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ABSTRACT

Bacillus Calmette-Guérin (BCG) is the standard treatment option for non-muscle-invasive bladder cancer (NMIBC). The mechanism of BCG relies on live mycobacteria inducing an immune response in the bladder wall and is therefore commonly associated with inflammatory side effects and, rarely, mycobacterial infectious complications.

We studied the diagnostic options of erythematous bladder lesions encountered during follow-up of BCG-treated patients. We found that 90% of erythematous bladder lesions were benign manifestations of an inflammatory response to BCG. However, 23% of erythematous lesions showed malignancy in the presence of positive urinary cytology. Most unnecessary biopsies of erythematous lesions can be avoided with the use of urine cytology, and routine biopsy is not recommended. In the second part of the thesis, we identified patients who had developed a BCG infection following BCG therapy in Finland over a 20-year period using nationwide registers, and we described the incidence, mortality, and clinical presentation of BCG infections. These studies showed BCG therapy to be associated with a 1.9% cumulative risk of BCG infections presenting either as systemic or local genitourinary infections. Emphasising the severity, BCG infections were associated with an overall mortality of 10%. BCG infections may also develop more than a year after administration of BCG, demonstrating variability in their clinical manifestations. Finally, we compared BCG maintenance schedules with monthly instillations and the Southwest Oncology Group (SWOG) protocol in terms of tolerability and oncological efficacy. We found that monthly instillations may be considered a comparable option to the SWOG protocol in terms of tolerability and efficacy.

These studies describe the challenges in the follow-up of BCG-treated patients and highlight the risk of potentially severe adverse effects of BCG therapy, which should be recognised when initiating BCG.

KEYWORDS: Bladder cancer, Bacillus Calmette-Guérin, urinary bladder neoplasms, tuberculosis, adverse effects

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TIIVISTELMÄ

Bacillus Calmette-Guérin (BCG) hoito on vakiintunut ei-invasiivisen virtsarakkosyövän hoitomuoto. BCG:n vaikutus perustuu mykobakteerien käynnistämään immuunivasteeseen ja siksi hoitoon usein liittyy myös tulehdusreaktion aiheuttamia haittavaikutuksia. BCG:n sisältämät mykobakteerit voivat levitä virtsarakon sisältä muualle elimistöön aiheuttaen tuberkuloosin kaltaisia infektioita, mutta näiden harvinaisten komplikaatioiden riski ja niiden ennuste tunnetaan melko huonosti.

Tässä väitöskirjassa tutkimme BCG-hoidettujen potilaiden seurannassa usein todettujen virtsarakon punoitusmuutosten diagnostiikkaa. Punoituksista otetuista koepaloista 90 % osoittautui hyvänlaatuisiksi BCG-hoidon immuunivasteeseen liittyviksi muutoksiksi. Punoituksista 23 % osoittautui pahanlaatuisiksi virtsan sytologian ollessa positiivinen. Valtaosa punoitusmuutoksista otetuista koepaloista kuitenkin voidaan välttää virtsan sytologisen tutkimustuloksen mukaisesti, ja rutiininomaista koepalan ottoa virtsarakon punoitusmuutoksista ei suositella. Toisessa osiossa tunnistimme Suomessa 20 vuoden aikana todetut BCG-hoidon jälkeiset BCG-infektiot kansallisten rekisterien avulla, ja kuvailimme niiden ilmaantuvuuden, kuolleisuuden sekä erilaiset ilmenemismuodot. Näissä osatöissä totesimme, että BCG-instillaatioihin liittyi 1,9 % riski BCG infektioille. BCG-infektioihin liittyi 10 % kuolleisuus kuvastaen niiden vakavuutta. Lisäksi totesimme, että BCG-infektioita todetaan hyvin vaihtelevalla viiveellä BCG-instillaatioiden jälkeen ja niitä voidaan todeta jopa yli vuosi BCG-hoidon päättymisen jälkeen. Viimeisessä osatyössä vertailimme kahta BCG-ylläpitohoitomuotoa: Kuukausittaista instillaatiohoitoa sekä Southwest Oncology Group (SWOG) mukaista hoitokaaviota. Näiden kahden hoitomuodon välillä emme todenneet tilastollisesti merkittäviä eroja siedettävyyden tai tehon suhteen ja näitä hoitokaavioita voidaan pitää verrannollisina hoitomuotoina.

Tämä väitöskirja kuvaa BCG-hoidon jälkeisen seurannan haasteita, sekä korostaa BCG-hoitoon liittyviä vakavien haittavaikutuksien riskiä, jotka tulee huomioida, kun potilaalle aloitetaan BCG-hoito.

AVAINSANAT: Virtsarakkosyöpä, Bacillus Calmette-Guérin, virtsarakon kasvaimet, tuberkuloosi, haittavaikutukset

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Abbreviations

ALA	Aminolevulinic acid
APC	Antigen-presenting cells
ASA	American Society of Anesthesiologists
AUA	American Urological Association
AUC	Atypical urothelial cells
BC	Bladder cancer
BCG	Bacillus Calmette-Guérin
CCI	Charlson comorbidity index
CI	Confidence interval
CIS	Carcinoma in situ
CT	Computed tomography
CUETO	Club Urológico Español de Tratamiento Oncológico
DM	Detrusor muscle
EAU	European Association of Urology
EMDA	Electromotive drug administration
EORTC	European Organisation for Research and Treatment of Cancer
ERBT	En-bloc resection of bladder tumour
FCR	Finnish Cancer Registry
FDA	Food and Drug Administration
FNIDR	Finnish National Infectious Diseases Register
HAL	Hexaminolevulinic acid
HG	High-grade
HGUC	High-grade urothelial carcinoma
HIVEC	Hyperthermic intravesical chemotherapy
HR	Hazard ratio
IBCG	International Bladder Cancer Group
ICD	International Classification of Diseases
IQR	Interquartile range
ISUP	International Society of Urological Pathology
LG	Low-grade
LVI	Lymphovascular invasion

MIBC	Muscle-invasive bladder cancer
MMC	Mitomycin C
MRI	Magnetic resonance imaging
NAC	Neoadjuvant chemotherapy
NBI	Narrow band imaging
NHGUC	Negative for high-grade urothelial carcinoma
NMIBC	Non-muscle-invasive bladder cancer
NPV	Negative predictive value
NSAID	Non-steroidal anti-inflammatory medication
PDD	Photodynamic diagnosis
PPV	Positive predictive value
PUNLMP	Papillary urothelial neoplasms of low malignant potential
RC	Radical cystectomy
RCT	Randomised controlled trial
RFS	Recurrence-free survival
RITE	Radiofrequency-induced thermochemotherapy
SHGUC	Suspicious for high-grade urothelial carcinoma
SI	Single instillation
SNP	Single nucleotide polymorphisms
SWOG	Southwest Oncology Group (now the Cancer Research Network)
TB	Tuberculosis
THL	Finnish Institute for Health and Welfare
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TNM	Tumour-, node-, metastasis
TUR-BT	Transurethral resection of bladder tumour
UC	Urothelial cancer
UICC	Union Internationale Contre le Cancer
US	Ultrasound
UTUC	Upper tract urothelial carcinoma
WHO	World Health Organization

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Nurminen P, Ettala O, Seppänen M, Taimen P, Boström PJ, Kaipia A. Urine cytology is a feasible tool for assessing erythematous bladder lesions after bacille Calmette-Guérin (BCG) treatment. BJU Int. 2019;123(2):246-51.

II Nurminen P, Ettala O, Uusitalo-Seppälä R, Nummi A, Järvinen R, Antti K, Boström PJ. Incidence of and mortality from Bacille Calmette-Guérin (BCG) infections after BCG instillation therapy. BJU Int. 2022 Jun;129(6):737-743.

III Nurminen P, Ettala O, Uusitalo-Seppälä R, Högerman M, Kaipia A, Boström PJ. Clinical presentation of bacille Calmette-Guérin (BCG) infections after BCG instillation therapy. BJU Int. 2023 Mar;131(3):306-312.

IV Nurminen P, Nummi A, Kesti O, Ettala O, Högerman M, Järvinen R, Sairanen J, Kaipia A, Boström PJ. Comparison of Bacillus Calmette-Guérin Maintenance Therapy with Monthly Instillations and the Southwest Oncology Group Protocol in the Treatment of Non-muscle-invasive Bladder Cancer. Eur Urol Focus. 2023 May 9:S2405-4569(23)00110-4.

1 Introduction

In Finland, bladder cancer (BC) is the fourth commonest cancer among men and the sixteenth among women. (Syöpärekisteri) Around 70–80% of bladder cancers present as non-muscle-invasive bladder cancer (NMIBC), in which the bladder tumour is confined to the urothelium and underlying lamina propria. (Pasin et al., 2008) NMIBC comprises a heterogeneous group of diseases with a divergent malignant potential, which is affected by several factors including patient age, tumour stage, grade, number and size of the tumours, previous recurrences, and presence of carcinoma in situ. The treatment of NMIBC comprises transurethral resection of the bladder tumour (TUR-BT) and intravesical instillation therapies, which are used for prevention of recurrence and progression of disease. (Babjuk et al., 2022)

Bacillus Calmette-Guérin (BCG) is an intravesically administered immuno-oncological agent containing a live, attenuated strain of *Mycobacterium bovis* bacilli. BCG was discovered over 100 years ago and was introduced as an intravesical treatment against BC in 1976. (Morales et al., 1976) BCG induces an inflammatory process in the bladder wall, helping the immune cells to identify and eliminate recurrent cancer cells and prevent tumour progression to invasive stages. Thus, BCG is indicated whenever there is a high risk of recurrence and progression of NMIBC.

BCG therapy consists of repeated intravesical instillations, starting with an induction period of six weekly instillations followed by a maintenance period of 1–3 years. The most used maintenance protocol is that of the Southwest Oncology Group (SWOG, now the Cancer Research Network), in which BCG is administered as 3-week cycles of weekly instillations repeated at 3, 6, and 12 months and every 6 months thereafter. (Lamm et al., 2000) However, after multiple Finnish multicentre trials applying BCG maintenance as monthly instillations showed comparable results, the monthly schedule was widely adopted in Finland. (Järvinen et al., 2009; Järvinen et al., 2015; Marttila et al., 2016)

Due to the inflammatory mechanism of BCG, many patients suffer from side effects either locally such as haematuria, dysuria, and urgency, or systemically such as fatigue, general malaise, and fever. Not uncommonly, erythematous bladder lesions are observed on cystoscopy that are related to inflammatory side effects.

Erythematous lesions may be hard to distinguish from recurrent disease and are often biopsied, further increasing the burden of disease and the risk of complications. Due to the toxicity of BCG, some 8–25% of patients discontinue instillations, which may compromise treatment efficacy. (Brausi et al., 2014; Nummi et al., 2019; van der Meijden et al., 2003)

As a rare complication, the mycobacteria may also disseminate from the bladder, causing tuberculous infections either in the genitourinary tract, such as cystitis or epididymitis, or as generalised infections, such as pulmonary infections, spondylodiscitis, or vascular infections, which may also be fatal. (Perez-Jacoiste Asin et al., 2014) BCG infections are treated with antituberculosis medication with an isoniazid-based regimen combined with rifampicin and ethambutol for 6–9 months, depending on the localisation of the infection. Although intravesical BCG has been used for several decades, the risk of BCG infection related to instillations is still unclear and its spectrum of manifestation is based mostly on case reports and small series. In addition, the mortality linked to BCG infections is unknown. It is important to acknowledge the risks associated with BCG while weighing the harms versus benefits of treatment.

