr>
<tr>
<td>Sex (male, female)</td>
</tr>
<tr>
<td>Race (white, other)</td>
</tr>
</tbody>
</table>

**Clinical staging and assessment**

- The most advanced clinical T stage documented prior to the initiation of definitive therapy (T2, T3-T4a)\(^1\)
- Any regional adenopathy (any description of enlarged lymph nodes on CT of the abdomen and pelvis, whether or not number and size were stated)\(^2\)
- Regional adenopathy >1cm (at least one regional node enlarged by CT size criteria, that is >1cm)\(^2\)
- Major cardiovascular disease (conditions documented in this field include myocardial infarction, heart surgery with or without myocardial infarction, stroke, congestive heart failure, and deep venous thrombosis not related to surgery)\(^3\)
- Major respiratory disease (conditions documented in this field include asthma, emphysema, and chronic obstructive pulmonary disease)\(^3\)
- Other malignancies (conditions documented in this field include all malignancies other than non-melanoma skin cancer and prostate cancer discovered at cystectomy)\(^3\)
- Smoking status (current, former, never)\(^4\)
- Number of smoking years\(^4\)
- Histologic type at TURBT (pure UC, UC with SD, UC with GD, …)
- Tumor grade (low, high)
- Creatinine clearance documented prior to the initiation of definitive therapy (mL/min)\(^5\)
- White blood cell count documented prior to the initiation of definitive therapy (cells/L)
- Platelet count documented prior to the initiation of definitive therapy (cells/L)

**Neo-adjuvant (pre-cystectomy) therapy**

- Systemic neo-adjuvant chemotherapy (GC vs. none)
- Number of systemic neo-adjuvant chemotherapy cycles completed (0, 1, 2, …)
- Intravesical therapy (BCG, mitomycin, alpha-interferon, …)

**Pathological staging and assessment**

- Pathological T stage at cystectomy
- Number of nodes examined
- Number of positive nodes
- Histologic type at cystectomy (pure UC, UC with SD, UC with GD, …)
- Lymphovascular invasion at cystectomy (present, absent)
- Ureteral margins (positive or negative)
- Urethral margins (positive or negative)
- Peripheral margins (positive or negative)

**Follow-up data**

- Time from initiation of definitive therapy until death from any cause or end of follow-up
- Underlying cause of death

**Other variables**

- Adjuvant (post-cystectomy) chemotherapy regimen (if any)
- Number of adjuvant chemotherapy cycles completed
- An indicator variable for the surgeon (this variable will take the same value for all patients treated by the same surgeon)\(^6\)

---

\(^1\) Based on TURBT pathology, pelvic CT report, and physical examination performed by urologist

\(^2\) Based on CT report of the abdomen and pelvis and physician correspondence / clinic notes

\(^3\) Based on “past medical history” section in the chart (cystectomy discharge summary, clinic notes / correspondence)

\(^4\) Based primarily on “social history” section in the chart

\(^5\) Computed according to the MDRD 4 variables equation, reference [131,132]

\(^6\) This variable is a numerical code (1,2,3,… ) not related to any personal identifying information of the patients or the physicians
The study subjects were identified through the Urology Billing Department using the CPT codes for cystectomy (51550, 51570, 51575, 51590, 51595, 51596, 51597, 51999). The medical record numbers of all patients treated with cystectomy at Strong Memorial Hospital during the years of 1999-2009 were obtained from the Urology Billing Department and clinical information was obtained from electronic medical records of these patients (Table 17).

The study database is stored in SAS format on secure password protected University of Rochester servers: the working database file is stored on Cerebro/(Q) server (Department of Community and Preventive Medicine) and the back-up database file is stored on the SSH Secure Shell Server. This study was approved by the Research Subjects Review Board at the University of Rochester.

4.2.2. Methods of Data Analysis

4.2.2.1 Tumor down-staging

The proportions of patients with stage pT0 at cystectomy were compared between the two treatment groups (neo-adjuvant GC vs. no neo-adjuvant chemotherapy) using the chi-square test or the Fisher’s exact test (as appropriate) in bivariable analysis, and the generalized linear model with Bernoulli distribution of the outcome and identity link function in multivariable analysis, with adjustment for clinical stage (H.2.1). Model parameters were estimated by maximizing the likelihood function with the Fisher scoring algorithm, as described in section 3.2.2.3.1 of Specific Aim 1 [113]. The same model was
used for exploratory analysis of the effect of mixed histology and the number of chemotherapy cycles completed on tumor down-staging to pT0 by neo-adjuvant GC.

For the purpose of these exploratory analyses, mixed histology was defined as a binary variable (pure UC or mixed tumors), and the number of cycles completed was defined as an ordinal variable (0, 1, 2, 3, 4). Other candidate covariates included regional adenopathy, the number of nodes examined, and the indicator variables for the surgeon. All main effects and the interaction of GC with clinical stage and histologic type (the most plausible interaction effects from the biological point of view) were tested at $\alpha = 0.05$. Other interactions were tested at $\alpha = 0.01$.

For sensitivity analysis, the estimated effect of neo-adjuvant GC on tumor down-staging was also controlled for clinical stage by direct standardization as described in section 3.2.2.3.2 of Specific Aim 1. The main difference in the application of this method in Specific Aim 2 is in the choice of the standard distribution of clinical stages. In randomized trials, it is often meaningful to estimate the causal effect of exposure in the entire study sample (exposed and unexposed subjects combined) because allocation of the study participants to intervention arms is determined by randomization process. In observational studies, estimation of the causal effect of exposure in the entire study cohort often has no biological meaning. For example, in the context of the proposed study, patients with inadequate renal function could experience extremely high mortality rates if they received neo-adjuvant GC. Therefore it is not very meaningful to ask, what would be the proportion of pT0s among these patients following chemotherapy with GC, since
these patients would not be able to tolerate such therapy. On the other hand, standardization of the down-staging effect of GC to the distribution of clinical stages among the exposed subjects corresponds to a biologically meaningful counterfactual contrast – the causal effect of exposure in the exposed cohort [117].

For the purpose of exploratory analyses, we also examined the effect of GC on alternative end-points of tumor down-staging. Ordinal logistic regression (the proportional odds model) was used for these exploratory analyses. This model is described in detail in section 4.2.2.3.2. The outcome variable was defined as an ordinal variable with the following ordered categories: (1) stage pT0, (2) stage pTa/Tis, (3) stage pT1, (4) stage pT2, (5) stage pT3/T4.

4.2.2.2 Survival analysis

The effect of neo-adjuvant GC on the hazard of death from all causes and the hazard of death from BC was estimated using the Cox model with adjustment for covariates (such as age, and clinical stage) that are associated with mortality but are not located in the causal pathway between the exposure (neo-adjuvant GC) and the outcome (survival). Note that no adjustment was performed for pathological stage and the use of adjuvant (post-cystectomy) chemotherapy. Pathological stage at cystectomy is clearly located in the causal pathway between the exposure (neo-adjuvant GC) and the outcome (survival) and is also strongly associated with the use of adjuvant therapy. Patients with stage pT0 almost never receive adjuvant therapy, while patients with residual disease in the cystectomy specimen, particularly those with gross extravesical tumor extension, positive
tissue margins, and/or positive nodes are very likely to receive some form of adjuvant therapy. In other words, the use of adjuvant therapy is a “marker” for extensive residual disease after cystectomy. According to the study hypothesis, neo-adjuvant GC improves survival by reducing the probability (and the extent) of residual disease after definitive surgery. Hence, when the effect of neo-adjuvant GC on mortality is conditioned on the use of adjuvant therapy, it is also conditioned on pathological stage at cystectomy and therefore is biased for the causal effect under investigation.

Survival time in the current study was measured from the initiation of definitive therapy until death from any cause. Patients who were alive at the end of follow-up were censored. Assumptions of the Cox model were tested as described in Specific Aim 1 (p56). In the analysis of BC-specific hazard, deaths from other causes were regarded as censoring events [111].

Information on vital status, the time of death, and the cause of death (if deceased) was obtained from the National Death Index (NDI) [115]. Information in the NDI is derived from the death certificate. Part I of the death certificate includes the immediate cause of death as well as the underlying cause (disease or injury that initiated events resulting in death). Part II of the death certificate includes other significant conditions contributing to death but not resulting in the underlying cause in Part I [115]. In the current study, BC was considered the cause of death only if it was listed in Part I of the death certificate.
The following information was submitted to the NDI for each subject: (1) first name, (2) last name, (3) date of birth, (4) social security number. These data items were matched to mortality records by the NDI staff. For the purpose of this study, matching was considered successful if it occurred on items (2), (3), and (4) and the first name in the NDI records either (i) matched the first name in the hospital records or (ii) could be recognized as a modified form of the first name in the hospital records (e.g., Michael = Mike) or (iii) matched the second name documented in the hospital records. For patients who died at Strong Hospital, the cause of death from the discharge notes / autopsy report (if available) was compared to the NDI records for quality assurance. In the event of disagreement, priority would be given to the discharge notes / autopsy report.

Accuracy of the NDI data has been examined in many studies, reviewed in reference [128]. With the use of the social security number, the sensitivity of the NDI (the probability of finding an NDI record given that the patient is deceased) was equal to 97% and the specificity exceeded 99% [128,129]. In a study comparing the underlying cause of death from the NDI records to the underlying cause of death assigned by expert review of the death certificates at the National Center for Health Statistics, the accuracy of the NDI records was equal to 99% for cancer causes and 96% for all causes of death combined [130].

Request for survival data was submitted to the NDI by express-mailing a CD-ROM with encrypted and password protected patient information (first and last name, social security number, and the date of birth). The password and the decryption key were submitted to
the NDI (by e-mail) separately from the data file. This process has been reviewed and approved by the Research Subject Review Board and the Chief Privacy Officer at the University of Rochester.

In the process of data collection, one may often find that information on certain variables may not be available for some patients. The following approach to missing data was followed in the current study.

[1] All patients with known information on exposure (GC vs. none), primary outcome (pT0 vs. residual disease) and clinical stage were included in the test of the primary hypothesis of the study (H.2.1) because adjustment for clinical stage is likely to be a sufficient condition for unbiased or approximately unbiased estimation of the effect of chemotherapy on tumor down-staging.

[2] All patients with known information on exposure (GC vs. none), clinical stage, age, sex, and comorbidities were included in survival analysis (H.2.2-H.2.3) because adjustment for these covariates is likely to be a necessary condition for unbiased or approximately unbiased estimation of the effect of chemotherapy on survival.

For the purpose of exploratory analysis, patients were included in the models if they had information on all relevant covariates.
4.2.2.3 Statistical models

4.2.2.3.1 Proportional odds model

The cumulative odds for the $j$th level of the ordinal outcome variable with $k$ levels may be defined as

$$O_c(Y = j) = \frac{P(Y \leq j)}{P(Y > j)} = \frac{P(Y \leq j)}{1 - P(Y \leq j)}$$

The model for the cumulative odds is written as

$$O_c(Y = j | x) = \frac{P(Y \leq j | x)}{1 - P(Y \leq j | x)} = \exp(\beta_{0j} + \beta_1x_1 + \beta_2x_2 + \ldots + \beta_px_p)$$

Or equivalently, in terms of the cumulative logit

$$\ln\left(\frac{P(Y \leq j | x)}{1 - P(Y \leq j | x)}\right) = \beta_{0j} + \beta_1x_1 + \beta_2x_2 + \ldots + \beta_px_p$$

Note that in this model, only the intercepts are level-specific. The slope coefficients are assumed to be the same for all levels (this is the proportional odds assumption). Hence, the regression coefficient for covariate $x_i$ may be interpreted as an additive change in the cumulative logit due to one unit increase in the value of $x_i$, holding other covariates constant. Similarly, the exponentiated regression coefficient for covariate $x_i$ may be interpreted as a multiplicative change in the cumulative odds due to one unit increase in the value of $x_i$, holding other covariates constant. In other words, the odds of $Y \leq j$ given $x_i + 1$ are ($\exp(\beta_i)$) times greater than the odds of $Y \leq j$ given $x_i$ [113,114].