2 Review of the Literature

2.1 Epidemiology and risk factors of bladder cancer

Globally, BC is the tenth commonest cancer, with around 573 000 new diagnoses and 213 000 related cancer deaths every year. (Sung et al., 2021) BC is four times more common among men than women, with an overall incidence and mortality rate of 9.5 and 3.3 per 100 000, respectively. (Sung et al., 2021) As most BCs appear as non-invasive bladder tumours with a good overall prognosis, BC is a now highly prevalent disease that continues to grow among increasingly aging populations. Additionally, due to the recurrent nature of the disease, BC is considered one of the most expensive cancers consuming healthcare resources. (Yeung et al., 2014)

In the Finnish Cancer Registry, BC is reported together with cancers of the urethra and upper urinary tract, of which the latter are the fourth commonest cancers in males and the eleventh in females. (Syöpärekisteri) While the number of diagnosed cancers of the urinary tract has continued to rise, the age-adjusted incidence rate has remained more stable over the last two decades, with around 10 and 40 new diagnoses per 100 000 person years in females and males, respectively, reflecting the role of aging of the population in the rising incidence of BC. Consequently, between 1980 and 2016, the incidence of BC continued to grow, with 1159 new diagnoses in 2016. **(Figure 1)**

Smoking is the most important risk factor for BC and accounts for roughly 50% of cases. (Burger, Catto, et al., 2013) Multiple case-control studies have suggested that smoking up to 15–20 cigarettes per day increases the risk of BC. (Brennan et al., 2000) The number of years of smoking increases the risk of BC linearly, suggesting that smoking has a cumulative effect on BC development. (Brennan et al., 2000) In addition, it has been shown that low-intensity smoking over a longer period has a more deleterious effect on the risk of BC than does short duration and high intensity. (van Osch et al., 2019) After cessation of smoking, the risk of BC gradually drops but does not reach that of never smokers, even 25–30 years or more after quitting. (Brennan et al., 2000; van Osch et al., 2019) As smoking status is also a risk factor for recurrences following TUR-BT, it could be hypothesised that successful

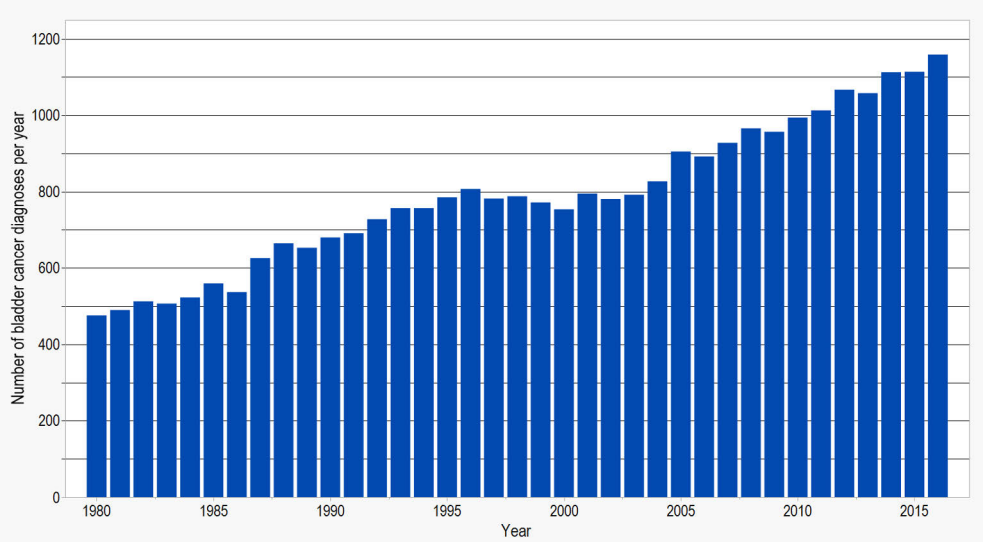

Figure 1. Annual incidence of bladder cancer (including all stages) in Finland between 1980 and 2016 (Published with permission from data retrieved from the Finnish Cancer registry).

cessation of smoking would lower the risk of recurrences following primary treatment, but this has not been confirmed. (Caini et al., 2022; Lammers, Witjes, Hendricksen, et al., 2011) However, counselling on quitting smoking should always be included as part of patient education during diagnosis and treatment of BC, as stopping smoking affects the overall health of the patient as well as comorbidities and treatment-related complications.

Occupational exposure to aromatic amines and polycyclic aromatic hydrocarbons is considered the second most important risk factor for BC. A person may be exposed to these agents while inhaling diesel engine exhausts, fumes from mineral oils, and painting-industry and hairdressing products. (Rushton et al., 2010) However, better awareness of these risk factors for multiple cancers has led to developments within industry to lower exposure, and thus to a lesser role of these occupational risk factors in recent decades.

BC may occur also as a secondary malignancy due to pelvic radiation therapy for prostate, rectal, or gynaecological cancers (Nieder et al., 2008; Warschkow et al., 2017; Wen et al., 2022) In addition, exposure to arsenic and some medications such as cyclophosphamide and pioglitazone have been associated with an increased risk of BC. (Burger, Catto, et al., 2013; Steinmaus et al., 2014; Tuccori et al., 2016) Chronic bladder infection due to schistosomiasis is associated with the risk of developing mainly squamous cell cancer, which is found in endemic areas in northern and sub-Saharan Africa. (Burger, Catto, et al., 2013)

2.2 Pathological classification of bladder cancer

The urinary bladder consists of three anatomical structures that act as landmarks in the classification of BC: the urothelium, the lamina propria, and the detrusor muscle. The innermost structure is the urothelium, consisting of three to seven rows of urothelial cells and creating an impermeable layer responsible for containing the urine and its micro-organisms. The next layer is the lamina propria, consisting of subepithelial connective tissue, an unmyelinated nerve fibre plexus, vasculature, and smooth muscle fibres (muscularis mucosae). The outermost structure is the detrusor muscle (muscularis propria) consisting of bundles of smooth muscle fibres oriented in random directions and able to contract and empty the bladder.

Most commonly, BC originates in urothelial cells and is referred to as urothelial cancer (UC), which is the focus of this thesis. Rare non-UC bladder neoplasms include squamous cell, glandular, urachal, and urethral accessory gland neoplasms and neoplasms of Mullerian type. (Netto et al., 2022)

UC includes a heterogeneous group of diseases harbouring a very divergent malignant potential. UC is characterised anatomically using the Tumour, Node, Metastasis (TNM) classification according to the Union Internationale Contre le Cancer (UICC) 8th edition, and morphologically by histological grading according to the World Health Organization (WHO) classification. (Brierley, 2016; Moch et al., 2016)

The most important factor guiding the treatment choices of UC is the division into NMIBC and muscle-invasive bladder cancer (MIBC). Approximately 75% of UCs appear as NMIBC, in which the tumour is confined to the urothelium and lamina propria. (Pasin et al., 2008) MIBC is concerned whenever the tumour reaches the detrusor muscle. This thesis focuses on NMIBC.

2.2.1 TNM classification

The TNM classification of BC adopted from the UICC 8th edition is presented in **Table 1.** (Brierley, 2016) Primary tumour (T-category) represents the anatomical invasion depth of the tumour within the bladder wall. Regional lymph node (N-category) represents the metastatic spread of disease into regional lymph nodes, which include the nodes surrounding the common, internal, and external iliac vessels and the obturator fossa. Distant metastasis (M-category) represents distant metastases in lymph nodes outside of regional areas, bone, or visceral metastases.

Table 1. TNM-classification of bladder cancer (Adopted from the UICC, 8th edition).

T- Primary tumour		**N – Regional Lymph Nodes**	
Tx	Primary tumour cannot be assessed	**Nx**	Regional lymph nodes cannot be assessed
T0	No evidence of primary tumour	**N0**	No regional lymph node metastasis
Ta	Non-invasive papillary carcinoma	**N1**	Metastasis in a single lymph node in the true pelvis (hypogastric, obturator, external iliac, or presacral)
Tis	Carcinoma in situ: 'flat tumour'	**N2**	Metastasis in multiple regional lymph nodes in the true pelvis (hypogastric, obturator, external iliac, or presacral)
T1	Tumour invades subepithelial connective tissue	**N3**	Metastasis in common iliac lymph node(s)
T2	Tumour invades muscle T2a Tumour invades superficial muscle (inner half) T2b Tumour invades deep muscle (outer half)	**M – Distant metastasis**	
T3	Tumour invades perivesical tissue T3a Microscopically T3b Macroscopically (extravesical mass)	**M0**	No distant metastasis
T4	Tumour invades any of the following: prostate stroma, seminal vesicles, uterus, vagina pelvic wall, abdominal wall T4a Tumour invades prostate stroma, seminal vesicles, uterus or vagina T4b Tumour invades pelvic wall or abdominal wall	**M1**	M1a Non-regional lymph nodes M1b Other distant metastases

In addition to the present UICC TNM classification, it has been suggested that dividing T1 tumours pathologically into substages either by histoanatomical or micrometric techniques might have a prognostic value guiding the treatment approach between bladder-sparing and radical cystectomy (RC). (Compérat et al., 2021; Kardoust Parizi et al., 2020) However, as the optimal classification between the two sub-staging techniques has not yet been defined, the use of T1 sub-staging has not been firmly adopted either globally or in Finland.

2.2.2 Histological grading

The tumour grade is determined by the tumour's architectural structure, cell morphology, and mitotic activity. It describes the aggressiveness of the tumour and its tendency to grow and infiltrate into surrounding tissues. As muscle-invasive tumours are mostly poorly differentiated and aggressive, grading is especially important in determining the treatment approach in NMIBC.

Traditionally, UC is graded according to the WHO 1973 classification, with a three-tier system of grades 1–3. (Mostofi et al., 1973) Grade 1 (G1) represents a tumour with the least cellular anaplasia compatible with a diagnosis of malignancy, mild architectural disorder, and low level of mitotic activity. Grade 3 (G3) indicates a tumour with the most severe cellular anaplasia, complete loss of architectural structure, and high level of mitotic activity. Grade 2 (G2) tumours are intermediate tumours that fall between G1 and G3. (Mostofi et al., 1973) In a pooled analysis, G1, G2, and G3 tumours were associated with a risk of recurrence of 33%, 42%, and 63%, respectively, and with a risk of progression of 3%, 10%, and 29%, respectively. (Soukup et al., 2017)

The International Society of Urological Pathology (ISUP) proposed a new grading system in 1998 aiming to align better with the evolving treatment approaches of NMIBC. (Epstein et al., 1998) The new grading was recommended by a WHO consensus group in 2004, whereupon malignant bladder tumours are divided into papillary urothelial neoplasms of low malignant potential (PUNLMP) and low grade (LG) or high grade (HG) carcinomas. (Eble et al., 2004; Epstein et al., 1998) Since then, the WHO 1973 and WHO 2004 grading systems have been used in parallel. Their relationship to each other is shown in **Figure 2.**

In the WHO 2004 classification, PUNLMP represents the most benign part of the G1 group with the mildest malignant features. Although a PUNLMP is considered nearly benign with a less than 2% risk of progression, and is not labelled as "cancer" in the new WHO 2004 classification, it is nonetheless associated with a recurrence risk of 12–28% and is therefore treated with a similar approach to LG tumours. (Babjuk et al., 2022; Hentschel et al., 2020; Soukup et al., 2017) LG tumours represent parts of the G1 and G2 tumours with mild to moderate cellular anaplasia and mitotic activity and are associated with a 4% risk of progression and 38% risk of recurrences. HG tumours include G3 tumours and G2 tumours with the most malignant features. However, G3 tumours have a worse prognosis than G2 HG tumours. Thus, using both systems in parallel may improve individualised treatment strategies.

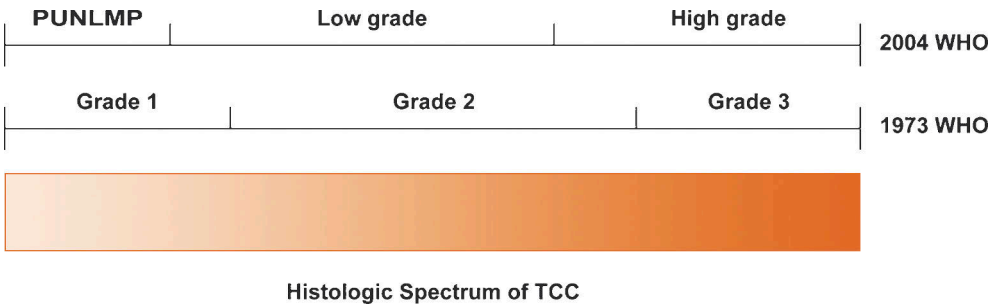

Figure 2. Stratification of the WHO 2004 and WHO 1973 grading systems. (Reproduced with permission from (MacLennan et al., 2007) Eur Urol 2007).

Variably in studies, agreement between pathologists using the WHO 1973 and WHO 2004 systems is achieved in 38–89% and 43–100% of tumours, respectively, suggesting that the WHO 2004 system has marginally better reproducibility. (Soukup et al., 2017) Despite some differences in the stratification, none of the grading systems have proven to be superior in predicting recurrence and progression. Therefore, both grading systems are usually included in the pathological analysis in Finland. Currently, the European Association of Urology (EAU) guidelines on NMIBC applies both grading systems in parallel, whereas the American Urological Association (AUA) guidelines are based on the WHO 2004 classification. (Babjuk et al., 2022; Chang et al., 2016)

2.2.3 Carcinoma in situ

Carcinoma in situ (CIS) is a flat urothelial tumour characterised histologically by high-grade urothelial carcinoma cells confined to the urothelium. (Lamm et al., 1998) Three different settings of CIS presentation may be distinguished: 1) primary, when diagnosed as an isolated lesion, 2) secondary, when diagnosed during follow-up after initial resection of a non-CIS TaT1 tumour, and 3) concomitant, when diagnosed together with a non-CIS TaT1 tumour. CIS is a clinically challenging entity for many reasons. First, it may present in a normal-appearing urothelium or resemble a benign inflammatory lesion during cystoscopy and can easily be missed if not biopsied. (Melamed et al., 1964) Second, more often than papillary tumours, CIS appears multifocal and involves the upper urinary tract and the prostatic ducts and may therefore progress to invasive stages after primary treatment of lesions in the bladder. (Sylvester, van der Meijden, Witjes, Jakse, et al., 2005) Finally, CIS is a precursor of high-grade invasive disease, with a 54% risk of progression to invasive disease, whereas the risk of progression of papillary tumours is approximately 10–20%. (Lamm et al., 1998; Sylvester et al., 2021) Therefore, it is important to

distinguish CIS from papillary tumours and report its presence in the pathological analysis, as it has strong implications for treatment.

2.2.4 Divergent differentiation, histologic variants of UC, and lymphovascular invasion

UC may present with histologically divergent differentiation along with another cancer type, such as squamous cell, glandular, or trophoblastic differentiation, or it may also present with fundamental characteristics of UC but with definitive variant histology, signifying also altered clinical behaviour and malignant potential. (Compérat et al., 2021) Different variants of UC histology include micropapillary, nested, plasmacytoid, and lymphoepithelioma-like, small-cell, sarcomatoid or neuroendocrine variants. (Moch et al., 2016) Of all differentiated UC and histologic variants, squamous cell with glandular differentiation is the most common and may be seen in up to 13–32% of UC tumours. (Shah et al., 2013) Squamous and glandular differentiation types also harbour similar malignant potential to that of pure UC, whereas other histological variants are present in fewer than 10% of UC tumours and present more often as invasive tumours with a worse prognosis than pure UC. (Compérat et al., 2021; Veskimäe et al., 2019)

Lymphovascular invasion (LVI) signifies the initial step in dissemination of UC into metastatic disease. Thus, tumours that show histological evidence of LVI in a TUR-BT specimen are more likely to be invasive, and lymph node metastases may be found in 41–45% of patients in radical cystectomy (RC) specimens. (Kim et al., 2014; Kunju et al., 2008) LVI predicts a poorer prognosis, even if RC and lymphadenectomy are performed and lymph node metastases are not found. (Kim et al., 2014; Tilki et al., 2013)

2.3 Diagnosis of bladder cancer

The most common symptom of BC is painless but visible haematuria. The estimated prevalence of BC among patients investigated for visible haematuria is 19–22%. (Edwards et al., 2006; Khadhouri et al., 2021) Irritative voiding symptoms such as dysuria, urgency, or nocturia may also be the initial manifestation of BC, especially in CIS, and should also be evaluated if no explanation for the voiding symptoms exists. (Cheng et al., 1999) Microscopic haematuria in a dipstick test, especially among asymptomatic patients, is not an absolute indication of invasive evaluation, as it rarely signifies BC and may be caused by several benign conditions. (Gonzalez et al., 2019) Variably in studies, the prevalence of BC among patients with microscopic haematuria is 1–5%. (Edwards et al., 2006; Gonzalez et al., 2019;

Jubber et al., 2020; Khadhouri et al., 2021) Symptoms such as flank pain, fatigue, and weight loss may signify advanced or metastasized BC.

2.3.1 Cytology and biomarkers

Urine cytology is the microscopic evaluation of exfoliated urinary cells and their cytological features. The results are categorised on a five-tier grading scale into classes 1–5, of which 1–2 indicate a negative cytology, class 3 indicates cells with abnormal features but not sufficiently pathognomonic for UC, and classes 4–5 indicate clusters of HG urothelial carcinoma cells in the urine and are interpreted as positive. (Owens et al., 2013) Currently the Paris reporting system is recommended, which uses the following diagnostic categories: negative for HG urothelial carcinoma (NHGUC); atypical urothelial cells (AUC); suspicious for HG urothelial carcinoma (SHGUC); and HG urothelial carcinoma (HGUC) (Rosenthal et al., 2016)

The overall sensitivity of cytological detection of UC is between 35% and 55% and the specificity between 86% and 94%, meaning that a positive cytology is highly predictive of UC but a negative result does not rule out a urinary tract tumour. (Glas et al., 2003; van Rhijn et al., 2005) Cytology is more sensitive in detecting HG tumours and CIS, with a sensitivity of 55–84%, than in detecting LG tumours, among which the sensitivity varies between 10% and 16%. (van Rhijn et al., 2005; Yafi et al., 2014; Yafi et al., 2015) In addition, cytology does not provide information on what level of the urinary tract the malignant or suspicious cells have exfoliated from. Another limitation is the need to train pathologists to achieve a reproducible level of expertise. (Raitanen et al., 2002; Reid et al., 2012)

Due to the low sensitivity of cytology, substantial efforts have been made to find new biomarkers for detection of UC with better sensitivity and negative predictive value (NPV), which could be used to replace cystoscopy especially during surveillance of BC. (Soria et al., 2018) Currently, multiple biomarkers for BC are commercially available that use novel genomic or transcriptomic techniques to detect the presence of UC. The most promising results have been observed with Cx-Bladder (Cx-Bladder™, Pacific edge, New Zealand), ADX-Bladder™ (Arquer diagnostics, United Kingdom), Xpert Bladder™ (Cepheid, United States), and Epicheck™ (Nucleix, Israel), with an overall NPV of 93–97% suggesting that some of these biomarkers could be used in part to replace some of the cystoscopies during follow-up of BC. (Laukhtina et al., 2021; Soria et al., 2018) However, data on novel biomarkers are better available for surveillance and are still insufficient for primary diagnostics of BC, which relies primarily on urinary cytology and cystoscopy. (Laukhtina et al., 2021)

2.3.2 Cystoscopy and transurethral resection

To date, no other method has been able to replace cystoscopy in the diagnosis or surveillance of BC. Diagnostic cystoscopy is performed as an outpatient procedure using a flexible scope with which the bladder tumour is ultimately visualised.

The stage (T-category) and grade of the tumour are established by a bladder biopsy or TUR-BT, which must therefore be performed meticulously to determine the prognosis and correct treatment approach. As only CIS can be properly diagnosed with a biopsy during flexible cystoscopy, most bladder tumours need to be eradicated with TUR-BT, while a biopsy is more of a useful initial step if suspicion of a tumour is not resolved on cystoscopy. However, an outpatient biopsy to confirm histologically a small recurrent bladder tumour before fulguration with electrocautery or laser is acceptable during surveillance of LG NMIBC.

To pathologically evaluate the depth of invasion and rule out MIBC, the detrusor muscle (DM) beneath the tumour base must be obtained as part of the histological specimen. The presence of DM in the specimen is a determinant of the quality of the TUR-BT and is essential for evaluation of muscle-invasive components in the tumour. An insufficient TUR-BT is a risk factor for understaging, recurrences, and progression. (Brausi et al., 2002; Mariappan et al., 2010; Naselli et al., 2018) Therefore, a second resection of the tumour bed should be performed 2–6 weeks after resection in case of incomplete resection of especially HG tumours, and always after a resection of T1 tumours, since understaging of T1 tumours can lead to potentially fateful undertreatment. (Babjuk et al., 2022) Variably in studies, residual tumour after resection of TaT1 tumour may be found in 16–71% of patients, and 0–32% of T1 tumours may be upstaged to T2, emphasising the importance of good quality of primary TUR-BT and performing a re-TUR-BT whenever indicated (Babjuk et al., 2022; Cumberbatch et al., 2018) The most common site for early recurrences is the primary tumour bed, suggesting that by improving the quality of TUR-BT early recurrences could be reduced. (Brausi et al., 2002)

To overcome these diagnostic and therapeutic challenges, new methods of tumour visualisation have been developed. Narrow band imaging (NBI) involves digital filtering of wavelengths of light, and only 540 nm (green) and 415 nm (blue) wavelengths are used. This enhances the visualisation of capillaries and blood vessels, and the tumour is identified when the capillary structures are interrupted by tumour growth. NBI has been shown to improve the diagnostic accuracy of cystoscopy during TUR-BT, but the evidence supporting the role of NBI in reducing recurrences is not as clear. (Chen et al., 2019; Gravestock et al., 2021) A recent Cochrane systematic review including eight studies assessing the benefit of NBI during TUR-BT concluded that NBI-enhanced TUR-BT is safe and may

reduce recurrences during follow-up with a low certainty of evidence. (Lai et al., 2022)

Photodynamic diagnosis (PDD) involves a single intravesical instillation of 5-aminolevulinic acid (ALA) or hexaminolevulinic acid (HAL) prior to cystoscopy or TUR-BT, which is then performed under blue light. ALA or HAL binds to the tumour cells, making small tumours fluorescent and easier to identify. Indeed, multiple meta-analyses have shown that PDD during TUR-BT improves the sensitivity of cystoscopy, decreases the number of residual tumours, and improves the recurrence-free survival (RFS) during follow-up. (Burger, Grossman, et al., 2013; Kausch et al., 2010; Veeratterapillay et al., 2021) The downside of PDD is its low specificity of around 62%, meaning that a normal urothelium may appear positive, for instance, due to inflammation. Recently, a randomised controlled trial (RCT) of 538 patients evaluating the PDD at TUR-BT showed conflicting results to previous studies and did not observe any benefit for RFS or cost-effectiveness during 3 years of follow-up (Heer et al., 2022) The underlying reason behind these conflicting results is unclear. Possibly, with the constant development of white-light cystoscopy technology and improved image quality and instruments, the additive value of visualisation techniques has become less important. A Finnish multicentre trial (FinnBladder 9) assessing the effect of PDD on recurrences among patients with recurrent or multiple LG tumours is ongoing and will provide updated knowledge on the value of PDD during TUR-BT over the past decade. (Trial ongoing. NCT01675219)

2.3.3 Imaging

Imaging is usually performed at the initial diagnostic phase of haematuria to visualise the bladder and upper urinary tract and kidneys to detect any tumours, urinary stones and hydronephrosis and, in case of malignant disease, perform staging of disease for lymph-node, visceral, or bone metastases. The primary aim of imaging, however, is not to rule out a bladder tumour, as this is eventually done with cystoscopy.

Ultrasound (US) is often performed already as part of primary care and is generally a useful tool in initial imaging, as it has good availability and does not cause radiation. The sensitivity and specificity of US in detecting BC are roughly 73–91% and 80–95%, respectively, but lower in detecting bladder tumours less than 15 mm in size. (Francica et al., 2008; Jiang et al., 2020; Kocakoc et al., 2008) For the evaluation of upper urinary tract tumours including kidney tumours in haematuria workup, US may provide fairly good overall accuracy as an initial evaluation tool (David et al., 2021), but it is insufficient to rule out small calyceal or pelvic upper-tract urothelial carcinoma (UTUC).