Note from $$\frac{P(Y \leq j | x)}{1 - P(Y \leq j | x)} = \exp(\beta_{0j} + \beta_1x_1 + \beta_2x_2 + \ldots + \beta_px_p)$$ that the probability of $Y \leq j$ (when $j \neq k$) may be written as
\[ P(Y \leq j \mid x) = \frac{\exp(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_p x_p)}{1 + \exp(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_p x_p)}, \]

which is very similar to binary logistic regression. In fact, for any given value of \( j \) (except \( j = k \)), the proportional odds model may be viewed as the binary logistic model with \( Y \leq j \) representing a “success”, and \( Y > j \) representing a “failure”. Note that when \( j = k \), \( P(Y \leq j) = P(Y \leq k) = 1 \). Hence the proportional odds model contains a total of \( k - 1 \) estimated intercept terms. The proportional odds assumption may be tested with a score test in PROC LOGISTIC in SAS.

4.2.3. Power and Sample Size Considerations

This section contains description of power analysis and sample size calculations. The power analysis is based on the test of the primary hypothesis of the proposed study (H.2.1), although sample size considerations for survival analysis are also discussed in this section.

Let \( P_1 \) denote the probability of stage pT0 at cystectomy among patients treated with neo-adjuvant GC and let \( P_2 \) denote the probability of stage pT0 at cystectomy among patients treated with immediate cystectomy. In the SWOG study, the conservatively estimated probability of stage pT0 at cystectomy in the chemotherapy arm was 30% (for pure UC and mixed tumors combined). The corresponding conservative estimate in the cystectomy-only arm was 11% [83]. Hence, for the purpose of power analysis, it is reasonable to specify the following conditions: \( P_1 = 0.3, P_2 = 0.1, \) and \( \alpha = 0.05 \) (two-sided test). The total number of patients treated without neo-adjuvant chemotherapy in
the proposed study is expected to be at least 100 and can be equal to 150 or more (we will denote this number by $n_2$). Table 18 shows power as a function of the number of patients treated with neo-adjuvant GC ($n_1$), assuming that H.2.1 will be tested with the chi-square test of significance and no adjustment for clinical stage will be required (power with adjustment for clinical stage will be considered later in this section). Power for a larger effect size ($P_1 = 0.35$, $P_2 = 0.10$, $\alpha = 0.05$) is shown in Table 19.

Table 18 Power analysis: chi-square test $P_1=0.30$, $P_2=0.10$, $\alpha = 0.05$

<table>
<thead>
<tr>
<th>$n_1$</th>
<th>$n_2 = 125$</th>
<th>$n_2 = 150$</th>
<th>$n_2 = 175$</th>
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<tr>
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<tr>
<td>31</td>
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<td>0.79</td>
<td>0.80</td>
</tr>
<tr>
<td>33</td>
<td>0.78</td>
<td>0.80</td>
<td>0.81</td>
</tr>
<tr>
<td>35</td>
<td>0.80</td>
<td>0.82</td>
<td>0.83</td>
</tr>
</tbody>
</table>

$n_1$ = number of patients treated with neo-adjuvant GC
$n_2$ = number of patients treated without neo-adjuvant chemotherapy
$P_1$ = probability of stage pT0 with chemotherapy, $P_2$ = probability of stage pT0 without chemotherapy

Table 19 Power analysis: chi-square test $P_1=0.35$, $P_2=0.10$, $\alpha = 0.05$

<table>
<thead>
<tr>
<th>$n_1$</th>
<th>$n_2 = 125$</th>
<th>$n_2 = 150$</th>
<th>$n_2 = 175$</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.84</td>
<td>0.86</td>
<td>0.87</td>
</tr>
<tr>
<td>27</td>
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<tr>
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<tr>
<td>31</td>
<td>0.89</td>
<td>0.90</td>
<td>0.91</td>
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<tr>
<td>33</td>
<td>0.90</td>
<td>0.91</td>
<td>0.92</td>
</tr>
<tr>
<td>35</td>
<td>0.91</td>
<td>0.92</td>
<td>0.93</td>
</tr>
</tbody>
</table>

$n_1$ = number of patients treated with neo-adjuvant GC
$n_2$ = number of patients treated without neo-adjuvant chemotherapy
$P_1$ = probability of stage pT0 with chemotherapy, $P_2$ = probability of stage pT0 without chemotherapy
These sample size and power calculations were obtained from PASS 97 (software for power analysis). Equivalently, power can be computed using the following equation [122]

\[
\text{Power} = \Phi\left(\Delta - z_{1-\alpha/2} \sqrt{\frac{\bar{P}(1-\bar{P})[(1/n_1) + (1/n_2)]}{\frac{P_1(1-P_1)}{n_1} + \frac{P_2(1-P_2)}{n_2}}}\right)
\]

where \( \Delta = |P_1 - P_2| \) and \( \bar{P} = \frac{n_1P_1 + n_2P_2}{n_1 + n_2} \)

For example, with \( \alpha = 0.05 \), \( n_1 = 30 \), \( n_2 = 150 \), \( P_1 = 0.30 \), and \( P_2 = 0.10 \), we have

\[
\Delta = 0.2, \quad \bar{P} = \frac{30 \times 0.3 + 150 \times 0.1}{180} = 0.133, \quad z_{1-0.05/2} = 1.96, \quad \text{and}
\]

\[
\text{Power} = \Phi\left(\frac{0.067}{\sqrt{0.007 + 0.0006}}\right) = \Phi(0.77) = 0.78
\]

In summary, the chi-square test of significance for the test of the primary hypothesis of the study (H.2.1) at \( \alpha = 0.05 \) would require approximately 30 patients treated with neo-adjuvant GC and 150 patients treated without neo-adjuvant chemotherapy to achieve a power of approximately 80% if the down-staging effect of chemotherapy is equal to 20% on the additive probability scale (an increase in the probability of stage pT0 from 10% to 30%).

As was discussed in the methods section (p62), the test of H.2.1 may also require adjustment for clinical stage. Although the distribution of clinical stages in the study cohort among patients who did and did not receive neo-adjuvant GC is not known in
advance (before data collection), it is possible that patients with more advanced clinical stages (>cT2) were more likely to receive neo-adjuvant chemotherapy compared to patients with less advanced disease (cT2). This unbalanced distribution of clinical stages between the levels of exposure need not necessarily be very substantial because in addition to clinical stage, the decision to administer neo-adjuvant chemotherapy is affected by other factors such as comorbidities and the use of adjuvant chemotherapy.

However, for the purpose of power analysis, we will consider a case of highly unbalanced distribution of clinical stages. We will let the proportion of deeply invasive tumors (cT3-T4a) among patients who received neo-adjuvant GC be greater than the proportion of deeply invasive tumors among patients who received no neo-adjuvant chemotherapy by 50% on the additive scale: \( \text{Prop}(cT3-T4a \mid GC^+) = 0.7 \), \( \text{Prop}(cT3-T4a \mid GC^-) = 0.2 \).

As before, we will assume that the crude (marginal with respect to the clinical stage) down-staging effect of GC is equal to 20% on the additive scale. Now to test H.2.1 with adjustment for clinical stage, we will fit the additive risk model as described in section 3.2.1.1.

\[
\Pr(pT0 = 1 \mid GC, C \_ \text{STAGE}) = \beta_0 + \beta_1 GC + \beta_2 C \_ \text{STAGE} ,
\]

where \( GC = 1 \) if neo-adjuvant GC was administered and \( GC = 0 \) otherwise,

\( C \_ \text{STAGE} = 1 \) if the clinical stage was cT3-T4a, and \( C \_ \text{STAGE} = 0 \) if the clinical stage was cT2. For the purpose of power analysis we will let
\[
\begin{align*}
\Pr(pT0 = 1 \mid GC = 1, C_{-STAGE} = 0) &= 0.40 \\
\Pr(pT0 = 1 \mid GC = 0, C_{-STAGE} = 0) &= 0.15 \\
\Pr(pT0 = 1 \mid GC = 1, C_{-STAGE} = 1) &= 0.30 \\
\Pr(pT0 = 1 \mid GC = 0, C_{-STAGE} = 1) &= 0.05
\end{align*}
\]

Hence, the model parameters are: \( \beta_0 = 0.15, \beta_1 = 0.25, \beta_2 = -0.10 \). Note that the marginal effect of GC is

\[
\begin{align*}
\Pr(pT0 = 1 \mid GC = 1) - \Pr(pT0 = 1 \mid GC = 0) &= (0.4 \times 0.3 + 0.3 \times 0.7) - (0.15 \times 0.8 + 0.05 \times 0.2) = \\
&= 0.33 - 0.13 = 0.2, \text{ or } 20\% \text{ as required.}
\end{align*}
\]

As was stated in the methods section, model parameters will be estimated by maximizing the Bernoulli likelihood function with the Fisher scoring algorithm. In the event of convergence failure, model parameters will be estimated by the method of least squares with robust covariance matrix estimator. Because there are no closed form expressions for the power of hypothesis tests based on these models, power was estimated by simulation using 1,000 realizations of the Bernoulli process.
Table 20 Power analysis: additive risk model \((\beta_0 = 0.15, \beta_1 = 0.25, \beta_2 = -0.10, n_1 = 30, n_2 = 150, \text{Prop}(cT3 - T4a | GC^+) = 0.7, \text{Prop} (cT3 - T4a | GC^-) = 0.2, \alpha = 0.05)\)

<table>
<thead>
<tr>
<th>Estimation method</th>
<th>Convergence</th>
<th>Mean</th>
<th>Coverage</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum likelihood</td>
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<td>0.24</td>
<td>0.93</td>
<td>0.77</td>
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<tr>
<td>Least squares</td>
<td>1.00</td>
<td>0.25</td>
<td>0.95</td>
<td>0.78</td>
</tr>
</tbody>
</table>

\(n_1\) = number of patients treated with neo-adjuvant GC; \(n_2\) = number of patients treated without neo-adjuvant chemotherapy; Convergence = estimated probability of model convergence; Mean = mean of the estimated regression coefficients for GC given model convergence; Coverage = estimated coverage probability of the 95% confidence intervals given model convergence; Power = estimated power given model convergence.

The sample size of \(n_1 = 30\) and \(n_2 = 150\) corresponds to the target sample size needed for the test of H.2.1 with a power of approximately 80% in bivariable analysis, given that \(P1 = 0.3\) and \(P2 = 0.1\) (Table 18). As shown in Table 20, this sample size would also provide reasonable power for the test of H.2.1 in multivariable analysis with adjustment for clinical stage.

In summary, the model-based test of significance for the test of the primary hypothesis of the study (H.2.1) at \(\alpha = 0.05\) with adjustment for clinical stage would require approximately 30 patients treated with neo-adjuvant GC and 150 patients treated without neo-adjuvant chemotherapy to achieve a power of approximately 80% if the marginal down-staging effect of chemotherapy is equal to 20% on the additive probability scale.

It may also be of interest to discuss power considerations for survival analysis. We will assume that the effect of chemotherapy on survival will be tested using the score test from the univariable Cox model, which is equivalent to the log-rank test. In the absence
of ties, the two tests are exactly identical. It is possible that the final model will also include covariates, such as age and clinical stage. However, their exact effect on the variance of the estimated coefficient for chemotherapy depends in a very complex way on several unknown quantities. Therefore, power calculations will be based on the univariable model. This approximation is reasonable because in practice, the variance of the estimated conditional effects in the Cox model is often similar to the variance of the estimated marginal effects [125].

The power of the score test depends on the effect size (the hazard ratio), the significance level (alpha), the proportion of exposed subjects (patients treated with neo-adjuvant GC), and the total number of deaths in the study sample. The relationship between these quantities can be expressed as [110]

\[ d = \frac{(z_{1-\alpha/2} + z_{1-\beta})^2}{\pi(1-\pi)(\ln HR)^2}, \]

where \( d \) is the total number of deaths, \( HR \) is the hazard ratio and \( \pi \) is the proportion of exposed subjects in the sample.

For the purpose of power analysis, we will assume that the restricted survival cohort will include 150 patients with documented adequate renal and hematologic function, and that 20% \( (n_i = 30) \) of patients in that cohort received neo-adjuvant GC \( (\pi = 0.2) \). The total number of deaths expected in the study cohort at the time of data analysis can be approximated as follows. First, the total number of subjects treated with cystectomy during each year from 1999 to 2009 is approximated using available information (second row of Table 21). It is known that more patients were treated in the recent years than in
the earlier years. This information is reflected in the unequal distribution of cases over time in Table 21. The estimated mortality proportion at the time of data analysis (approximately January 2010) for each sub-cohort defined by the year of definitive surgery was obtained from the SEER registry using SEER patients with stage T2-T4a BC treated with cystectomy. For example, among SEER patients treated in a given year, 10% are deceased by the end of the year, 30% are deceased by the end of the second year, 44% are deceased by the end of the third year, etc. Note that some of these patients had AJCC stage group IV due to positive nodes discovered at cystectomy. This also applies to patients treated at Strong Hospital. The expected number of deaths in each sub-cohort defined by the year of cystectomy was computed by multiplying the number of patients in the sub-cohort by the estimated mortality.

Table 21 Estimated numbers of deaths

<table>
<thead>
<tr>
<th>YOC</th>
<th>#Patients</th>
<th>Mortality</th>
<th>E(#Deaths)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>20</td>
<td>0.1</td>
<td>2</td>
</tr>
<tr>
<td>2008</td>
<td>20</td>
<td>0.3</td>
<td>6</td>
</tr>
<tr>
<td>2007</td>
<td>20</td>
<td>0.44</td>
<td>8.8</td>
</tr>
<tr>
<td>2006</td>
<td>20</td>
<td>0.5</td>
<td>10</td>
</tr>
<tr>
<td>2005</td>
<td>10</td>
<td>0.55</td>
<td>5.5</td>
</tr>
<tr>
<td>2004</td>
<td>10</td>
<td>0.59</td>
<td>5.9</td>
</tr>
<tr>
<td>2003</td>
<td>10</td>
<td>0.62</td>
<td>6.2</td>
</tr>
<tr>
<td>2002</td>
<td>10</td>
<td>0.64</td>
<td>6.4</td>
</tr>
<tr>
<td>2001</td>
<td>10</td>
<td>0.67</td>
<td>6.7</td>
</tr>
<tr>
<td>2000</td>
<td>10</td>
<td>0.69</td>
<td>6.9</td>
</tr>
<tr>
<td>1999</td>
<td>10</td>
<td>0.71</td>
<td>7.1</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>0.48</td>
<td>71.5</td>
</tr>
</tbody>
</table>

YOC = year of cystectomy; # Patients = approximate number of patients in the study sample treated during this year; mortality = SEER-based estimate of mortality proportion at the time of data analysis (approximately January 2010); E(# Deaths) = expected number of deaths

Given a total of 72 deaths, the minimum detectable hazard ratio with $\pi = 0.2$, alpha = 0.05, and power = 80% can be computed as
\[ HR = \exp\left[\frac{(z_{1-\alpha/2} + z_{1-\beta})^2}{\pi(1-\pi)d}\right] = 2.28 \]

This is equivalent to the protective hazard ratio of \( 1 / 2.28 = 0.44 \). Note that this effect is almost equal in magnitude to the hazard ratio that was estimated in the secondary analysis of the SWOG trial among patients with mixed tumors (MVAC-plus-cystectomy vs. cystectomy alone, HR = 0.46). Hence the effect of this magnitude is not completely unrealistic. Power would be lower in the analysis of BC-specific hazard (given the HR of similar magnitude) because deaths from other causes are coded as censoring events.

### 4.3 Results

Between January 1, 1999 and December 31, 2009, a total of 543 cystectomy procedures were performed at Strong Memorial Hospital. A total of 68 of these procedures were performed for conditions such as pure non-urothelial primary cancers of the bladder, malignancies of other organs involving the bladder by direct extension or metastases, and certain non-neoplastic conditions. Of the remaining 475 procedures, 57 were partial cystectomies. Partial cystectomies were excluded from analysis because none of the patients treated with partial cystectomy received neo-adjuvant chemotherapy. Among the remaining 418 patients, muscle invasion prior to cystectomy was documented in 173 patients (41%), of which 38 received neo-adjuvant chemotherapy and the remaining 135 were treated without neo-adjuvant chemotherapy (Figure 4). Ten of the 38 patients treated with neo-adjuvant chemotherapy received drug combinations other than GC and were excluded from analysis. Three of the 28 patients treated with neo-adjuvant GC were
found to be ineligible after complete chart review (one received neo-adjuvant radiation along with GC, one received a taxane in addition to GC, and one had a diagnosis of distant metastases before the first cycle of GC was administered). Hence, the study cohort included 160 eligible patients, of which 25 received neo-adjuvant GC and 135 did not receive neo-adjuvant chemotherapy (Figure 4). Baseline characteristics of these patients documented before the initiation of definitive therapy for BC are shown in Table 22.

Figure 4 Application of inclusion / exclusion criteria
Table 22 Baseline characteristics of the study cohort

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>GC+RC (n=25)</th>
<th>RC (n=135)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years (median)</td>
<td>64 (65)</td>
<td>70 (70)</td>
<td>0.01</td>
</tr>
<tr>
<td>Females, No. (%)</td>
<td>7 (20%)</td>
<td>27 (28%)</td>
<td>0.42</td>
</tr>
<tr>
<td>White, No. (%)</td>
<td>24 (96%)</td>
<td>130 (96%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Clinical T3/T4, No. (%)</td>
<td>19 (76%)</td>
<td>29 (21%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any regional adenopathy, No. (%)</td>
<td>9 (36%)</td>
<td>15 (11%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Regional adenopathy &gt; 1 cm, No. (%)</td>
<td>5 (20%)</td>
<td>10 (7%)</td>
<td>0.06</td>
</tr>
<tr>
<td>MCVD – MRD –</td>
<td>19 (76%)</td>
<td>72 (53%)</td>
<td>0.05</td>
</tr>
<tr>
<td>MCVD + MRD –</td>
<td>4 (16%)</td>
<td>36 (27%)</td>
<td></td>
</tr>
<tr>
<td>MCVD – MRD +</td>
<td>2 (8%)</td>
<td>18 (13%)</td>
<td></td>
</tr>
<tr>
<td>MCVD + MRD +</td>
<td>0 (0%)</td>
<td>9 (7%)</td>
<td></td>
</tr>
<tr>
<td>Other malignancies, No. (%)</td>
<td>3 (12%)</td>
<td>21 (16%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL, mean (median)</td>
<td>0.95 (0.9)</td>
<td>1.04 (1.0)</td>
<td>0.44</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min, mean (median)</td>
<td>85.3 (83.9)</td>
<td>78.5 (76.7)</td>
<td>0.21</td>
</tr>
<tr>
<td>WBC, 1000 cells / microL, mean (median)</td>
<td>9.3 (7.5)</td>
<td>8.4 (7.7)</td>
<td>0.69</td>
</tr>
<tr>
<td>Platelets, cells / microL, mean (median)</td>
<td>274 (253)</td>
<td>264 (251)</td>
<td>0.98</td>
</tr>
<tr>
<td>Never smokers, No. (%)</td>
<td>7 (29%)</td>
<td>21 (17%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Former smokers, No. (%)</td>
<td>12 (50%)</td>
<td>70 (56%)</td>
<td></td>
</tr>
<tr>
<td>Current smokers, No. (%)</td>
<td>5 (21%)</td>
<td>35 (28%)</td>
<td></td>
</tr>
<tr>
<td>No. of smoking years, mean (median)*</td>
<td>33 (35)</td>
<td>36 (39)</td>
<td>0.30</td>
</tr>
<tr>
<td>Intravesical therapy, No. (%)</td>
<td>6 (24%)</td>
<td>26 (19%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Mixed histology, No. (%)</td>
<td>9 (36%)</td>
<td>30 (22%)</td>
<td>0.20</td>
</tr>
<tr>
<td>High grade, No. (%)</td>
<td>25 (100%)</td>
<td>132 (98%)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

RC = radical cystectomy; potential confounders are in bold font (p<0.1)
1 Wilcoxon test; 2 Fisher’s exact test
* computed for former and current smokers combined;
MCVD = major cardiovascular disease, MRD = major respiratory disease (see Table 17 for definition of these variables)

Compared to patients treated without neo-adjuvant chemotherapy, those who received neo-adjuvant GC were on average younger (64 vs. 70 y.o) and had fewer comorbidities.

In particular, the proportions of patients who had neither major cardiovascular disease (MCVD) nor major respiratory disease (MRD) were 76% in the GC+RC group and 53% in the RC group (Table 22). In addition, no one in the GC+RC group had a combination of MCVD and MRD, while in the RC group 7% of patients had such combination. On the other hand, compared to patients in the RC group, patients in the GC+RC group were more likely to have direct extravesical extension of BC based on clinical staging (76% vs.
21% and were more likely to have regional adenopathy (any 36% vs. 11%, >1 cm 20% vs. 7%). Chemotherapy group also included a higher proportion of mixed tumors, although this difference was not statistically significant. The distribution of other covariates was fairly similar in the two treatment groups (Table 22).

Missing values were uncommon in these series. Serum creatinine, white counts and platelet counts before the initiation of definitive therapy for BC were unknown on 7 patients. These patients were excluded from the calculation of the average serum creatinine and creatinine clearance and average cell counts in Table 22, but were included in other analyses. Smoking status was unknown on 10 patients. These patients were excluded from the calculation of proportions of current, former, and never smokers in Table 22, but were included in other analyses. Among 122 current or former smokers, the number of smoking years was unknown on 3 patients. These 3 patients were excluded from calculation of the average number of smoking years in current and former smokers in Table 22, but were included in other analyses.

In these series, neo-adjuvant GC was administered in 3-week cycles according to the following protocol: gemcitabine 1,000 mg/m² per cycle and cisplatin 70 mg/m² per cycle. During the course of treatment, doses were adjusted as deemed necessary by medical oncologists based on cell counts and other considerations. The numbers of cycles completed in these series were as follows: 3 patients completed two cycles, 8 patients completed three cycles, and 14 patients completed four cycles. The effect of neo-adjuvant GC on tumor down-staging is described in the next section.
4.3.1. *Tumor down-staging*

There was evidence of tumor down-staging in the bladder following neo-adjuvant chemotherapy (Table 23). Stage pT0 at cystectomy was found in 5 of 25 patients (20%) in the GC+RC group and in 7 of 135 patients (5%) in the RC group (model-based risk difference adjusted for clinical stage = 16%, $p = 0.03$; Table 24, first row; this is Hypothesis 2.1). Same results were obtained after direct standardization for clinical stage (standardized risk difference = 16%, $p = 0.03$).