Computed tomography (CT) urography is the standard imaging modality for BC and may be used for assessment of extravesical invasion, metastases, and concomitant UTUC. CT urography detects bladder tumours with 86% sensitivity and 92% specificity and UTUC with 67–100% sensitivity and 89–99% specificity. (Grahn et al., 2017; Rud et al., 2020; Trinh et al., 2018) However, screening of UTUC at BC diagnosis is debatable, as concomitant UTUC is rare at presentation of BC and has an observed overall probability of only 1.8%, although this can rise to 7.4% if the tumour is located in the trigone. (Palou et al., 2005)

Multiparametric magnetic resonance imaging (MRI) may be used for more accurate assessment of local invasion of NMIBC. (Panebianco et al., 2018) However, the role of MRI is still unclear and is not yet a part of routine imaging of BC.

2.3.4 Risk groups and predicting disease recurrence and progression of NMIBC

The malignant potential of NMIBC is highly heterogeneous; therefore the treatment must be risk-adapted. To estimate individual prognoses, the EAU NMIBC guidelines panel performed a multicentre retrospective analysis of 3401 patients for whom the risk of progression was individually estimated using factors such as patient age at diagnosis, tumour stage and grade, number of tumours and diameter of the largest tumour, and presence of concomitant CIS. (Babjuk et al., 2022; Sylvester et al., 2021) Based on these factors, patients were classified into low, intermediate, high, and very high risk groups. The stratification of patients is shown in **Table2.**

The probability of progression at 5 years in the low, intermediate, high, and very high risk groups is around 0.57–93%, 3.6–4.9%, 9.6–11%, and 40–44%, respectively.

The limitation of the EAU risk tables is that patients with primary CIS, previous recurrences, variant histology, and LVI were excluded from the analyses, and the treatment of patients with these risk factors needs to be evaluated individually based on the EAU risk tables. In addition, patients treated with BCG were also excluded, and their risk of progression following BCG treatment cannot be directly drawn from the EAU risk tables. To estimate the risk of recurrences and progression after BCG treatment, a designated nomogram and risk estimation tool have been introduced by the European Organisation for Research and Treatment of Cancer (EORTC) and the Club Urológico Español de Tratamiento Oncológico (CUETO). (Cambier et al., 2016; Fernandez-Gomez et al., 2009) Using these data, it was shown that the 2016 update of the EORTC/EAU risk tables overestimates the risk of progression, especially among patients with high-risk disease treated with BCG therapy. (Fernandez-Gomez et al., 2011)

Table 2. Risk groups of NMIBC, adopted from the Sylvester et al 2021, Eur urol.

Risk group	
Low risk	A primary, single, Ta LG/G1 tumor ≤3 cm in diameter without CIS in a patient ≤70 yrs A primary LG/G1 tumor with at most ONE of the following additional clinical risk factors: • Age >70 yr • Multiple tumors • Tumor diameter ≥3 cm • Stage T1
Intermediate risk	Patients without CIS who are not included in either the low-, high-, or very high-risk groups
High risk	All T1 HG/G3 without CIS, EXCEPT those included in the very high-risk group All CIS patients, EXCEPT those included in the very high-risk group. Stage, grade with additional clinical risk factors • Ta LG/G2 or T1 G1, no CIS with all 3 risk factors • Ta HG/G3 or T1 LG, no CIS with at least 2 risk factors • T1 G2 no CIS with at least 1 risk factor
Very high risk	Stage, grade with additional clinical risk factors • Ta HG/G3 and CIS with all 3 risk factors • T1 G2 and CIS with at least 2 risk factors • T1 HG/G3 and CIS with at least 1 risk factor • T1 HG/G3 no CIS with all 3 risk factors
Additional risk factors: Age >70 yr, multiple tumours, tumour diameter ≥3 cm	

2.4 Treatment of NMICB

2.4.1 TUR-BT

Complete eradication of tumour and satellites is an important initial step in the management of NMIBC. As discussed earlier, the quality of TUR-BT and sufficient depth of resection to reach the DM under the tumour base is imperative to achieve definitive pathological analysis and reduce the risk of understaging and early recurrences. TUR-BT is performed with a resectoscope, preferably using bipolar energy.

In conventional TUR-BT, the exophytic part and base of the tumour should be resected in separate vials for the pathologist, to facilitate identification of the tumour

base when evaluating the depth of invasion. *En bloc* resection of the bladder tumour (ERBT) is performed with a conventional resectoscope by opening the urothelium around the tumour down to the level of the DM, then disintegrating the muscle bundles beneath the tumour and detaching the tumour in one piece. Increasing evidence has shown that with ERBT the quality of pathological specimens is better, with 94% presence of DM and fewer bladder perforations and similar operation time, but the superiority of ERBT in reducing recurrence rate has not been firmly established despite some promising results. (Yanagisawa et al., 2022; Zhang et al., 2020) ERBT is technically successful mostly in tumours less than 3 cm in diameter but may be more difficult in tumours located at the bladder dome. (Teoh et al., 2020)

2.4.2 Cystectomy

Immediate cystectomy in NMIBC is a clinical challenge, as the majority of NMIBC tumours may be completely eradicated with TUR-BT and many patients with CIS and high-risk disease will respond to BCG therapy. Currently, RC is considered a standard first-line option for patients with BCG-unresponsive NMIBC, since—despite intensive research in this field—effective bladder-preserving treatment options are still lacking. (Babjuk et al., 2022; Kamat et al., 2017)

Indications for immediate RC are not as clear. EAU guidelines recommend discussing immediate RC as a first-line treatment option for those at very high risk, considering the substantial baseline risk of 40% for progression to MIBC. (Babjuk et al., 2022) However, it has been estimated that following adequate BCG, the risk of progression in the very-high risk group is approximately 14%, suggesting that abandoning BCG and categorically performing RC may mean that some patients are overtreated. (Lobo et al., 2022) However, particularly in the case of multiple T1 tumours with concomitant CIS, cystectomy should be considered. In addition, histologically substaged T1 tumours with extensive pT1, presence of LVI, and variant histology are associated with a substantial risk of upstaging and poor prognosis, and therefore immediate cystectomy should be considered among these patients. (Abufaraj et al., 2019; D Andrea et al., 2018; de Jong et al., 2021; Kim et al., 2014; van Rhijn et al., 2012)

It has been suggested that prognosis of MIBC after RC might be worse if the disease has progressed after previously treated NMIBC compared to treatment-naïve primary MIBC. (Moschini et al., 2016) However, the evidence from retrospective studies is conflicting and a meta-analysis including 11 analyses and 4102 patients did not observe any differences between survival outcomes of primary and progressive MIBC. (J. Chen et al., 2018) While neoadjuvant chemotherapy (NAC) has become the mainstay in the treatment of MIBC, recent reports have suggested that NAC plays an important role also in the treatment of progressive MIBC, as

prognosis of primary and progressive MIBC appears comparative if NAC has been applied. (Benidir et al., 2022; Hensley et al., 2021) However, there are concerns that the response to NAC may be inferior among patients with progressive MIBC. (D'Andrea et al., 2022) Thus, new tools are needed for choosing the optimal treatment for patients with NMIBC at particularly high risk of progression. Ultimately, the choice between a bladder-preserving strategy or immediate RC is based on shared decision-making between the patient and the physician.

2.4.3 Intravesical instillation of chemotherapy

NMIBC is a recurrent disease, and 30–80% of patients may experience recurrences after TUR-BT. (Sylvester et al., 2006) The nature of transurethral surgery in NMIBC is a predisposing factor for recurrences, since TUR-BT may be incomplete, small tumours may be overlooked, and circulation of tumour cells in the bladder during surgery may result in seeding and re-implantation of tumour cells. (Brausi et al., 2002; Soloway & Masters, 1980) Despite efforts to improve surgical techniques and optical enhancement tools, surgery alone is insufficient to control recurrences, and all patients benefit from adjuvant treatments. Intravesical instillations of a chemotherapeutic agent as a topical cytotoxic treatment has an important role especially in reducing recurrences of NMIBC.

Single instillation of chemotherapeutic agent

Single instillation (SI) of chemotherapeutic agent aims to destroy the circulating tumour cells and small tumour residuals after TUR-BT. As re-implantation occurs within a few hours, SI should be administered either immediately after surgery or preferably within 2 hours. (Pode et al., 1986) Mitomycin C (MMC), epirubicin, and pirarubicin have all shown consistent beneficial results in preventing recurrences compared to instillation of water, saline, or TUR-BT alone (Abern et al., 2013; Perlis et al., 2013; Sylvester et al., 2016). Trials using gemcitabine give more conflicting results, although gemcitabine may cause fewer side effects. (Böhle et al., 2009; Koimtzis et al., 2022; Messing et al., 2018) In Finland, epirubicin is the most widely adopted following its promising results in the Finnbladder 3 trial. (Rajala et al., 2002)

The role of SI has been well established by multiple meta-analyses suggesting that it results in a roughly 12–14% absolute reduction in recurrence rate during follow-up, with seven patients needing treatment to avoid one recurrence. (Abern et al., 2013; Perlis et al., 2013; Sylvester et al., 2016; Sylvester et al., 2004)However, a meta-analysis by Sylvester et al. using individual patient data showed that patients with a history of more than one recurrence per year and an EORTC score of ≥5 according to the 2016 EORTC recurrence risk scoring system did not benefit from

SI, and no effect on progression or BC-related death was observed among these patients. (Sylvester et al., 2016; Sylvester et al., 2006) Therefore, SI is the most beneficial in primary LG tumours and selected recurrent LG tumours in which recurrence is detected more than 1 year after TUR-BT. (Babjuk et al., 2022; Babjuk et al., 2018) In low-risk NMIBC, SI is considered sufficient adjuvant treatment. (Babjuk et al., 2022)

Around 20–25% of patients may suffer from adverse effects such as dysuria and lower abdominal pain during or after instillation. (Bosschieter et al., 2018) In addition, SI may rarely cause serious complications if a bladder perforation during TUR-BT has been unnoticed, resulting in extravasation of chemotherapeutic agent and possible serious damage to the bladder. (Elmamoun et al., 2014) Therefore, among patients with highly recurrent diseases, SI should not be used in order to avoid unnecessary risks. Promising comparative results to SI have also been observed using continuous irrigation with saline or sterile water 15–20 hours after TUR-BT, and may be considered an option if SI is not available, postoperative bleeding continues after TUR-BT, or in case of a suspected bladder perforation. (Mahran et al., 2018)

Adjuvant intravesical chemotherapy

Among patients with highly recurrent disease, SI is inefficient in reducing recurrences, and patients may benefit from further adjuvant instillations of chemotherapy. MMC is the most studied agent, but also epirubicin, adriamycin, mitoxantrone, gemcitabine, docetaxel, and thiotepa have been investigated with no conclusive evidence of differences between them. (Chou et al., 2017) Overall, trials estimating intravesical chemotherapy suffer from substantial heterogeneity, since many different agents with various treatment protocols have been used.

Intravesical chemotherapy includes an induction period of six weekly instillations followed by a maintenance period. Comparison of an induction period of MMC versus induction plus monthly maintenance for up to 3 years showed a recurrence-free rate of 68% in the induction arm and 86% in the maintenance arm ($p=0.001$), suggesting superiority of the maintenance protocol. (Friedrich et al., 2007) Given the substantial effort needed for 3 years of maintenance, the benefit and cost effectiveness of maintenance is debatable. In a meta-analysis by Sylvester et al., the authors concluded that a short maintenance period of 3–4 months may be sufficient and that exceeding 1 year does not provide additional benefit. (Sylvester et al., 2008) The efficacy of intravesical chemotherapy may be improved by optimising the urine pH and drug concentration, which has been studied using MMC. Optimised MMC treatment includes 1) a dose increase from 20 mg to 40 mg, 2) reduction of the dosing volume from 40 ml to 20 ml, 3) minimisation of residual

urine at the time of treatment, 4) reduction of urine production during treatment by fluid restriction, and 5) alkalinisation of the urine. A phase 3 trial allocating 230 patients to receive either optimised or conventional MMC treatment demonstrated a 19% unit decrease (76% vs 57%) in the 5-year risk of recurrence in favour of optimised MMC treatment. (Au et al., 2001) Although dysuria was observed 15% more in the optimised MMC arm, other adverse effects did not differ between the two instillation protocols.

Overall, intravesical chemotherapy as an adjuvant treatment following TUR-BT has been shown to be effective in reducing recurrences compared to TUR-BT alone, with 38% reduction of recurrences at 1 year, corresponding to an absolute difference of 13–14% in the number of patients with recurrence. (Huncharek et al., 2000; Huncharek et al., 2001)

When intravesical chemotherapy is compared with BCG therapy, the results show some heterogeneity. A meta-analysis by the Cochrane group showed that BCG is superior in reducing recurrences only in the high risk group, and conclusions on other risk groups are unclear due to substantial heterogeneity between studies. (Shelley et al., 2004) However, Bohle et al. found that BCG is superior to MMC in terms of recurrences regardless of the baseline risk group (OR 0.56, 95% CI 0.38–0.84, $P = 0.005$) and that BCG is superior for progression over MMC if maintenance BCG is applied. (Bohle & Bock, 2004; Böhle et al., 2003) A similar result was found in a meta-analysis by Malmström et al. utilising individual patient data of studies comparing MMC and BCG. The authors concluded that intravesical chemotherapy applied with maintenance and a BCG induction period results in similar reduction of recurrences, but that BCG is more effective when maintenance BCG is applied. (Malmstrom et al., 2009) No differences in progression or long-term outcomes were observed between MMC and BCG. Finally, a recent meta-analysis specifically on intermediate-risk patients found that MMC with maintenance provided an even lower risk of recurrences than did BCG. (Laukhtina et al., 2022) Thus, given the low baseline risk of progression in intermediate-risk patients, intravesical chemotherapy may provide comparative efficacy in reducing recurrences compared to BCG, with a more favourable toxicity profile, and could therefore be considered a valid adjuvant treatment option for intermediate-risk patients at low risk of progression. Driven by a BCG shortage, intravesical chemotherapy consisting of sequential gemcitabine and docetaxel has been used instead of BCG, with promising results showing comparable or even superior efficacy to BCG in reducing recurrences, but the evidence is based on small series and retrospective analyses and will need further validation with randomised controlled studies. (Kawada et al., 2022; McElree et al., 2023)

Intravesical chemotherapy may also be used as a chemoablative treatment. The rationale for chemoablation is that the patient may avoid surgical intervention and that it may also induce less bladder scarring, since it affects tumour cells more

specifically than TUR-BT and electrocautery, especially in the case of multiple small tumours. Most chemoablation protocols include weekly instillations over 4–8 weeks, but shorter and more intensive courses have been investigated. (Colombo et al., 2011) In a meta-analysis estimating the efficacy and safety of chemoablation, a complete response was achieved in 51% (95% CI: 45.9–55.9) and 64% (95% CI: 56.2–72.3) of patients with the weekly schedule and intense schedules, respectively. Adverse effects in these trials included cystitis in 9%, dysuria in 16%, frequency in 24%, haematuria in 14%, and incontinence in 8%. Compared to surgical treatment, comparable complete response rates have been achieved with an intense protocol including maintenance treatment, but a 4-weekly chemoablation protocol was inferior to surgical treatment in a phase 2 trial. (Mostafid et al., 2020; Racioppi et al., 2019) Further investigation is needed to find an optimal chemoablation protocol. For selected patients, chemoablation may be considered to replace TUR-BT especially in case of small, multiple LG recurrences.

Device-assisted therapies

Device-assisted therapies use mechanical techniques to enhance intravesical chemotherapy. Currently, there are three main types of device-assisted therapies: Hyperthermic intravesical chemotherapy (HIVEC), radiofrequency-induced thermochemotherapy (RITE), and electromotive drug administration (EMDA). Device-assisted therapies have been developed to work in parallel or as an alternative to BCG therapy among patients with intermediate- and high-risk disease. Since MMC is the most preclinically studied agent in combination with hyperthermia and electromotive administration, MMC is most widely used in device-assisted therapies.

In HIVEC, MMC is heated extracorporeally and then circulated through the bladder during instillation. Hyperthermia is shown to increase the penetration of chemotherapeutic agent deeper into the bladder wall by causing transient damage to the cytoskeleton of uroepithelial cells and inducing opening of pores between the cells, allowing cell-to-cell drug delivery. (Coss & Linnemans, 1996; Kong et al., 2000) However, two recent randomised controlled trials failed to demonstrate the efficacy of high-temperature MMC with HIVEC in reducing recurrences of NMIBC compared to room temperature MMC. (Angulo et al., 2022; Tan et al., 2022) HIVEC was overall well tolerated but induced slightly more dysuria and bladder spasms than conventional MMC treatment.

RITE is based on radiofrequency-induced hyperthermia, which is delivered by an energy source at the end of a commercially available catheter during instillation of MMC (Synergo®, Netherlands). Studies using RITE have shown more promising results regarding efficacy. A systematic review suggested that RITE is associated with a 59% relative reduction in NMIBC recurrences compared to standard MMC

treatment. (Lammers, Witjes, Inman, et al., 2011) The main adverse effects during the treatment include bladder spasms in 21.6% and bladder pain in 17.5% of patients, and after the treatment irritative storage symptoms in 26% and hematuria in 6%. (Lammers, Witjes, Inman, et al., 2011) MMC treatment using RITE was compared to BCG and showed even superior results compared to the BCG arm in the per-protocol analysis. However, the study was closed prematurely and no difference in recurrence rate between the RITE and BCG groups could be demonstrated in the intention-to-treat analysis; therefore, further randomised controlled trials are needed to confirm the results. (Arends et al., 2016)

EMDA involves an electric current delivered to the bladder with a commercially available catheter and an energy source (PHYSIONIZER®, Physion Srl, Mirandola, Italy) during instillation of MMC. The mechanism of EMDA is based on electro-osmosis, in which the electric current between the catheter and electrodes in the lower abdomen drags Na+ ions of the MMC-NaCl solution into the bladder wall, creating an osmotic drag of water and non-ionized MMC molecules, increasing the concentration of MMC in the urothelium and underlying tissues. (Di Stasi et al., 1999) In a three-arm trial comparing EMDA-MMC monotherapy, conventional MMC, and BCG therapy, EMDA-MMC was superior to passively administered MMC and the efficacy was similar to BCG therapy. (Di Stasi et al., 2003) Another trial by the same group of authors allocating 212 high-risk NMIBC patients to either a sequential instillation schedule of EMDA-MMC and BCG or standard BCG therapy for 1 year demonstrated a 48-month longer disease-free interval (69 mo. vs. 21 mo., p=0.0012), 12.6% lower risk of progression (9.3% vs. 21.9%, p=0.0047), and 10.9% higher overall survival rate (78.5% vs. 67.6%, p=0.045) in the sequential BCG/EMDA-MMC arm. Toxicity profiles were similar, and treatment was terminated due to toxicity in 3% of patients in both arms. (Di Stasi et al., 2006) A prospective single-arm trial also found comparable results to those of the Di Stasi group. (Gan et al., 2016) However, despite the exceptionally good results observed with sequential EMDA-MMC and BCG therapy, EMDA-MMC is not widely popular, most likely due to the lack of confirmatory randomised trials.

2.4.4 Bacillus Calmette-Guérin instillation therapy

2.4.4.1 History of BCG

Today, BCG is the first-line treatment option for intermediate- and high risk NMIBC. The journey of BCG in attaining this status is unique and started over a 100 years ago. Since the discovery of *Mycobacterium tuberculosis* by Robert Koch in 1882, there has been an urgent need to develop a vaccine against tuberculosis (TB). (Koch, 1932)

In 1908, French physician Albert Calmette and veterinary surgeon Camille Guérin began their work at the Pasteur Institute in Lille, France with a virulent strain of *Mycobacterium bovis*, which had been isolated in 1902 from a cow with tuberculous mastitis. Calmette and Guérin observed that culturing the strain in a medium with bile and potato resulted in attenuation of virulence. After 13 years and 230 subcultures, they finally succeeded in producing a live, attenuated strain of *Mycobacterium bovis* that did not produce progressive disease in animals. (Calmette et al., 1921)

Following the first oral administrations of BCG vaccine in 1921 and the declaration of BCG vaccine to be safe by the League of Nations in 1928, the Pasteur Institute began the mass production of BCG, which became widely adopted globally. In the Lübeck disaster of 1930, 230 infants were accidentally vaccinated with vials of BCG contaminated with a virulent strain of *Mycobacterium tuberculosis,* resulting in the death of 73 infants. (Lange, 1931) The origins of this disaster were later traced, and it was found that the contaminated vials included different strains of *Mycobacterium tuberculosis* with various levels of virulent potential. (Fox et al., 2016) While some infants who received the most virulent strain remained asymptomatic, others who received the least virulent strain developed lethal TB, demonstrating the individual variability in susceptibility to TB. Following the catastrophe, the reputation of BCG collapsed for nearly 20 years, until the international tuberculosis campaign initiated by the WHO in 1948 became a central part of national immunisation programs globally, dramatically changing the course of the TB pandemic. (Comstock, 1994; Daniel, 2006) In Finland, BCG was part of the national vaccination program from 1941 to 2006; since then, BCG has only been given to children considered at high risk of contracting TB. (THL, 2020)

The role of BCG as a cancer immunotherapy arose from post-mortem observations, when it was noticed that cadavers with TB lesions had less malignant disease. (Pearl, 1929) The concept of attenuated BCG as an antitumour agent was later demonstrated by Lloyd and colleagues in 1959, who showed that mice injected with BCG were more resistant to transplanted tumours. (Old et al., 1959) After several series of animal tests, BCG was tested in humans with intralesional injections against lymphoblastic leukaemia, and subsequently with transurethral intralesional injections of melanoma metastasis in the bladder, which resulted in complete eradication of the tumour. (deKernion et al., 1975; Silverstein et al., 1974) These observations led to the idea of intracavitary administration of BCG.

Eventually, Alvaro Morales began his work with BCG, and demonstrated a 12-fold reduction in BC recurrences following intravesical instillations BCG against urothelial bladder tumours in an eminent report on nine patients in 1976. (Morales et al., 1976) The BCG regimen that Morales et al. were using apparently originated from a practical point of view, since Armand Frappier's BCG package included six

separate vials. The first observations of toxicity of BCG also affected the design of the schedule, since the irritative symptoms of BCG typically lasted less than 1 week, leading to today's standard concept of an "induction period of six weekly instillations". (Morales et al., 1976)

Subsequently, clinical trials were initiated demonstrating that six weekly BCG reduces both recurrences and progression compared to tumour resection and fulguration of recurrences. (Lamm et al., 1980; Pinsky et al., 1985) Probably the most revolutionary effect of the introduction of BCG was in the treatment of CIS, which usually warranted radical cystectomy before the BCG era but is now routinely managed using BCG. (Brosman, 1985) In light of the positive results of clinical trials, approval of BCG for the treatment of NMIBC was granted by the Food and Drug administration (FDA) in 1990.

In Finland, the first national Finnbladder 1 trial randomising patients to receive either MMC or BCG started recruiting in 1984 (Rintala et al., 1991). Since then, the national Finnbladder study group has published multiple clinical trials assessing the efficacy of BCG in various comparisons to multiple competitive agents, which has been a key element of the Finnish Urologists adopting standardised practices in BCG therapy.

2.4.4.2 BCG strains

BCG is no longer a single vaccine or intravesical agent. During the worldwide mass production and in-vitro passages, BCG has undergone genetic alterations resulting in multiple distinguishable strains. Although there are over 20 genetically different strains of BCG, a handful of strains including BCG TICE, BCG Moreau, BCG Connaught, BCG Tokyo-172, and BCG RIVM strains with different quantities of colony-forming units are available for intravesical use. The availability also varies by continent and has altered over time. In Finland, BCG TICE was primarily available from its granted sales permission in 1991 until 2006, after which it was restricted to special-permit use only, and its use has gradually declined. Since then, RIVM has largely been the only available strain, although some batches of TICE were delivered to Finland during a BCG shortage in 2015–2016.