Table 23 Tumor down-staging: crude analysis

<table>
<thead>
<tr>
<th>End-point</th>
<th>GC+RC</th>
<th>RC</th>
<th>cRD</th>
<th>95% CI$^1$</th>
<th>P$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pT0</td>
<td>20% (5/25)</td>
<td>5% (7/135)</td>
<td>15%</td>
<td>(2%, 33%)</td>
<td>0.02</td>
</tr>
<tr>
<td>&lt; pT1</td>
<td>40% (10/25)</td>
<td>11% (15/135)</td>
<td>29%</td>
<td>(10%, 49%)</td>
<td>0.001</td>
</tr>
<tr>
<td>&lt; pT2</td>
<td>44% (11/25)</td>
<td>16% (21/135)</td>
<td>28%</td>
<td>(9%, 49%)</td>
<td>0.003</td>
</tr>
<tr>
<td>&lt; pT3</td>
<td>60% (15/25)</td>
<td>37% (50/135)</td>
<td>23%</td>
<td>(2%, 42%)</td>
<td>0.045</td>
</tr>
<tr>
<td>Margins</td>
<td>88% (22/25)</td>
<td>89% (120/135)</td>
<td>-1%</td>
<td>(-18%, 10%)</td>
<td>0.99</td>
</tr>
<tr>
<td>pN0</td>
<td>56% (14/25)</td>
<td>57% (77/135)</td>
<td>-1%</td>
<td>(-22%, 19%)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

pT0 = stage pT0; <pT1 no residual invasion of lamina propria (this implies pT0 or pTis because there were no pTa cases); <pT2 = no residual muscle-invasion; <pT3 no extravesical extension; Margins = negative margins; pN0 = negative nodes; cRD = crude risk difference; $^1$Likelihood ratio; $^2$Fisher’s exact test
Table 24 Tumor down-staging: adjusted analysis

<table>
<thead>
<tr>
<th>End-point</th>
<th>Variable</th>
<th>aRD</th>
<th>95% CI</th>
<th>P^1</th>
</tr>
</thead>
<tbody>
<tr>
<td>pT0</td>
<td>Treatment (GC+RC vs. RC)</td>
<td>16%</td>
<td>(1%, 35%)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Clinical stage (T3/T4 vs. T2)</td>
<td>-2%</td>
<td>(-9%, 9%)</td>
<td>0.67</td>
</tr>
<tr>
<td>&lt; pT1</td>
<td>Treatment (GC+RC vs. RC)</td>
<td>30%</td>
<td>(10%, 51%)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Clinical stage (T3/T4 vs. T2)</td>
<td>-2%</td>
<td>(-12%, 12%)</td>
<td>0.77</td>
</tr>
<tr>
<td>&lt; pT2</td>
<td>Treatment (GC+RC vs. RC)</td>
<td>30%</td>
<td>(9%, 51%)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Clinical stage (T3/T4 vs. T2)</td>
<td>-3%</td>
<td>(-15%, 13%)</td>
<td>0.70</td>
</tr>
<tr>
<td>&lt; pT3</td>
<td>Treatment (GC+RC vs. RC)</td>
<td>31%</td>
<td>(8%, 51%)</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Clinical stage (T3/T4 vs. T2)</td>
<td>-15%</td>
<td>(-31%, 3%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Margins</td>
<td>Treatment (GC+RC vs. RC)</td>
<td>8%</td>
<td>(-12%, 28%)</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Clinical stage (T3/T4 vs. T2)</td>
<td>-12%</td>
<td>(-16%, 2%)</td>
<td>0.11</td>
</tr>
<tr>
<td>pN0</td>
<td>Treatment (GC+RC vs. RC)</td>
<td>4%</td>
<td>(-19%, 27%)</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Clinical stage (T3/T4 vs. T2)</td>
<td>-3%</td>
<td>(-18%, 22%)</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Adenopathy (any vs. none)</td>
<td>-25%</td>
<td>(-47%, -2%)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>No. nodes examined (1, 2, ..)</td>
<td>-0.1%</td>
<td>(-1%, 1%)</td>
<td>0.83</td>
</tr>
</tbody>
</table>

pT0 = stage pT0; <pT1 no residual invasion of lamina propria (this implies pT0 or pTis because there were no pTa cases); <pT2 = no residual muscle-invasion; <pT3 no extravesical extension; Margins = negative margins; pN0 = negative nodes; aRD = adjusted risk difference; ^1Likelihood ratio; Note: in these models, interaction of treatment (GC+RC vs. RC) with clinical stage was not statistically significant for all down-staging end-points (all interaction p > 0.1).

Compared to patients treated without neo-adjuvant chemotherapy, those treated with neo-adjuvant GC were also significantly less likely to have residual muscle-invasive disease in the bladder at cystectomy (adjusted risk difference 30%, p = 0.004; Table 24) or direct extravesical extension from the bladder at cystectomy (adjusted risk difference 31%, p = 0.008; Table 24).

We also examined the effect of neo-adjuvant GC on pathological T stage defined as an ordinal variable (pT0, pTis/pTa, pT1, pT2, pT3/T4), as explained in the methods section. The estimated cumulative odds ratio for the effect of chemotherapy on tumor down-
staging obtained from the proportional odds model adjusted for clinical stage was equal to 4.54 (\( p = 0.001 \), Table 25). Hence, the estimated cumulative odds of achieving a given down-staging end-point in the bladder (\( pT0, <pT1, <pT2, <pT3 \)) were on the average more than 4 times greater in patients treated with neo-adjuvant GC compared to those who did not receive neo-adjuvant chemotherapy. The proportional odds assumption did not seem violated (\( p=0.47 \)). These findings suggest that neo-adjuvant chemotherapy with GC is capable of down-staging the tumors in the bladder.

### Table 25 Proportional odds model

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Cumulative odds ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (GC+RC vs. RC-only)</td>
<td>4.54</td>
<td>(1.81, 11.42)</td>
<td>0.001</td>
</tr>
<tr>
<td>Clinical stage (T3/T4 vs. T2)</td>
<td>0.56</td>
<td>(0.26, 1.24)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

In contrast, there was no evidence of a significantly beneficial effect of chemotherapy on the risk of positive nodes at cystectomy, even after adjustment for clinical stage, regional adenopathy, and the number of nodes examined (adjusted risk difference 4%, \( p = 0.74 \); Table 24). The average number of nodes examined was slightly greater in the GC+RC group than in the RC group (21.4 vs. 16.1, \( p=0.02 \)). The corresponding medians were 20 and 15 respectively. Among patients with positive nodes, the average node density (the ratio of the number of positive nodes to the number of nodes examined) was 0.24 in the GC+RC group and 0.30 in the RC group (\( p = 0.44 \)).
Positive nodes after chemotherapy were primarily, although not exclusively, associated with direct extravesical extension at cystectomy (stage \( \geq \) pT3) (Table 26). Among 10 patients with stage \( \geq \) pT3 after neo-adjuvant GC, positive nodes were present in 8 patients (80%). Among 15 patients without direct extravesical extension after neo-adjuvant GC (stage < pT3), positive nodes were found in 3 patients (20%). Each of these 3 patients had stage pTis and only one positive node at cystectomy. Nodal metastases were also associated with direct extravesical extension in the RC group, where positive nodes at cystectomy were found in 54% of 85 patients with stage \( \geq \) pT3 and in 24% of 50 patients with stage < pT3 (Table 26).

<table>
<thead>
<tr>
<th></th>
<th>&lt; pT3</th>
<th>&gt;= pT3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>GC+RC</td>
<td>3/15</td>
<td>20%</td>
<td>8/10</td>
</tr>
<tr>
<td>RC</td>
<td>12/50</td>
<td>24%</td>
<td>46/85</td>
</tr>
</tbody>
</table>

Proportions of patients with negative surgical margins at cystectomy were similar in the two treatment groups (Table 23). After adjustment for clinical stage, the estimated risk difference was equal to 8%, favoring chemotherapy; however this was not statistically significant (p=0.41; Table 24). Indicator variable for the surgeon was not significantly associated with the outcome in this comparison and in comparisons with other down-staging end-points (all p > 0.1).
The number of chemotherapy cycles completed when treated as an ordinal variable (2, 3, 4) was not significantly associated with tumor down-staging in these series (p > 0.1 for all down-staging end-points in the crude analysis and after adjustment for clinical stage, adenopathy, and the number of nodes examined).

We also examined the association of mixed histology with tumor down-staging in these series. Among the 25 patients in the GC+RC group, mixed histology, as determined from TURBT specimens, was found in 9 patients, of which 1 (11%) had stage pT0, 2 (22%) had stage pTis, and 6 (67%) had muscle-invasive disease. Among the 135 patients in the GC+RC group, mixed histology (based on TURBT specimens), was found in 29 patients, of which no one had stage pT0, 2 (7%) had stage pTis or pT1, and 27 (93%) had muscle-invasive disease. Because of small number of mixed histology cases in the GC+RC group, analysis of the interaction of treatment (GC+RC vs. RC) with histologic type was highly imprecise. However, there was no clear evidence that the down-staging effect differed by histologic type. For the primary down-staging end-point (stage pT0), the estimated risk difference for the treatment effect adjusted for clinical stage was 0.19 for pure UC and 0.12 for mixed tumors, interaction p=0.64).

4.3.2. Survival analysis

The median potential follow-up time in these series was 32 months. Approximately 55% of the patients were treated in the last three years (2007, 2008, 2009) and therefore had less than 3 full years of potential follow-up; the remaining 45% were treated in 1999-2006 and had at least 3 full years of potential follow-up. A total of 66 of 160 patients
(41%) died during the study period. There were 8 deaths among 25 patients (32%) in the GC+RC group (all from BC) and 58 deaths among 135 patients (43%) in the RC group (76% from BC, 24% from other causes, most frequently cardiovascular disease). We did not observe any discrepancies between the underlying cause of death obtained from the NDI and information available from the chart.

Crude survival curves for all-cause mortality in each treatment group are shown in Figure 5. The estimated cumulative survival function in the GC+RC group was fairly imprecise due to small number of events (8 deaths).

Figure 5 All-cause mortality by treatment: crude analysis (log-rank p=0.4)

Note that these survival curves do not estimate the treatment effect because the two treatment groups were very different with respect to certain baseline characteristics related to mortality (Table 22). Compared to patients in the RC group, those in the GC+RC group were on average 5 years younger, and had fewer co-morbidities, but were
more likely to have direct extravesical extension of BC based on clinical staging and were more likely to have regional adenopathy (Table 22).

In order to estimate the treatment effect with adjustment for these baseline characteristics, patients who had both MCVD and MRD were excluded from analysis because no one in the GC+RC group had both MCVD and MRD, while 7% of patients in the GC+RC group had this combination (Table 22). Model-based adjustment for comorbidities would be difficult to interpret without this restriction (due to complete separation on the MCVD+ MRD+ status).

The restricted cohort included 25 patients (8 events) in the GC+RC group and 126 patients (55 events) in the RC group. Treatment effect on all-cause mortality was estimated using the Cox model stratified on MCVD/MRD comorbidity indicator and clinical stage, and adjusted for regional adenopathy, and age.

The estimated treatment effect on all-cause mortality was HR=0.61, 95%CI: 0.26, 1.42, p=0.25 (Hypothesis 2.2). The interaction of treatment with histologic type was not statistically significant (p=0.82). The estimated treatment affect on the hazard of death from BC with adjustment for the same covariates was HR=0.76, 95%CI: 0.31, 1.83, p=0.5 (Hypothesis 2.3). These results are summarized in Table 27. The interaction of treatment with histologic type was not statistically significant (p=0.81). The proportional hazards assumption for covariates not involved in stratification and the assumption of
linearity of the effect of age on the log-hazard scale were tested as described in the methods section. No evidence that the assumptions were violated was detected (all p>0.1).

Table 27 Adjusted hazard ratios (treatment, adenopathy, and age)

<table>
<thead>
<tr>
<th>Mortality</th>
<th>Variable</th>
<th>HR</th>
<th>95%CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All causes</td>
<td>Treatment (GC+RC vs. RC)</td>
<td>0.61</td>
<td>(0.26, 1.42)</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Adenopathy (any vs. none)</td>
<td>1.47</td>
<td>(0.65, 3.32)</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Age (years)</td>
<td>1.01</td>
<td>(0.98, 1.03)</td>
<td>0.89</td>
</tr>
<tr>
<td>BC</td>
<td>Treatment (GC+RC vs. RC)</td>
<td>0.76</td>
<td>(0.31, 1.83)</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Adenopathy (any vs. none)</td>
<td>1.71</td>
<td>(0.71, 4.13)</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Age (years)</td>
<td>1.00</td>
<td>(0.96, 1.02)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Stratification variables: clinical stage and comorbidity indicator

Model-based survival curves (GC+RC vs. RC) for the most frequent covariate pattern (age 65 years, clinical stage T2, no adenopathy and no major cardiovascular or respiratory comorbidities) are shown in Figure 6.

Figure 6 All-cause mortality by treatment: adjusted analysis with the proportional hazards assumption for the treatment effect

![Survival Curve](image)
These curves (corresponding to the estimated hazard ratio of 0.61) assume proportional hazards for the treatment effect. Although this assumption was tested and could not be rejected, we also performed sensitivity analysis by constructing model-based survival curves with treatment as a stratification variable. This approach does not involve the proportional hazards assumption for the treatment effect and does not produce a point estimate of the hazard ratio. The corresponding survival curves for the most frequent covariate pattern (age 65 years, clinical stage T2, no adenopathy and no major cardiovascular or respiratory comorbidities) are shown in Figure 7.

Figure 7 All-cause mortality by treatment: adjusted analysis without the proportional hazards assumption for the treatment effect

Baseline covariates such as creatinine clearance, white count, platelet count, other malignancies, smoking years (zero for never smokers), gender, and indicator variable for the surgeon were not significantly associated with survival (all p>0.1) in the Cox models
conditioned on treatment (GC+RC vs. RC), clinical stage, comorbidities indicator, and age, and were not included in the final models.

Of note, pathological variables documented at cystectomy such as the T stage, nodal status, lymphovascular invasion and surgical margins were independently associated with survival in this study (Table 28; these are established prognostic factors for BC). The cut-offs for the nodal categories (1, 2-3, >3) were based on tertiles of positive nodes among patients with nodal metastases.

Table 28 Effects of pathological variables at cystectomy on all-cause mortality (multivariable Cox model; unrestricted cohort)

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR</th>
<th>95%CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathological stage (&gt;=T3 vs. &lt;T3)</td>
<td>2.79</td>
<td>(1.42, 5.50)</td>
<td>0.003</td>
</tr>
<tr>
<td>Lymphovascular invasion (present vs. absent)</td>
<td>2.14</td>
<td>(1.19, 3.85)</td>
<td>0.01</td>
</tr>
<tr>
<td>Surgical margins (positive vs. negative)</td>
<td>2.04</td>
<td>(1.03, 6.07)</td>
<td>0.04</td>
</tr>
<tr>
<td>Number of nodes examined (0, 1, 2, …)</td>
<td>0.96</td>
<td>(0.93, 0.99)</td>
<td>0.02</td>
</tr>
<tr>
<td>Number of positive nodes (1 vs. 0)</td>
<td>1.13</td>
<td>(0.52, 2.45)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Number of positive nodes (2-3 vs. 0)</td>
<td>1.36</td>
<td>(0.64, 2.89)</td>
<td></td>
</tr>
<tr>
<td>Number of positive nodes (&gt;3 vs. 0)</td>
<td>2.02</td>
<td>(1.04, 3.90)</td>
<td></td>
</tr>
</tbody>
</table>

* test for trend

A total of 7/25 (28%) of patients in the neo-adjuvant GC group and 42/135 (31%) of patients in the control group received adjuvant chemotherapy (AC) after cystectomy.

The most frequently used AC regimens were gemcitabine plus cisplatin (36% of all AC cases), gemcitabine plus Taxol (19% of all AC cases), and gemcitabine plus carboplatin (14% of all AC cases). The numbers of cycles completed were as follows: 4 patients received 1-2 cycles, 8 patients received 3 cycles, 25 patients received 4 cycles, 7 patients received >4 cycles, and for the remaining 5 patients the number of cycles was unknown.
The use of AC was strongly associated with pathological stage. Among the 49 patients in the AC group, 46 (96%) had either direct extravesical extension or positive nodes at cystectomy. In contrast, among the 111 patients treated without AC, 64 (58%) had either direct extravesical extension or positive nodes at cystectomy. Among patients who had either direct extravesical extension or positive nodes at cystectomy, the use of AC was associated with improved survival (HR=0.40, 95%CI: 0.21, 0.78, p=0.007; adjusted for pathological variables in Table 28 as well as age, and comorbidities). This comparison however was not based on a randomized treatment assignment and could be confounded by the patients’ ability to tolerate chemotherapy after cystectomy (which may not be adequately reflected by comorbidities documented at baseline). Most patients who had direct extravesical extension and/or positive nodes at cystectomy and did not receive AC were not able to tolerate such therapy. Hence the observed association of AC with improved survival may not represent the true treatment effect.

4.4 Discussion

The purpose of this study (Specific Aim 2 of the dissertation project) was to investigate the effect of neo-adjuvant chemotherapy with GC on pathological down-staging and survival of patients with locally advanced BC. Our findings suggest that this chemotherapy regimen is capable of down-staging the tumors in the bladder. In particular, stage pT0 at cystectomy (the primary down-staging end-point) was documented in 20% (5/25) of patients in the GC+RC group and in 5% (7/135) of patients in the RC group. Both of these proportions were somewhat lower than the corresponding proportions in the
SWOG study, where stage pT0 at cystectomy was found in approximately 30% and 11% of patients in the MVAC+RC and RC arms respectively.

The difference between the proportion of pT0s after GC (20%) in our series and the corresponding proportion after MVAC (30%) in the SWOG study does not necessarily indicate that GC is inferior to MVAC. It must be recognized that in our series, one of the main factors that influenced the decision to administer neo-adjuvant chemotherapy was clinical evidence of extravesical tumor extension and concerns about resectability, while in the SWOG study treatment was randomized. Therefore, patients treated with GC in our series on average had more advanced disease than patients treated with MVAC in the SWOG trial. Hence, if GC and MVAC were equally effective in terms of tumor down-staging, GC would be expected to produce a smaller proportion of pT0s in these settings. In addition, the estimated down-staging effect of GC in our series was relatively imprecise due to small sample size (although statistically significant at the conventional level of significance).

The proportion of pT0s among patients treated without neo-adjuvant chemotherapy in our series was only 5%, which is somewhat smaller than the corresponding proportion in the SWOG trial (11%), although similar to what has been reported in other institutional series [68,102]. For example, Stein et al reviewed 1,054 cystectomies performed at the University of Southern California for biopsy confirmed muscle-invasive and high grade non-muscle-invasive BC and reported that 6% of patients had stage pT0 at cystectomy.
In our series, all patients had documented muscle-invasion before the initiation of definitive therapy for BC, which may explain the relatively small proportion of pT0s.

Variation in the distribution of pathological stages in different studies may also be attributed to differences in referral patterns. According to several reports from the Surveillance Epidemiology and End Results (SEER) database [133,134], a substantial proportion (perhaps >50%) of patients diagnosed with muscle-invasive BC in the US do not receive cystectomy as initial treatment. It is possible that these patients are eventually referred for cystectomy to high volume centers (like Strong Hospital or University of Southern California) after they fail more conservative initial therapies. At the time of referral, these patients may already have fairly advanced disease, with a near zero chance of complete resection by endoscopic surgery alone and with substantial risk of extravesical extension.

Although in our series neo-adjuvant GC appeared to shrink the tumors in the bladder, we did not observe a clear beneficial effect of GC on disease in the nodes. The proportions of patients with positive nodes were about the same in both treatment groups in the crude analysis (GC+RC = 44%, RC = 43%). Adjustment for adenopathy and clinical stage shifted this balance slightly in favor of GC, but the adjusted effect was small in magnitude (a risk difference of 4%) and not statistically significant.

One hypothetical explanation for this is that tumor’s ability to metastasize through the lymphatic system may be associated with resistance to chemotherapy (as would be the
case if for example these tumor characteristics arise from the same genetic pathway). In this case, down-staging following chemotherapy would occur primarily for node-negative tumors. It is also possible that the concentration of gemcitabine and/or cisplatin in the lymphatic tissue is on average lower than in the bladder, which may explain potentially lower activity of GC in the nodes. These possibilities, however, are purely hypothetical at this time, since no data are currently available to support or refute them.

Another possible explanation for lack of a clearly beneficial effect of GC on disease in the nodes is residual confounding, which is a general limitation of observational studies of therapeutic interventions. It may be the case that the proportion of truly node-positive patients in the GC+RC group before the initiation of neo-adjuvant chemotherapy was higher than the proportion of node-positive patients in the RC group. Although we made adjustment for adenopathy in the analysis, it is possible that compared to patients in the RC group, those in the GC+RC group had more extensive adenopathy that could not be completely accounted for in the analysis. Despite these considerations, it appears that if GC had any beneficial effect on disease in the nodes, it was not very large in magnitude, since as many as 44% (11/25) of patients in our series had positive nodes after neo-adjuvant chemotherapy. Of note, absence of adenopathy on pre-cystectomy pelvic CT scans was not an accurate predictor of the nodal status at cystectomy, since in the RC group, only 11% of patients had any adenopathy, but 43% were found to have positive nodes at cystectomy.
The effect of other regimens such as MVAC and CMV on the risk of nodal metastases at cystectomy was not previously reported because nodal status by itself was not an end-point in these trials. In Weight et al’s series (2009) [97], positive nodes at cystectomy were found in 38 of 88 patients (43%) treated without neo-adjuvant chemotherapy and in 11 of 29 patients (38%) treated with neo-adjuvant chemotherapy (of which 20 patients received GC and 9 received other regimens). These proportions were very similar to proportions observed in our series. Hence, although neo-adjuvant GC may down-stage the tumors for a sizable proportion of patients, many patients have very advanced disease at cystectomy despite neo-adjuvant chemotherapy and have a substantial risk of death from BC. In our series, 8 of 25 patients (32%) in the GC+RC group died from BC.

The proportions of patients with positive surgical margins in our series were similar in the two treatment groups (GC+RC = 12%, RC = 11%). After adjustment for clinical stage and adenopathy, the risk difference was equal to 8% favoring GC, but this was not statistically significant. In the SWOG trial, positive margins were found in 7% of patients in the MVAC+RC arm and in 14% of patients in the RC arm (risk difference 7% favoring MVAC, p=0.1) [79]. Because positive margins are relatively uncommon even without neo-adjuvant chemotherapy, a definitive reduction in the risk of this outcome following chemotherapy is generally difficult to demonstrate.

In our series, observed survival was better on the average in the GC+RC group than in the RC group. However, this difference was not statistically significant. Estimated cumulative survival in the RC group in our series was very similar to the estimated
cumulative survival of patients in the RC arm of the SOWG trial (estimated 3-year survival was approximately 50% in both studies).

4.4.1. *Strengths and limitations*

The strengths of our study include direct access to all medical records, which allowed for accurate documentation of all relevant information, and the use of the National Death Index which eliminated the problem of loss to follow-up. The most important limitations of the current study are lack of randomization in the assignment of treatment regimens and relatively small sample size.

Lack of randomized treatment assignment is a general limitation of observational studies of therapeutic interventions because the decision to administer a particular treatment may often be influenced by factors that also affect the outcome. If all such factors are documented and accounted for in the analysis, the treatment effect can be estimated without bias. The feasibility of this may depend to some extent on the choice of the end-point.

For tumor down-staging end-points, adjustment for clinical T stage, adenopathy, and the number of nodes examined may be sufficient. Although the possibility of residual confounding cannot be entirely excluded here, its magnitude is likely to be small and its direction is predictable because those who receive neo-adjuvant chemotherapy on average have more extensive tumors than those who are treated without neo-adjuvant chemotherapy. Hence, if any residual confounding occurred in our study in the analysis
of tumor down-staging in the bladder, it could not account for the observed treatment effect because any such residual confounding would produce bias in the opposite direction.