There is preclinical evidence that some strains may trigger different immunological reactions, which suggests that strains could also differ in their protective effect against NMIBC. (Ritz et al., 2008) Although some studies have suggested differences in efficacy between the strains, the studies have been relatively small, and limitations such as differences in the use of maintenance therapy have prevented firm conclusions from being drawn. Consequently, a network meta-analysis could not demonstrate clinically significant differences between strains,

leaving the preclinically hypothesised question on clinical differences unresolved (Boehm et al., 2017)

Over the past decade, BCG has suffered from periods of global shortage, starting in 2013 following the suspension of BCG Connaught in Canada due to suspected fungal contamination of the production line. Given the low economic benefit derivable from an old drug, the complicated production process involved, and strict regulatory hurdles in the production of live bacteria, re-organising the supply of BCG for the global market was slow and led to an ongoing global shortage. The BCG shortage has affected countless BC patients and is estimated to have had a major effect on the treatment of many NMIBC patients. (Ourfali et al., 2021)

2.4.4.3 Mechanism of BCG

The biological mechanism underlying the tumour-mediating mechanism of BCG is still not completely understood. Preclinical and clinical studies have shown that a robust inflammatory process is initiated in the bladder wall after BCG instillation. (Pettenati & Ingersoll, 2018)

Based on animal models, the initial step involves attachment of BCG to the surface of the urothelium, followed by internalisation. Attachment is believed to be mediated by fibronectin, a molecule found in fibrin clots formed in areas where the urothelium is disrupted, e.g. due to cauterisation during TUR-BT. (Kavoussi et al., 1990) However, in humans this theory has not been supported by studies using fibrin clot inhibitors (aspirin), in which aspirin did not reduce the efficacy of BCG; therefore the precise mechanism of how BCG attaches to the bladder wall is still unclear. (Witjes et al., 1993) *In vitro* models and animal studies, as well as studies on *Escherichia coli* infections, suggest that BCG is internalised into the urothelial cells, urothelial cancer cells, and macrophages. This is presumed to occur also during intravesical instillation in humans. (Pettenati & Ingersoll, 2018)

Once BCG has been introduced into the bladder wall, antigen-presenting cells (APC)—such as dendritic cells and macrophages—identify the antigens on the surface of the bacilli and start producing cytokines and chemokines to attract granulocytes and mononuclear cells to the bladder. This process is the causative mechanism of granuloma formation typically seen in the bladder after instillations. Next, through the production of multiple interleukins and tumour necrosis factor (TNF), immune cells such as neutrophils, monocytes, macrophages, T cells, B cells, and natural killer cells are recruited which are ultimately responsible for the tumour cell-killing effect of this complex cascade. Increasing the complexity, also the adaptive immune system plays a key role in the inflammatory process. (Ratliff et al., 1987) APCs that have identified the mycobacteria interact with T cells, which are differentiated into T-helper-1 cells, which are then responsible for activating

cytotoxic T-cells to assist with the elimination of tumour cells. (Pettenati & Ingersoll, 2018)

2.4.4.4 Efficacy of BCG

Multiple meta-analyses have established that BCG is superior in reducing recurrences when compared to TUR-BT alone and is therefore considered the treatment of choice for patients at high risk of recurrences. (Chou et al., 2017; Han & Pan, 2006; Shelley et al., 2001) A meta-analysis including six trials and 585 patients demonstrated an overall 56% relative reduction of hazard of recurrence when comparing TUR-BT followed by BCG adjuvant therapy with TUR-BT alone (lnHR -0.83 (95% CI -1.08 to -0.57, $p<0.001$). (Shelley et al., 2001) Compared with intravesical chemotherapy, BCG has better efficacy when applied as maintenance therapy but not if applied with an induction course only. (Böhle et al., 2003; Chou et al., 2017; Malmstrom et al., 2009; Morales et al., 1976) It has also been shown that BCG reduces the risk of disease progression compared to TUR-BT alone or intravesical chemotherapy, but the benefit may be only observed among patients treated with BCG maintenance therapy. (Bohle & Bock, 2004; Chou et al., 2017; Sylvester et al., 2002) Interestingly, unlike previous meta-analyses, a large meta-analysis including individual patient data of 2820 patients could not confirm the statistically significant benefit of BCG in terms of progression compared to intravesical chemotherapy ($p=0.141$). (Malmstrom et al., 2009) The inconsistency in the evidence supporting the efficacy of BCG against progression probably arises from most trials being primarily designed to measure recurrences, and since progression occurs in a small subset of patients, there is limited data on progression for comparison. In addition, Malmström and colleagues did not observe a statistically significant difference in either disease specific survival ($p=0.133$) or overall survival ($p=0.414$). However, BC-related deaths did occur less among BCG-treated patients, with a disease-specific mortality rate of 5.9%, but the disease-specific mortality rate in the MMC group was 9.3%, suggesting that BCG also may have a meaningful protective effect on the risk dying from BC. (Malmstrom et al., 2009).

BCG is considered especially beneficial for patients with CIS due to its obscure appearance and high risk of progression to muscle-invasive disease when treated with TUR-BT only. A meta-analysis comparing intravesical chemotherapy and BCG, covering nine trials and 700 patients, showed that 68% of patients receiving BCG had a complete response (CR) at 3–6 months. Of the complete responders, 34% developed recurrence during a median follow-up of 3.6 years, resulting in 47% of patients remaining completely disease-free during follow-up. (Sylvester, van der Meijden, Witjes, & Kurth, 2005) Although the odds of a treatment response was superior for BCG compared to intravesical chemotherapy (OR 0.53, 95% CI 0.38-

0.74, p=0.0002), a considerably large proportion of patients with CIS treated with BCG will still develop a treatment failure initially or during follow-up. The risk of progression was 15% among patients treated with BCG, which is, however, substantially lower when compared to historical reports of the natural course of CIS with 54% risk of progression. (Lamm et al., 1998; Sylvester, van der Meijden, Witjes, & Kurth, 2005)

2.4.4.5 BCG schedule

Based on existing evidence, a maintenance period of 1–3 years should always be included in the regimen to achieve maximal therapeutic efficacy of BCG. (Bohle & Bock, 2004; Böhle et al., 2003; S. Chen et al., 2018; Zhu et al., 2013) Although the induction course of six weekly instillations has remained similar that demonstrated by Morales et al. in 1976, the use of a maintenance schedule is more varied. (Morales et al., 1976) In the last few decades, various maintenance schedules have been introduced including repeating the six weekly instillations every 6 months (Palou, Laguna, et al., 2001), single monthly instillations (Akaza et al., 1995; Badalament et al., 1987), single instillations every 3 months (Martínez-Piñeiro et al., 2015), and the maintenance schedule introduced by the Southwest Oncology Group (SWOG) administering three weekly instillations at 3, 6, and 12 months and every 6 months thereafter for 3 years. (Lamm et al., 2000) The landmark paper by Lamm et al. demonstrated significantly superior results in the maintenance treatment group compared to the no-maintenance group, with respective 5-year RFS and 5-year worsening free survival of 60% vs. 41% (p=<0.001) and 76% vs 70% (p=0.040). (Lamm et al., 2000) Attributed to this trial, the SWOG protocol has become the most used maintenance schedule and is considered a standard. In fact, in randomised controlled trials using maintenance with monthly instillations, a repeated induction period, or a single instillation every 3 months, none were able to demonstrate a significant benefit in terms of recurrences compared to an induction period alone. (Zhu et al., 2013)

Despite the limitations of previous studies, the Finnbladder group has shown durable and comparable long-term results to those of the SWOG group by comparing 1–2 years of BCG using monthly instillations to mitomycin C, interferon α2b, and a combination of epirubicin and interferon α2a, all resulting in durable superiority of monthly maintenance BCG with a reported 5-year, 7.4-year, and 20-year recurrence rate of 38%, 39%, and 53%, respectively. (Järvinen et al., 2009; Järvinen et al., 2015; Marttila et al., 2016) These results suggest that monthly maintenance may also be considered a valid option, and therefore monthly maintenance is widely adopted by many centres in Finland. However, only a few studies have directly compared the SWOG protocol and monthly maintenance, and

they suffer from small number of patients and events for comparison. (Gupta et al., 2020; Lashay et al., 2021)

The duration and dose of BCG, however, has been more meticulously studied. A large RCT 1355 patients allocated patients to receive BCG either with a full dose for 1–3 years or reduced dose of one-third for 1–3 years. (Oddens et al., 2013) This four-arm study showed that the reduced dose for 1 year was inferior, in terms of RFS, to 3 years of full-dose treatment (HR 0.75, 95% CI 0.59–0.94; p=0.01) and that intermediate-risk patients did not receive additional benefit from 3 years of BCG compared to 1 year of treatment. However, among the high-risk patients, 3 years of treatment at full dose yielded better efficacy than did 1 year (HR: 1.61; 95% CI 1.13–2.30; p=0.009). As a result, 3 years of maintenance therapy is recommended for high-risk patents in the EAU guidelines (Babjuk et al., 2022; Oddens et al., 2013). However, meta-analyses have demonstrated an increased risk of recurrence, but no differences in progression rates, with reduced-dose BCG compared to full dose treatment. (S. Y. Choi et al., 2022; Quan et al., 2017) Finally, in an RCT by Grimm et al. that sought to further minimise the number of needed BCG instillations while maintaining a therapeutic effect, patients were randomised either to standard full-dose BCG maintenance according to the SWOG protocol for 1 year or to a reduced instillation schedule consisting of an induction period of three full-dose instillations every 2 weeks and a maintenance schedule of two biweekly instillations at 3, 6, and 12 months. (Grimm et al., 2020) However, it was found that the standard treatment arm yielded significantly superior RFS at interim analysis (HR 0.40, 95% CI 0.24–0.68) and the recruitment was therefore terminated. These results suggest that 1 year of full-dose treatment with BCG is the minimum, and for high-risk patients 3 years of maintenance should be pursued. (Babjuk et al., 2022)

2.4.4.6 Combination of BCG and intravesical chemotherapy

It has been hypothesised that BCG and MMC might have synergistic efficacy when administered together or as an alternating schedule. (Rajala et al., 1992) However, a Nordic study allocating patients with CIS to receive either BCG monotherapy for up to 1 year with monthly instillations or six weekly MMC instillations followed by alternating BCG and MMC instillations on a monthly basis showed that the combination arm yielded inferior disease-free survival (log rank p=0.03) (Kaasinen et al., 2003) Another randomised controlled trial by the CUETO group used a different schedule and allocated patients to receive either standard BCG monotherapy or a combination arm, in which a single MMC instillation was administered 1 day before each BCG instillation and which demonstrated significantly improved disease-free survival. (HR: 0.57; 95% CI 0.39–0.83; p=0.003) (Solsona et al., 2015) However, toxicity was a major issue and grade 3–4

adverse events were observed in 55% of patients allocated to the combination arm, with a 32% withdrawal rate, whereas grade 3–4 adverse events were observed in 11% of patients in the BCG arm, with a 4% withdrawal rate. As a result, the dose of MMC was reduced to 10 mg after interim analysis demonstrated the severe toxicity profile of combination treatment. Interestingly, two meta-analyses found that combination therapy with BCG and MMC yields improved RFS among patients with papillary tumours but not with CIS, and they even demonstrated a benefit in disease-specific survival, but not progression-free survival. (Cui et al., 2016; Huang et al., 2019) Despite these results, combination treatments are not widely used, likely due to the lack of standardised schedules, toxicity issues, and heterogeneity in the results.

2.4.4.7 Adverse effects of BCG

Although BCG is an effective treatment against NMIBC, the downside of the treatment is the frequently encountered side effects that affect most patients receiving BCG therapy and may sometimes lead to treatment discontinuation. Most commonly, inflammatory bladder symptoms including dysuria, bladder pain, and haematuria occur during the first 12–24 hours after instillation, when the accumulation of mononuclear cells and granulocytes in the bladder wall is induced. During this time, the patient may also suffer from systemic symptoms such as fever and general malaise, which usually resolve spontaneously within a few days. However, the mycobacteria in the BCG solution may also disseminate from the bladder wall and cause infectious complications with a clinical course of TB infection, with various manifestations either locally in the genitourinary tract or as systemic infections, referred to as “BCG infections”, which may at worst be fatal.

In the landmark paper by Lamm et al., in which the 3-year maintenance regimen known as the SWOG protocol was introduced, only 16% of patients were able to complete the whole 3-year course of maintenance therapy, demonstrating that many patients are not able to finish the assigned maintenance period, although many courses were interrupted due to inefficacy and not toxicity. (Lamm et al., 2000) The EORTC 30911 phase 3 trial analysed 487 patients receiving BCG with or without isoniazid, specifically investigating the occurrence of adverse effects during maintenance therapy. The results showed that 75% of patients are affected by local side effects such as chemical cystitis (47%), macroscopic haematuria (35%), urinary frequency (34%), or bacterial cystitis (26%). (van der Meijden et al., 2003) Systemic side effects were observed in 39% of patients and commonly presented as general malaise (23%) or fever (15%). Altogether, 19% of patients had to stop BCG due to local or systemic side effects, while 29% completed the full 3-year course in this trial. (van der Meijden et al., 2003) Later, the EORTC 30962 trial sought to reduce the toxicity of BCG by allocating patients to receive either a full dose or a one-third

dose for 1 or 3 years. (Brausi et al., 2014) In that study, only 7.8% of patients discontinued the treatment due to toxicity, and 36% of patients receiving full-dose BCG for 3 years completed their maintenance course. The main conclusion of the EORTC 30926 trial was that either reducing the dose of BCG or duration of maintenance decreased the percentage of patients who stopped the pursued treatment. (Brausi et al., 2014) A survey conducted in Europe and North America included 971 patients and showed that in clinical practice, around 15% of patients discontinued treatment due to toxicity. (Witjes et al., 2013) Increasing age does not seem to increase the side effects of BCG in a clinical trial setting or in observational studies. (Krajewski et al., 2020; Oddens et al., 2016)

Most side effects resolve spontaneously during the first 2–3 days and may be eased with non-steroidal anti-inflammatory (NSAID) medication. (Kamali et al., 2020) In addition, there are three trials suggesting that administering fluoroquinolones prior to each instillation may reduce symptoms of chemical cystitis and class III adverse events and lower the risk of treatment discontinuation due to toxicity, one trial even reporting a preliminary result of improved disease-free survival. (Colombel et al., 2006; Damiano et al., 2009; Numakura et al., 2022) The effect of fluoroquinolones in reducing adverse effects may act through their antimycobacterial effect. Interestingly, however, the EORTC 30911 trial did not observe any benefit from adding isoniazid prophylaxis to BCG instillations in reducing toxicity. (Vegt et al., 1997) In addition, oxybutynin, phenazopyridine, propantheline bromide, and intravesical instillations of anaesthetic solutions have been proposed as treatment options for BCG-induced cystitis, but none of them have been standardised in clinical practice due to limited available evidence on their efficacy. (Palou, Rodríguez-Villamil, et al., 2001; Witjes et al., 2008) If irritative side effects persist until the next BCG instillation date, the instillation is usually postponed and continued after 1 or 2 weeks, but in the case of burdensome symptoms, continuation of treatment needs to be balanced with the NMIBC baseline risk group, patient age, comorbidities, and co-operation. However, if signs of severe adverse events occur, such as fever over 38°C lasting over 48 hours, and no alternative explanation is found, BCG needs to be terminated permanently since it may signify an emerging BCG infection.

2.4.4.8 BCG infections

The most severe adverse event related to BCG may occur when *Mycobacterium bovis* is absorbed from the urothelium and causes an actual tuberculous infection, which may at worst be fatal. (Rawls et al., 1990) BCG infections are a clinical and diagnostic challenge since they are rare, may present in many forms, and may also present after a substantial latency following instillation therapy. (Cabas et al., 2021)

Much of the knowledge on BCG infections has long been based only on case reports, patient series, and pooled analyses combining these; thus many unclarities remain about BCG infections, including their incidence, mortality, diagnosis, and treatment despite the prolonged history of BCG instillation therapy. Due to the risk of dissemination of BCG, contraindications to BCG instillation include administration within 2 weeks after TUR-BT, active macroscopic haematuria, traumatic catheterisation, and symptomatic urinary tract infection, which are believed to predispose to BCG infections via disruption of the urothelium. (Babjuk et al., 2022)

The reported incidences of BCG infections related to BCG instillations are listed in **Table 3.** In addition to these, complications associated with BCG were investigated in a multicentre retrospective study by Lamm et al., in which an overall 7.6% rate of BCG-related complications was reported, but it was not definitively demonstrated which of the complications were treated with antituberculosis medications and thus represent an overall complication rate of BCG. (Lamm et al., 1992) Among the retrospective cohort analyses, the incidence of BCG infections related to instillations varies between 1.3% and 4.3%. (Gonzalez-Del Vecchio et al., 2016; Nummi et al., 2019; Perez-Jacoiste Asin et al., 2014; Steg et al., 1989) Quite recently, a Danish nationwide register-based study reported a lower incidence and identified 66 BCG infections among 6753 patients treated with BCG, representing a roughly 1% incidence of BCG infections. (Larsen et al., 2019) However, the authors stated that the study may substantially underestimate the incidence due to reporting bias linked to the study design. Overall, reported incidences of BCG infections show heterogeneity due to the rarity of infections and variability in reporting them. For this reason, the individual risk of BCG infections related to BCG instillation therapy is still unclear but may be estimated as less than 5%.

Table 3. Reported incidence proportions of BCG Infections in published cohort studies estimating the risk of BCG infection related to BCG instillations.

Reference	Study design	Country	Patients treated with BCG	Incidence of BCG infections % (n)
(Nummi et al., 2019)	Retrospective, single center cohort	Finland	418	3.0 (12)
(Perez-Jacoiste Asin et al., 2014)	Retrospective, single center cohort	Spain	256	4.3 (11)
(Gonzalez-Del Vecchio et al., 2016)	Retrospective, single center cohort	Spain	786	1.3 (11)
(Steg et al., 1989)	Retrospective, single center cohort	France	169	3.0 (5)
(Larsen et al., 2019)	Nationwide register study	Denmark	6753	1.0 (66)

BCG infections may present either as local infections of the genitourinary tract, such as BCG cystitis, granulomatous prostatitis, and epididymitis, or as systemic infections which may present as pulmonary infections, spondylodiscitis, mycotic aneurysms, and generalised infections referred to as "BCG sepsis". (Lamm et al., 1992; Perez-Jacoiste Asin et al., 2014) In addition, other rarely reported BCG complications include reactive arthritis, uveitis, BCG meningitis, BCG peritonitis, and Guillain-Barré syndrome. (Golub et al., 2011; Larsen et al., 2019; Nielsen et al., 2021; Perez-Jacoiste Asin et al., 2014; Toohey et al., 2022; Webb & Venkatesan, 2018) In the Danish study, local infections including chronic bladder ulceration and BCG cystitis, epididymitis, and granulomatous prostatitis represented 44% of infections, while 48.5% and 7.6% of infections were systemic infections or reactive arthritis, respectively. (Larsen et al., 2019) Among the retrospective studies, 66–100% of identified infections were systemic, representing a larger proportion of infections among these studies. (Gonzalez-Del Vecchio et al., 2016; Nummi et al., 2019; Perez-Jacoiste Asin et al., 2014; Steg et al., 1989)

The microbiological diagnosis of BCG infection relies on a culture or nucleic acid test of *Mycobacterium bovis* or histological diagnosis of caseous necrosis, granulomatous inflammation, and identification of acid-fast bacilli. However, even if the mycobacterial samples are negative, treatment may have to be initiated based on the clinical diagnosis. In previous reports, microbiological evidence of *Mycobacterium bovis* was obtained in 36–48% of patients, meaning that most of the BCG infection diagnoses were based on clinical diagnosis. (Gonzalez-Del Vecchio et al., 2016; Nummi et al., 2019; Perez-Jacoiste Asin et al., 2014) In systemic infections, radiological foci such as a pulmonary cavitation, miliary pattern, and spinal or vascular lesions may be observed.

The treatment of disseminated BCG infection is based on a combination regimen consisting of isoniazid, rifampicin, and ethambutol for 6–9 months, to which corticosteroids may be added in case of severe septicaemia. (Babjuk et al., 2022; Lamm et al., 1992) The treatment depends on the localisation and severity of infection, and the treatment algorithms in various BCG infections adopted from the EAU Guidelines are presented in **Table 4**. (Gontero et al., 2023) Surgery may be needed in the treatment of epididymitis, vascular infections, and occasionally after BCG cystitis, if a contracted bladder has developed. (Babjuk et al., 2022; Medina-González et al., 2022; Perez-Jacoiste Asin et al., 2014). Long-term complications such as hydronephrosis or contracted bladder may develop in fewer than 1% of patients. (Lamm et al., 1992) Mortality related to BCG infections has been estimated mostly in pooled analyses combining published case reports and series. (Cabas et al., 2021; Perez-Jacoiste Asin et al., 2014) These studies suggest that BCG infections are associated with an overall mortality risk of 5% and a risk of 10% among systemic infections, with vascular involvement being identified as the most lethal form of

BCG infection with an observed mortality risk of 10–15%. (Cabas et al., 2021; Ho et al., 2022)

Table 4. Management options for BCG Infections as adapted from the EAU Guidelines 2022 edition. (Gontero et al., 2023)

Infection type	Infection site	Treatment
Local infections		
	BCG Cystitis	Phenazopyridine, propantheline bromide, NSAIDs Empirical antibiotics (Fluoroquinolones) If symptoms persist, anti-tuberculosis drugs + corticosteroids. Contracted bladder: radical cystectomy.
	Granulomatous prostatitis (symptomatic)	Fluoroquinolones Isoniazid and rifampicin for 3 months if no response
	Epididymitis	Fluoroquinolones Orchidectomy if abscess or no response to treatment.
Systemic side effects		
	Persistent high-grade fever (>38.5 8C for >48 h)	Prompt treatment with three antimicrobial agents (Isoniazid, rifampicin, ethambutol) Permanent cessation of BCG instillation therapy
	BCG sepsis	Isoniazid, rifampicin, and ethambutol daily for 6 months. Early, high-dose corticosteroids for as long as symptoms persist.
	Arthralgia and/or arthritis	NSAIDs. If no/partial response, proceed to corticosteroids, high-dose quinolones or antituberculosis drugs

2.5 Follow-up of NMIBC

As with the treatment approach, follow-up of NMIBC also needs to be risk-adapted in line with the individual risk of recurrences and progression. The first cystoscopy check-up is performed at 3 months following TUR-BT in all risk groups, whereafter the follow-up schedule is individualised by the NMIBC risk group. The follow-up visit consists of urinary cytology and cystoscopy and may include imaging. In addition, surveillance of bladder function and adverse effects related to instillation therapies and bladder procedures is an important part of the follow-up.

2.5.1 Cystoscopy, cytology, and imaging in NMIBC

The optimal schedule for follow-up of NMIBC is based on a low level of evidence, since there are a limited number of studies comparing different follow-up schedules of NMIBC. (Babjuk et al., 2022; Dreyer et al., 2022) The risk-adopted follow-up schedule based on the EAU Guidelines panel is shown in **Table 5**. Strict follow-up is important for patients with a history of HG tumours, as they are at risk of progression. Early detection of the LG tumour, however, is not as crucial, and the surveillance may be tailored individually in line with age, comorbidities, and patient cooperation. Usually, intermediate-risk disease is followed every 6 months for 1–2 years and yearly thereafter.

Table 5. Cystoscopy follow-up of NMIBC adapted from the EAU Guidelines.