With nodal status at cystectomy, we observed no significantly beneficial treatment effect, and this could theoretically be attributed at least to some extent to residual confounding. However, it is unlikely that confounding played a major role because this comparison was controlled for the main predictors of nodal metastases that could also affect the decision to administer neo-adjuvant chemotherapy. Survival analysis could also theoretically be influenced by residual confounding because complete documentation by retrospective chart review of all factors that affect treatment decisions and survival may not be feasible. Nevertheless, we were able to document the major comorbidities (defined in Table 16) and incorporate them in the models. Hence, it is unlikely that confounding played a very major role in survival analysis.

In addition to lack of randomization in treatment assignment, the current study was limited by a relatively small sample size in the GC+RC group. The target sample size for this group was approximately 30 patients. We initially identified 28 patients who received neo-adjuvant GC; however, three of these patients were found to be ineligible for inclusion in the study after complete chart review (one received neo-adjuvant radiation along with GC, one received a taxane in addition to GC, and one had a diagnosis of distant metastases before the first cycle of GC was administered). Hence, all analyses were based on 25 patients in the GC+RC group.
Despite small sample size, there was significant evidence of tumor down-staging in the bladder by GC. The p-value for the test of the primary hypothesis (H.2.1) was less than the pre-determined level of significance ($\alpha = 0.05$, $p = 0.03$). For other down-staging end-points ($<pT2$ and $<pT3$) the p-values were even smaller ($p=0.004$ and $p=0.008$ respectively). Hence, since the null hypotheses were rejected, power is not technically an issue in the analysis of tumor down-staging in the bladder. Nevertheless, small sample size limits the precision of estimation irrespective of significance testing, and this must be acknowledged as a limitation. In survival analysis in particular, the estimated treatment effect (neo-adjuvant GC+RC vs. RC) was fairly imprecise because there were only 8 events in the GC+RC group. Limited precision and power in survival analysis were recognized a priori at the design stage. For this reason (and because of concerns about residual confounding) survival analysis was considered mostly a descriptive technique, while the primary end-point of the study was defined as tumor down-staging to pT0.

4.4.2. Future studies

Ideally, questions addressed in this study could be further examined in a well-powered randomized trial. However, it is unlikely that the use of GC in the neo-adjuvant setting in such a trial would be appealing to many urologists, particularly if patients without clinical evidence of extravesical disease (i.e., clinical stage T2, N0) are to be included. Many clinicians avoid routine use of neo-adjuvant chemotherapy for muscle-invasive BC because a substantial proportion of patients have organ-confined (stage $<pT3$, pN0) disease at cystectomy even without neo-adjuvant chemotherapy. Hence, if neo-adjuvant
chemotherapy was administered to all patients with muscle-invasive BC, it is possible that many patients would be over-treated. The preferred approach is often to proceed with cystectomy and administer adjuvant chemotherapy only to patients with stage >pT2 and/or positive nodes.

One difficulty associated with this otherwise very reasonable approach is that the survival benefit of adjuvant chemotherapy in BC has not been firmly established because only 2 of 5 randomized trials showed a significant improvement in over-all survival following AC (compared to observation) [86]. It must be recognized however that the two trials that did show survival advantage from AC used multi-drug combinations (cisplatin plus two or three other drugs) and included primarily high risk patients with extravesical (T3/T4) tumors and/or positive nodes [85]. In contrast, out of 3 trials with no difference in overall survival, one trial used cisplatin as a single agent and another trial enrolled primarily lower risk patients (no one had positive nodes) [85]. Thus existing evidence from randomized trials suggests that multi-drug AC may potentially improve survival of high risk patients; however, this question is still very controversial and clear evidence-based guidelines on this matter are lacking. It must also be noted that none of the 5 trials of AC used gemcitabine plus cisplatin or gemcitabine plus taxane – the AC regimens that are now most commonly used in practice.

Further trials of AC for BC may be difficult to organize because patients with pathologically organ-confined disease on average do relatively well and may not need chemotherapy, while patients with direct extravesical extension and/or positive nodes at
cystectomy are at high risk for micro-metastases and it may be unethical to randomize them to observation with no treatment until recurrence (currently, treatment at the time of recurrence is highly ineffective and almost never results in cure). If a trial of adjuvant GC vs. observation is to be designed, it will likely have to include intermediate risk patients whose risk of recurrence after cystectomy is not so low that they are unlikely to benefit from chemotherapy and is not so high that their randomization to observation becomes unethical. This intermediate risk group may potentially include node-negative patients with stage pT2 who also have lymphovascular invasion, or (arguably) pT3 N0 disease.

It must be recognized however that even if GC and similar regimens improve survival of patients with muscle-invasive BC relative to observation, the efficacy of these regimens is still fairly limited because many patients experience recurrence and die from BC despite systemic chemotherapy (GC, MVAC, or other). Hence, more effective therapies for muscle-invasive and metastatic BC are desperately needed.

4.5 Conclusion

In summary, our findings suggest that neo-adjuvant chemotherapy with gemcitabine and cisplatin is capable of down-staging the tumors in the bladder, although we did not observe a clearly beneficial effect of chemotherapy in the nodes. The use of neo-adjuvant chemotherapy was also associated with improved survival; however, this association was not statistically significant. Future studies may explore the effect of adjuvant GC on survival in a well-powered randomized design. At the same time, search for more effective BC chemotherapy regimens must continue.
SUMMARY

The aims of this dissertation project were: (1) To determine whether the effect of neo-adjuvant MVAC on pathological down-staging and survival of patients with locally advanced BC is influenced by the presence of non-urothelial components in the tumor (Specific Aim 1) and (2) To investigate the effect of neo-adjuvant chemotherapy with GC on pathological down-staging and survival of patients with locally advanced BC (Specific Aim 2).

To address Specific Aim 1, we performed a secondary analysis of the Southwest Oncology Group trial 8710 of neo-adjuvant MVAC followed by cystectomy versus cystectomy alone for treatment of locally advanced UC of the bladder. For the purpose of these analyses, tumors were classified based on the presence of non-urothelial components as either pure UC (n=236) or mixed tumors (n=59). Non-urothelial components included squamous and/or glandular differentiation. Additive probability models and Cox regression analysis were used to estimate the effect of chemotherapy on pathological down-staging and survival of patients with pure UC and patients with mixed tumors and to test for the interaction of treatment with histologic type. We found evidence of tumor down-staging to pT0 following chemotherapy among patients with mixed tumors (adjusted risk difference = 27%, p=0.004) and among patients with pure UC (adjusted risk difference = 15%, p=0.004; interaction p=0.17). There was evidence of a survival benefit from chemotherapy in patients with mixed tumors (HR=0.46, p=0.02). Patients with pure UC had improved survival on the chemotherapy arm however the survival benefit was not statistically significant (HR=0.90, p=0.48). There was marginal
evidence that the survival benefit of chemotherapy in patients with mixed tumors was greater than it was for patients with pure UC (interaction p=0.09).

To address Specific Aim 2, we conducted a retrospective cohort study of 160 patients treated with cystectomy for locally advanced BC at Strong Memorial Hospital during the years of 1999-2009. A total of 25 of these patients received neo-adjuvant GC and the remaining 135 patients were treated without neo-adjuvant chemotherapy. Additive probability models and Cox regression analyses were used to estimate the effect of neo-adjuvant GC on pathological down-staging and survival of these patients with adjustment for confounding factors. We found evidence of tumor down-staging to pT0 following neo-adjuvant chemotherapy (risk difference adjusted for clinical stage = 16%, p = 0.03). The use of neo-adjuvant chemotherapy was associated with improved survival; however this association was not statistically significant (adjusted hazard ratio 0.61, 95%CI: 0.26, 1.42, p=0.25).

In summary, the findings of this research investigation (Specific Aims 1 and 2) suggest that: (1) Presence of squamous or glandular differentiation in locally advanced UC of the bladder does not confer resistance to MVAC and in fact may be an indication for the use of neo-adjuvant chemotherapy prior to radical cystectomy and (2) Neo-adjuvant chemotherapy with GC is capable of down-staging the tumors in the bladder. However, its effect on survival of patients with locally advanced BC remains uncertain. Future studies may address this question in a well-powered randomized design.
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Appendices

Appendix A. AJCC/TNM Stages for Bladder Cancer

Primary tumor (T)

The suffix "m" should be added to the appropriate T category to indicate multiple lesions. The suffix "is" may be added to any T to indicate the presence of associated carcinoma in situ.

- TX: Primary tumor cannot be assessed
- T0: No evidence of primary tumor
- Ta: Noninvasive papillary carcinoma
- Tis: Carcinoma in situ: "flat tumor"
- T1: Tumor invades subepithelial connective tissue
- T2: Tumor invades muscle
  - T2a: Tumor invades superficial muscle (inner half)
  - T2b: Tumor invades deep muscle (outer half)
- T3: Tumor invades perivesical tissue
  - T3a: microscopically
  - T3b: macroscopically (extravesical mass)
- T4: Tumor invades any of the following: prostate, uterus, vagina, pelvic wall, or abdominal wall.
  - T4a: Tumor invades the prostate, uterus, vagina
  - T4b: Tumor invades the pelvic wall, abdominal wall

Nodal involvement (N)

Regional lymph nodes are those within the true pelvis; all others are distant nodes.

- NX: Regional lymph nodes cannot be assessed
- N0: No regional lymph node metastasis
- N1: Metastasis in a single regional lymph node
- N2: Metastasis in multiple regional lymph nodes
- N3: Metastasis in the common iliac chain

Distant metastasis (M)

- MX: Presence of distant metastasis cannot be assessed
- M0: No distant metastasis
- M1: Distant metastasis
AJCC Stage Groups

**Stage 0a:** Ta, N0, M0

**Stage 0is:** Tis, N0, M0

**Stage I:** T1, N0, M0

**Stage II:** T2a, N0, M0
  - T2b, N0, M0

**Stage III:** T3a, N0, M0
  - T3b, N0, M0
  - T4a, N0, M0

**Stage IV:** T4b, N0, M0
  - Any T, N1, M0
  - Any T, N2, M0
  - Any T, N3, M0
  - Any T, any N, M1
Appendix B. American Urological Association Guideline for the Management Of Nonmuscle Invasive Bladder Cancer (Stages Ta, T1, and Tis): 2007 Update

Treatment Guideline Statements

The Panel based the majority of the following guideline statements on a careful analysis of comparative outcomes from randomized controlled trials. Included were data published after the previous guideline was completed as well as results from previous studies involving TURBT and intravesical therapies. These statements apply to the treatment of patients with nonmuscle invasive transitional cell carcinoma of the bladder including Tis as well as stages Ta and T1 tumors. Inherent in these guideline statements is the importance of individualizing patient diagnostic evaluation and therapy. Some of the treatment paradigms addressed below were not based on data but on Panel experience alone.

In an attempt to recognize commonly encountered clinical variations, the Panel has designated certain example settings as “index patients.” In establishing these index patients, the Panel closely examined pressing questions involving the use of intravesical chemotherapy versus immunotherapy and the role of maintenance therapy. Each guideline statement addresses a specific index patient.

For All Index Patients

Standard: Physicians should discuss with the patient the treatment options and the benefits and harms, including side effects, of intravesical treatment.
[Based on Panel consensus.]

Although a variety of the adjuvant intravesical treatments studied decrease the probability of bladder cancer recurrence when compared with TURBT alone, published data do not support the conclusion that the rate of progression to muscle invasive disease is necessarily significantly altered, especially with low-risk tumors. Physicians should discuss these potential benefits as well as the possible complications with the patient. Currently, there is little evidence defining and/or verifying the optimal dose, number of doses, and timing of instillations for either induction or maintenance intravesical therapy. This lack of uniformity renders the establishment of a guideline statement regarding specific regimens impossible and increases the difficulty of comparing therapy types.

For Index Patient No. 1: A patient who presents with an abnormal growth on the urothelium but who has not yet been diagnosed with bladder cancer.

Standard: If the patient does not have an established histologic diagnosis, a biopsy should be obtained for pathologic analysis.
[Based on Panel consensus.]

Although laboratory diagnoses can indicate the likelihood of bladder cancer, the definitive diagnosis is established by pathologic examination of tissue removed by TURBT or biopsy. Transitional cell carcinoma of the bladder often has a characteristic appearance, but other conditions can mimic the gross appearance of bladder cancer.
Standard: Under most circumstances, complete eradication of all visible tumors should be performed.
[Based on Panel consensus.]

When feasible, surgeons should attempt to resect all tumors. The size and/or multiplicity of tumors or obvious deep muscle invasion may prevent complete resection. Also, comorbid conditions must be considered and may occasionally influence a decision about whether or not to attempt entire endoscopic removal of bladder tumors. Tumor resection can be accomplished with electrocautery resection, fulguration, or application of laser energy. Adequate tissue should be available for determination of clinical stage, but in some cases endoscopic ablative techniques may not permit submission of all material for histologic evaluation.

Standard: If bladder cancer is confirmed, periodic surveillance cystoscopy should be performed.
[Based on Panel consensus.]

Neither the ideal interval nor the duration of follow-up cystoscopy has been defined. Given the variable risk of recurrence and progression, a risk-adapted approach should be considered. Patients with high-risk disease should undergo more intensive followup.

Option: An initial single dose of intravesical chemotherapy may be administered immediately postoperatively.
[Based on Panel consensus.]

The immediate use of intravesical chemotherapy was considered an option and not a standard by the Panel because of potential cost issues, uncertainty of pathology, side effects, and patient preference. In addition, the use of immediate intravesical chemotherapy would not be beneficial for bladder tumors that are most likely muscle invasive. In cases where the tumor appears to be papillary (Ta) by visual inspection and there are no contraindications to therapy, such as bladder perforation, immediate intravesical chemotherapy should be considered.

For Index Patient No. 2: A patient with small volume, low-grade Ta bladder cancer.

Recommendation: An initial single dose of intravesical chemotherapy may be administered immediately postoperatively.
[Based on review of the data.]

Although outcomes data pertaining specifically to patients with low-grade, Ta bladder cancer are limited, the risk of recurrence and more importantly progression is relatively low. Metaanalyses including our own, do confirm, however, for nonmuscle invasive cancer, single postoperative instillation does decrease recurrence. In our comparison, the combination of TURBT and single-dose mitomycin C resulted in 17% (95% confidence interval [CI]: 8, 28) fewer recurrences than TURBT alone when all patient risk groups were considered. There is no evidence that multiple adjuvant instillations of either BCG or chemotherapy have additional benefit in patients at initial diagnosis of Ta Grade 1 bladder cancer.

For Index Patient No. 3: A patient with multifocal and/or large volume, histologically confirmed, low-grade Ta or a patient with recurrent low-grade Ta bladder cancer.
Recommendation: An induction course of intravesical therapy with bacillus Calmette-Guérin or mitomycin C is recommended for the treatment of these patients with the goal of preventing or delaying recurrence. [Based on review of the data.]

Adjuvant intravesical therapy is useful for nonmuscle invasive tumors. The Panel identified BCG and mitomycin C because they are the most widely available of the intravesical therapies and are used in the United States. The results of the analysis demonstrated a decreased probability of recurrence with either BCG or mitomycin C when compared to TURBT alone. In our meta-analysis of randomized controlled trials, regardless of patient risk, recurrences were reduced by 24% (95% CI: 3, 47) with the combination of TURBT and BGG induction only and by 3% (95% CI: -10, 16) with TURBT and mitomycin C induction only compared with TURBT alone. While it may appear from these data that BCG is superior to mitomycin C, the wide confidence intervals do not permit this conclusion.

Option: Maintenance bacillus Calmette-Guérin or mitomycin C may be considered. [Based on review of the data.]

Maintenance therapy with BCG or mitomycin C is more effective in decreasing recurrences, when compared to induction alone. However, when considering cost, possible side effects, lack of a uniform and accepted dosing schedule and, importantly, the low risk of progression in this index patient, the Panel believes that routine maintenance therapy is an option. The Panel’s metaanalysis of randomized controlled trials published between 1990 and 2006 demonstrated that compared to TURBT alone, recurrences are decreased by 31% (95% CI: 18, 42) with TURBT and BGG maintenance and by 18% (95% CI: 6, 30) with TURBT and mitomycin C maintenance. It is unclear whether any intravesical therapy affects the ultimate rate of progression to muscle invasive disease in these low-risk patients. The progression rate estimate in all patient risk groups was 8% (95% CI: 0, 15) with TURBT and BCG maintenance and 4% (95% CI: -26, 32) with TURBT and mitomycin C maintenance. Although maintenance therapy reduces recurrence and may reduce progression, the side effects and discomfort of the treatment and possibly the costs of the treatment may outweigh the benefits for some patients. Thus, discussion of the tradeoffs and consideration of patient preferences are important before beginning or continuing maintenance therapy. The optimal maintenance schedule and duration has yet to be determined. However, the best available evidence supports the use of the SWOG regimen, a six-week induction course of BCG followed by a three-week maintenance course at 3, 6, 12, 18, 24, 30, and 36 months (if tolerated by the patient). This regimen was used in, by far, the largest trial that demonstrated the benefit of maintenance BCG therapy.

For Index Patient No. 4:

A patient with initial histologically confirmed high-grade Ta, T1, and/or carcinoma in situ bladder cancer.

Standard: For patients with lamina propria invasion (T1) but without muscularis propria in the specimen, repeat resection should be performed prior to additional intravesical therapy. [Based on review of the data and Panel consensus.]

Disease-appropriate therapy is predicated on accurate staging. Despite continued attempts to improve clinical staging, however, a significant percentage of patients are understaged. In the absence of muscularis propria in the specimen, data suggests that 20% to 40% of patients will have either residual tumor and/or unrecognized muscle invasive disease. With the lack of accurate noninvasive clinical staging modalities, efforts should be focused on acquiring a
definitive tissue diagnosis. Repeat resection may also be appropriate for patients with high-grade Ta tumors as well as patients with T1 tumors and muscularis propria in the specimen to increase the accuracy of clinical staging.

**Recommendation:** An induction course of bacillus Calmette-Guérin followed by maintenance therapy is recommended for treatment of these patients. [Based on review of the data.]

As with Index Patient No. 3, both BCG and mitomycin C are intravesical therapies that can favorably prolong recurrence-free rates. However, in this high-risk group, maintenance BCG is superior to mitomycin C with or without maintenance. In our single-arm meta-analysis of randomized controlled trials of high-risk patients, the estimated five-year recurrence rate was 34% in patients receiving TURBT and BCG maintenance and 62% with mitomycin C maintenance. The meta-analysis of all risk groups found that, compared with TURBT and mitomycin C maintenance, TURBT and BCG maintenance therapy reduced recurrence by 17% (95% CI: 7, 26). In addition, there are limited data suggesting a trend to preventing progression with maintenance BCG. The progression in one study of 380 patients was reduced by 5% (95% CI: -1, 11) with TURBT plus BCG maintenance when compared with TURBT plus mitomycin C maintenance. Although maintenance therapy reduces recurrence and may reduce progression, the side effects and discomfort of the treatment and possibly the costs of the treatment may outweigh the benefits for some patients. Thus, discussion of the tradeoffs and consideration of patient preferences is important before beginning or continuing maintenance therapy.

**Option:** Cystectomy should be considered for initial therapy in select patients. [Based on review of the data and Panel consensus.]

Because there is risk of initially understaged muscle invasive disease or progression to muscle invasive disease even after intravesical therapy, cystectomy may be considered as an initial treatment option in certain cases. It is not certain whether intravesical therapy alters this risk of progression. In addition, the high cure rate associated with patients undergoing cystectomy further justifies this decision choice. Among factors associated with increased risk of progression are large tumor size, high-grade, tumor location in a site poorly accessible to complete resection, diffuse disease, the presence of carcinoma in situ, infiltration of lymphatic or vascular spaces, and prostatic urethral involvement. Cystectomy, however, is not without its possible complications and morbidity. Physicians should present specific information about the risks of cystectomy and methods for urinary reconstruction to patients who are contemplating bladder removal.

**For Index Patient No. 5:** A patient with high-grade Ta, T1, and/or carcinoma in situ bladder cancer which has recurred after prior intravesical therapy.

**Standard:** For patients with lamina propria invasion (T1) but without muscularis propria in the specimen, repeat resection should be performed prior to additional intravesical therapy. [Based on review of the data and Panel consensus.]

This guideline statement is the same for Index Patient 4. In this setting, accurate clinical staging is crucial for appropriate therapy.

**Recommendation:** Cystectomy should be considered as a therapeutic alternative for these patients.
Even more so than patients who initially present with high-risk disease, those who fail initial intravesical therapy should be considered for cystectomy. There is a substantial risk of progression to muscle invasive cancer in these patients. The high likelihood of intravesical treatment failure and adverse consequences of delaying cystectomy make cystectomy the preferred treatment for these patients.

**Option: Further intravesical therapy may be considered for these patients.**

[Based on review of the data and Panel consensus.]

There is some evidence that select patients will respond to second induction regimens, particularly with BCG. Repeat intravesical therapy may be appropriate in patients who develop a late recurrence after previous complete response to an intravesical agent. However, in patients at high risk for progression, further intravesical therapy puts the patient at risk for muscle invasion and/or metastasis. Data are insufficient, however, to support conclusions about the role of drug combination regimens or the beneficial effect of alternating therapies.

Definitions:

Levels of Evidence

1a Evidence obtained from meta-analysis of randomized trials
1b Evidence obtained from at least one randomized trial
2a Evidence obtained from one well-designed controlled study without randomization
2b Evidence obtained from at least one other type of well-designed quasi-experimental study
3 Evidence obtained from well-designed non-experimental studies, such as comparative studies, correlation studies and case reports
4 Evidence obtained from expert committee reports or opinions or clinical experience of respected authorities

Grades of Recommendation

A. Based on clinical studies of good quality and consistency addressing the specific recommendations and including at least one randomized trial
B. Based on well-conducted clinical studies, but without randomized clinical trials
C. Made despite the absence of directly applicable clinical studies of good quality

Guidelines on Assessment of Tumour Specimens

Mandatory Evaluations

• Depth of invasion (categories pT2 vs pT3a, pT3b or pT4)
• Margins with special attention paid to the radial margin
• Histological subtype, if it has clinical implications
• Extensive lymph node representation (more than eight)

Optional Evaluations

• Bladder wall blood vessel invasion
• Pattern of muscle invasion

Diagnosis and Staging

Diagnosis

Recommendations for Primary Assessment of Presumably Invasive Bladder Tumours

• Renal and bladder ultrasonography, intravenous urography (IVU) or computed tomography (CT) prior to transurethral resection (TUR) (Grade of recommendation: B).
• Cystoscopy with description of the tumour (site, size, number and appearance) and mucosal abnormalities. A bladder diagram is recommended (Grade of recommendation: C).
• TUR in one piece for small tumours (less than 1 cm), including a part from the underlying bladder muscle wall (Grade of recommendation: B).
• TUR infractions (including muscle tissue) for larger tumours (Grade of recommendation: B).
• Biopsies of abnormal-looking urothelium, biopsies from normal-looking mucosa when cytology is positive or when exophytic tumour is of non-papillary appearance or in case of fluorescence if photodynamic diagnosis (PDD) is used (Grade of recommendation: C).
• Biopsy of the prostatic urethra in the case of bladder neck tumour, when bladder carcinoma in situ (CIS) is present or suspected or when abnormalities of prostatic urethra are visible (Grade of recommendation: C).
• Careful inspection with histological evaluation of the bladder neck and urethral margin, either prior to or at the time of cystoscopy in women undergoing a subsequent orthotopic neobladder (Grade of recommendation: C).
• A second TUR at 2 to 6 weeks after the initial resection when it was incomplete or when a high-grade or T1 tumour was detected (Grade of recommendation: B).
• The pathological report should specify the grade, the depth of tumour invasion and whether the lamina propria and muscle are present in the specimen (Grade of recommendation: C).

Imaging for Staging in Verified Bladder Tumours

Conclusions

• Diagnosis of invasive bladder cancer is made by cystoscopy and biopsy.
• Imaging is used for formal staging only if it will make a difference to the selection of treatment options.
• In all T1 tumours considered for conservative treatment, a second TUR is recommended before deciding on definite treatment (Grade of recommendation: B).

Recommendations for Staging

• For optimal local staging, either MRI with fast dynamic contrast-enhancement or MDCT with contrast enhancement are recommended for patients considered suitable for radical treatment (Grade of recommendation: B).
• For patients with confirmed muscle-invasive bladder cancer, multidetector-row CT(MDCT) of the chest, abdomen and pelvis is the optimal form of staging, including MDCT urography for complete examination of the upper urinary tracts. If MDCT is not available, lesser alternatives are excretory urography and a chest X-ray (Grade of recommendation: B).

Neoadjuvant Chemotherapy

Conclusions

• Neoadjuvant cisplatin-containing combination chemotherapy improves overall survival by 5 to 7% at 5 years (Level of evidence: 1a), irrespective of the type of definitive treatment used.
• Neoadjuvant chemotherapy has its limitations regarding patient selection, current development of surgical technique, and current chemotherapy combinations.
**Recommendations**

- Neoadjuvant cisplatin-containing combination chemotherapy should be considered in muscle-invasive bladder cancer, irrespective of definitive treatment (Grade of recommendation: A).
- Neoadjuvant chemotherapy is not recommended in patients with performance status (PS) ≥2 and impaired renal function (Grade of recommendation: B).

**Radical Surgery and Urinary Diversion**

**Conclusions**

- Cystectomy is the preferred curative treatment for localised bladder neoplasm (Level of evidence: 2)
- Radical cystectomy includes removal of regional lymph nodes, the extent of which has not been sufficiently defined (Level of evidence: 3)
- Radical cystectomy in both sexes must not include the removal of the entire urethra in all cases, which may then serve as outlet for an orthotopic bladder substitution (Level of evidence: 3)
- Terminal ileum and colon are the intestinal segments of choice for urinary diversion (Level of evidence: 3)
- The type of urinary diversion does not affect oncological outcome (Level of evidence: 3)

**Recommendations for Radical Cystectomy**

- Radical cystectomy in T2-T4a, N0-NX, M0, and high risk non-muscle invasive bladder cancer (BC) (see Treatment Failure of Non-Muscle-Invasive Bladder Tumors, above) (Grade of recommendation: B)
- No preoperative radiotherapy (Grade of recommendation: A)
- Lymph node dissection should be an integral part of cystectomy, extent not established (Grade of recommendation: B)
- Preservation of the urethra is reasonable if margins are negative. If no bladder substitution is attached the urethra must be checked regularly (Grade of recommendation: B)
- Laparoscopic and robot assisted laparoscopic cystectomy may be an option. Current data, however, have not sufficiently proven its advantages or disadvantages (Grade of recommendation: C).

**Recommendations for Urinary Diversion**

- Treatment is recommended at centers experienced in major types of diversion techniques and postoperative care (Grade of recommendation: B)
- Before cystectomy, the patient should be counselled adequately regarding all possible alternatives, and the final decision should be based on a consensus between patient and surgeon (Grade of recommendation: B).
- An orthotopic bladder substitute should be offered to male and female patients lacking any contraindications and who have no tumour in the urethra and at the level of urethral dissection (Grade of recommendation: B).
Non-Resectable Tumours

Conclusions

- Primary radical cystectomy in T4b bladder cancer is not a curative option.
- If there are symptoms, radical cystectomy may be a therapeutic/palliative option.
- Intestinal or non-intestinal forms of urinary diversion can be used with or without palliative cystectomy.

Recommendations

- For patients with inoperable locally advanced tumours (T4b), primary radical cystectomy is not a curative option (Grade of recommendation: B).
- The indication for performing a palliative cystectomy is symptom relief.
- Morbidity of surgery and quality of life should be weighed against other options (Level of evidence: 3; Grade of recommendation: B/C).

Neo-Adjuvant Radiotherapy

Conclusions

- It is not proven that pre-operative radiotherapy for operable muscle-invasive bladder cancer increases survival (Level of evidence: 2).
- It is shown that pre-operative radiotherapy for operable muscle-invasive bladder cancer, using a dose of 45 to 50 Gy in fractions of 1.8 to 2 Gy results in down-staging after 4 to 6 weeks (Level of evidence: 2).
- Pre-operative radiotherapy with a dose of 45 to 50 Gy/1.8 to 2 Gy does not seem to significantly increase toxicity after surgery (Level of evidence: 3).
- There are suggestions in older literature that pre-operative radiotherapy will result in a decrease in local recurrence of muscle-invasive bladder cancer (Level of evidence: 3).

Recommendations

- Pre-operative radiotherapy is not recommended to improve survival (Grade of recommendation: B).
- Pre-operative radiotherapy for operable muscle-invasive bladder cancer results in tumour downstaging after 4 to 6 weeks (Grade of recommendation: A-C).

Bladder-Sparing Treatments

Transurethral Resection

Conclusion and Recommendation

TUR alone is not a curative treatment option in most patients (Level of evidence: 2a; Grade of recommendation: B).
External Beam Radiotherapy

Conclusions

- External beam radiotherapy alone should only be considered as a therapeutic option when the patient is unfit for cystectomy or a multimodality bladder-preserving approach (Level of evidence: 3).
- Radiotherapy can also be used to stop bleeding from the tumour when local control cannot be achieved by transurethral manipulation because of extensive local tumour growth (Level of evidence: 3).

Recommendation

- There is evidence that radiotherapy alone is less effective than curative therapy (surgery or trimodality treatment) (Grade of recommendation: B).

Chemotherapy

Conclusion

- With cisplatin-based chemotherapy as primary therapy for locally advanced tumours in highly selected patients, complete and partial local responses have been reported (Level of evidence: 2b).

Recommendation

Chemotherapy alone is not recommended as primary therapy for localized bladder cancer (Grade of recommendation: A).

Multimodality Treatment

Conclusions

- A multimodality treatment approach shows a long-term survival rate comparable to that of primary treatment with radical cystectomy (Level of evidence: 3).
- Delay in surgical therapy can compromise survival rates. (Level of evidence: 2b).

Recommendations

- TUR alone is not a curative treatment option in most patients (Grade of recommendation: B).
- Radiotherapy alone is less effective than surgery (Grade of recommendation: B).
- Chemotherapy alone is not recommended as primary therapy for localized bladder cancer (Grade of recommendation: B).
- Multimodality treatment is an alternative in selected, well-informed and compliant patients where cystectomy is not considered for clinical or personal reasons (Grade of recommendation: B).

Adjuvant Chemotherapy

Conclusion
Adjuvant chemotherapy is under debate. Neither randomized trials nor a meta-analysis have provided sufficient data to support the routine use of adjuvant chemotherapy (Level of evidence: 1a).

Recommendation

Adjuvant chemotherapy is advised within clinical trials, but not for routine use because it has not been studied sufficiently (Grade of recommendation: A).

Metastatic Disease

Conclusions

Urothelial carcinoma is a chemosensitive tumour.

Performance status and the presence or absence of visceral metastases are independent prognostic factors for survival. These factors are at least as important as the type of chemotherapy administered (Level of evidence: 3).

Cisplatin-containing combination chemotherapy is able to achieve a median survival of up to 14 months, with long-term disease-free survival reported in about 15% of patients with nodal disease and good PS (Level of evidence: 1b).

Single-agent chemotherapy provides low response rates of usually short duration (Level of evidence: 2a).

Carboplatin-combination chemotherapy is less effective than cisplatin-based chemotherapy in terms of (complete response) CR and survival (Level of evidence: 2a).

Non-platinum combination chemotherapy has produced substantial responses in first- and second-line use, but has not been tested against standard chemotherapy in fit patients or in a purely unfit patient group (Level of evidence: 2a).

To date, there is no defined standard chemotherapy for 'unfit' patients with advanced or metastatic urothelial cancer (Level of evidence: 2b).

Small-sized phase II trials provide evidence of moderate response rates for single agents or nonplatinum combinations at second-line use (Level of evidence: 2a).

Post-chemotherapy surgery after a partial or complete response may contribute to long-term disease-free survival (Level of evidence: 3).

Recommendations

Prognostic factors guide treatment selection (Grade of recommendation: B).

First-line treatment for fit patients: use cisplatin-containing combination chemotherapy with gemcitabine plus cisplatin (GC), methotrexate/vinblastine/adriamycin/cisplatin (MVAC), preferably with granulocyte colony-stimulating factor (GCSF), or high-dose MVAC with GCSF (Grade of recommendation: A).

Carboplatin and non-platinum combination chemotherapy as first-line treatment in patients fit for cisplatin is not recommended (Grade of recommendation: B).

First-line treatment in patients unfit for cisplatin: use carboplatin combination chemotherapy or single agents (Grade of recommendation: C).

Second-line treatment: consider single agents or paclitaxel/gemcitabine if the patient has a good PS (Grade of recommendation: C).
Appendix D. Chemotherapeutic Agents

**Cisplatin**

*Chemical structure*

\[ 
\text{Cl} \quad \text{Pt} \quad \text{NH}_3 \\
\text{Cl} \quad \text{NH}_3 
\]

*Main mechanism of action*

Blocks DNA replication by forming covalent bonds between the complementary strands of the DNA [135]

*Typical dose per cycle (as neo-adjuvant chemotherapy for locally advanced BC)*

70 mg/m\(^2\) in combination with gemcitabine (GC regimen) or in combination with doxorubicin, methotrexate, and vinblastine (MVAC regimen) [98,135]

*Dose-limiting toxicity*

Renal insufficiency [135]

**Doxorubicin (Adriamycin)**

*Chemical structure*
**Main mechanism of action**

Doxorubicin is inserted (intercalated) into the DNA double helix inhibiting the action of topoisomerase II, an enzyme that normally breaks and rejoins the DNA strands during DNA replication to prevent excessive twisting of the double helix [135].

**Typical dose per cycle (as neo-adjuvant chemotherapy for locally advanced BC)**

30 mg/m² in combination with methotrexate, vinblastine, and cisplatin (MVAC regimen) [135]

**Dose-limiting toxicity**

Myelosuppression (particularly leukopenia); cardiotoxicity with congestive heart failure [135]

**Gemcitabine**

**Chemical structure**

![Gemcitabine Chemical Structure](image)

**Main mechanism of action**

Nucleoside analog, competes with deoxycytidine triphosphate for incorporation into the DNA strand during replication [135]

**Typical dose per cycle (as neo-adjuvant chemotherapy for locally advanced BC)**

2000 mg/m² in combination with cisplatin (GC regimen) [98]
Dose-limiting toxicity

Myelosuppression [135]

Methotrexate

Chemical structure

![Chemical structure of Methotrexate]

Main mechanism of action

Antimetabolite, resembles folic acid in structure and can therefore bind to and inhibit dihydrofolate reductase, an enzyme that normally catalyzes the formation of a reduced (hydrogenated) form of folic acid, which is required for the synthesis of the DNA bases [135]

Typical dose per cycle (as neo-adjuvant chemotherapy for locally advanced BC)

90 mg/m² in combination with vinblastine, doxorubicin, and cisplatin (MVAC regimen) [135]

Dose-limiting toxicity

Myelosuppression [135]
Vinblastine

Chemical structure

![Chemical structure of Vinblastine]

Main mechanism of action

Antimicrotubule agent, inhibits formation of the mitotic spindle apparatus [135]

Typical dose per cycle (as neo-adjuvant chemotherapy for locally advanced BC)

9 mg/m\(^2\) in combination with methotrexate, doxorubicin, and cisplatin (MVAC regimen) [135]

Dose-limiting toxicity

Myelosuppression (neutropenia) [135]