Risk group	Cystoscopy schedule
Low risk	• At 3 months • At 1 year after TUR-BT • Yearly until 5 years post TUR-BT
Intermediate risk	• Individualised schedule
High to Very High risk	• Every 3 months for 2 years • Every 6 months during years 2-5 • Yearly thereafter

The role of cytology is less important in the follow-up of LG tumours, as the sensitivity and specificity among these is only 10–16% and the risk of HG recurrences is low. However, cytology should be included in the follow-up of intermediate and high-risk disease. (van Rhijn et al., 2005; Yafi et al., 2015) Cytology plays an essential role in the surveillance of BCG-treated patients. BCG often induces erythematous lesions or granulomas of the bladder wall, which is a normal phenomenon resulting from the inflammatory process initiated by BCG. However, these lesions may be difficult to distinguish from a recurrent tumour or

CIS. Urinary cytology is an important tool in assessing the need to take a biopsy of such lesions. A cumulative analysis of studies assessing the need for bladder biopsy after an induction course of BCG, when erythematous lesions are often present, found that 9% of erythematous lesions harboured malignancy in the presence of negative cytology and 59% in the presence of positive cytology. (Swietek et al., 2012) The downside of cytology is that its reliability depends on the experience of the pathologist, and BCG especially may cause cytological changes that hamper interpretation. (Takashi et al., 2000)

Imaging in the follow-up of NMIBC is not routinely needed for all patients. The probability of UTUC recurrence during surveillance of low, intermediate, and high-risk NMIBC is 0.6%, 1.8%, and 4.1%, respectively. Therefore, low- and intermediate-risk patients do not need routine imaging surveillance, but for high-risk patients especially with a history of multiple tumours, yearly CT urography is recommended. (Babjuk et al., 2022; Millán-Rodríguez et al., 2000)

2.5.2 Treatment options for recurrences or progression after intravesical instillation therapy

Whenever a recurrent tumour is detected during follow-up, the risk group needs to be re-evaluated and treatment adjusted accordingly. NMIBC recurrence after intravesical chemotherapy may be treated according to normal treatment principles for NMIBC, since previous intravesical chemotherapy does not affect the efficacy of BCG therapy. (Malmstrom et al., 2009) Usually, BCG is the treatment of choice for these patients.

A recurrence during or after BCG therapy should be evaluated more meticulously, as BCG failure has a poor prognosis and RC should be considered. A low-grade recurrence is not considered to be failure of BCG, since only high-grade recurrences are considered to have a significant risk of progression, which is known to predict poor prognosis. (van den Bosch & Alfred Witjes, 2011) To identify which patients with HG recurrences are unlikely to benefit from further BCG therapy, two factors need to be considered: 1) the adequacy of the BCG course and 2) the duration of recurrence following the last instillation. To standardise future studies, the FDA defines adequate BCG therapy as having the following criteria:

- At least five of six doses of an initial induction course plus at least two of three doses of maintenance therapy
- At least five of six doses of an initial induction course plus at least two of six doses of a second induction course (Administration, 2018).

Based on these factors, the International Bladder Cancer Group (IBCG) categorises patients with HG recurrences during or after BCG as shown in **Table 6.** (Roumiguié et al., 2022).

Table 6. Categorization of high grade (HG) recurrences during or after BCG instillation therapy adapted from Romiguie et al, Eur Urol 2022.

Disease state	Definition
BCG resistant	• Ta/cis at 3 months after an adequate induction period
BCG unresponsive	• T1 tumour after adequate induction of BCG • HG TaT1 tumour after 6 months of adequate BCG • CIS ≤12 months after the last instillation of adequate BCG
BCG relapsing after adequate BCG	• Any high-risk recurrence 12-24 months after adequate BCG
BCG relapsing after inadequate BCG	• Any high-risk recurrence 6-24 months after inadequate BCG

Patients with BCG-unresponsive tumours are not likely to benefit from further BCG, and currently RC should be offered as first-line treatment. (Babjuk et al., 2022; Roumiguié et al., 2021) Other groups may be characterised as "BCG exposed" and further BCG treatment may be considered. (Babjuk et al., 2022; Roumiguié et al., 2021) However, it should be noted that in other groups the evidence on BCG efficacy, especially in regards to progression, is weaker than for the treatment of BCG-naïve NMIBC, and the choice between BCG re-challenge and radical surgery must be weighed in light of the patient's age and comorbidities and discussed with the patient. (Babjuk et al., 2022) BCG may be inadequate due to either intolerance or e.g., poor compliance. If BCG treatment was terminated due to BCG infection, further BCG should not be offered owing to the risk of fatal complications, but in case of irritative symptoms a re-challenge of BCG may sometimes be considered along with preventive medication such as fluoroquinolones. (Witjes et al., 2008)

Multiple efforts have been made to investigate effective bladder-sparing options in BCG-unresponsive disease, including device-assisted therapies, gene therapy, and immune-oncological treatments, which are currently undergoing a single-arm phase 2 study. Currently, there are two agents with FDA approval for BCG-unresponsive disease: a checkpoint inhibitor, pembrolizumab, and an adenovirus vector-based gene therapy, nadofaragene firadenovec-vncg. (Balar et al., 2021; Boorjian et al., 2021) Pembrolizumab showed a 40% complete response rate among patients with CIS either solitary or concomitant with TaT1 tumours, which was maintained in 48% of patients up to 12 months in a single-arm phase 2 trial (Balar et al., 2021) Nadofaragene firadenovec-vncg showed a 60% overall complete-response rate, with

45–50% of patients remaining disease free at 12 months. (Boorjian et al., 2021) Evolving evidence will most likely provide further risk stratification tools and treatment recommendations in both the BCG-exposed and BCG-unresponsive disease states.

3 Aims

The follow-up of many patients treated with BCG poses several challenges due to the BCG mechanism aiming to induce an inflammatory response in the bladder wall. Follow-up may be complicated due to inflammatory lesions on cystoscopy that may be hard to distinguish from recurrent disease, and, not uncommonly, patients suffer from inflammatory side effects which may lead to treatment being terminated prematurely, compromising its efficacy. Besides irritative side effects, there is a rare but potentially serious underlying risk of developing an actual tuberculous BCG infection, of which the incidence, risk factors, management, and ultimately prognosis are still poorly described, and which therefore may be overlooked while balancing the risks versus benefits of BCG instillations. The general aim of this thesis is to characterise the various manifestations of adverse effects related to BCG treatment and the factors affecting the toxicity of BCG.

The specific aims of the current study were:

1. To clarify the nature of, and diagnostic approach to, inflammatory erythematous bladder lesions encountered during follow-up of patients treated with BCG.
2. To characterise the BCG infections diagnosed in Finland over a 20-year period and to describe their
    a. incidence and mortality, and
    b. clinical presentation and treatment of various types of BCG infections.
3. To compare the tolerability and oncological efficacy of BCG maintenance therapy applied either with monthly instillations or according to the SWOG protocol.

4 Materials and Methods

4.1 Data sources

Institutional medical records database

In studies I and IV, patients were identified from institutional medical record databases. In study I, patients were identified from the databases of Turku University Hospital and Satakunta Central hospital. We identified patients with NMIBC who had been treated with BCG instillations during 2009–2015 by identifying patients with a diagnosis of bladder cancer (International Classification of Diseases, 10th Revision ICD-10: C67) and the procedure code used for intravesical BCG instillations (TKW99). In study IV, patients were identified using an identical method at both Turku University Hospital and Helsinki University Hospital during 2009–2018. Patients in study I identified at Turku University hospital were included also in study IV, and these two cohorts overlap during 2009–2015. Basic patient demographics and clinical data related to the treatment of NMIBC and BCG instillations were collected from the medical charts of identified patients.

The Finnish Cancer Registry

The Finnish Cancer Registry (FCR) maintains a nationwide register of all cancer diagnoses in Finland. The FCR was founded in 1952 and is maintained by the Cancer Society of Finland. Data are provided to the FCR by pathology laboratories and clinicians from all heath care units in Finland, including public and private facilities. Notification of cancer diagnoses to the FCR became mandatory in 1961.The reported information includes the date of diagnosis, primary tumour site by diagnosis code, histological type of cancer, TNM stage, tumour grade, and information on the treatment. The data are regularly validated by linking them to the Cause of Death register maintained by Statistics Finland.

Finnish National Infectious Diseases Register

The Finnish National Infectious Diseases Register (FNIDR) maintains a nationwide register on infectious diseases in Finland based on the Communicable Diseases Act and Decree. The registry is maintained by the Finnish Institute for Health and Welfare (THL). Based on the communicable disease act and decree, the FNIDR maintains data on tuberculous infections in Finland. (*Government Decree on Communicable Diseases*) The data on any microbiologically verified positive test of *Mycobacterium tuberculosis complex*, based either on culture or a nucleic acid test, are provided by the laboratory to the FNIDR. Since 2007, data on also clinically diagnosed tuberculosis has been notified by the treating physician if microbiological verification was not obtained. Since *Mycobacterium bovis* belongs to the *Mycobacterium tuberculosis* complex, microbiologically verified BCG infections are thus all registered in the FNIDR, but registration of clinically diagnosed BCG infections without microbiological verification is not mandatory. The registered information includes the identification data, date of diagnosis, site and type of the microbiological sample, name of the bacterium, site and diagnosis of infection, and the hospital district responsible for the treatment.

Linking of registries and data retrieval in studies II & III

In studies II and III, data in the FCR and FNIDR were used to identify patients with NMIBC treated with BCG, and a subsequent BCG infection related to instillation therapy. The FCR and FNIDR data were linked in co-operation with the data management staff of THL and the FCR. Patients who had a registered diagnosis of urothelial carcinoma of the bladder and urinary tract (ICD-10: C65-68, D09.0-1, D41.1-9) in the FCR and a mycobacterial infection based on either microbiological verification or clinical diagnosis in the FNIDR were identified. A list of all identified patients was received from THL. All basic demographic and clinical data were collected from the medical charts of the identified patients and double-checked against the information of infection type and site provided on the FNIDR data sheet.

Finnish Medicines Agency

The Finnish Medicines Agency (Fimea) is the national competent authority for regulating pharmaceuticals in Finland. Data on all medicinal products including BCG vials ordered and distributed by hospital pharmacies throughout the nation are registered and regulated by Fimea. In study 2, we estimated the number of patients who had been treated with BCG based on the number of consumed BCG vials and received data on all consumed BCG vials on a monthly basis in Finland during 2000–2016.

Use of data sources in studies I–IV

The patient identification used in studies I–IV from the above-described data sources is summarised in **Table 7.**

Table 7. Summary of patient identification used in the studies in this thesis.

Identification terms	**Study I**	**Studies II & III**	**Study IV**
Diagnosis (ICD-10)	C67	C67	C67
Procedure	Intravesical BCG instillation	Intravesical BCG instillation	Intravesical BCG instillation
Other condition	-	Tuberculosis or detected *Mycobacterium bovis* BCG	-
Population	Hospital districts Turku University Hospital Satakunta central Hospital	Finland	Hospital districts Turku University Hospital Helsinki University Hospital
Data source	Institutional medical record database	The Finnish cancer registry The Finnish National Infectious diseases register	Institutional medical record database
Study period	2009–2015	1996–2016	2009–2018
Number of identified patients	**206**	**100**	**802**

4.2 Study settings

4.2.1 Study I

Study I was designed to investigate retrospectively the risk of malignancy in erythematous bladder lesions biopsied or resected during follow-up after BCG instillation therapy, and to calculate the accuracy of urinary cytology in detecting malignancy in these lesions.

In this study, we identified 206 consecutive patients with NMIBC who had been treated with at least one or more instillations of BCG in Satakunta Central Hospital and Turku University hospital during 2009–2015. Medical charts of the identified patients were reviewed and the data included in retrospective analyses. Basic

demographic and clinical data of the included patients were collected including age, gender, smoking history, American Society of Anesthesiologists (ASA) score, and details of bladder cancer and BCG instillations.

All patients had been assigned to receive an induction course of six weekly BCG instillations followed by maintenance therapy. Maintenance protocols applied with monthly instillations and according to the SWOG protocol were used during the study period. (Lamm et al., 2000) The follow-up was performed regularly according to the EAU guideline principles with outpatient clinic cystoscopies and voided urine cytology evaluation. Suspicious bladder lesions were investigated with a biopsy or removed with TUR-BT as decided by the treating urologist. There was no standard practice to retrieve random biopsies from a normal-looking bladder.

The clinical and histopathological data on all biopsies or TUR-BT specimens taken during follow-up were collected. Investigated bladder lesions were categorised according to their described appearance as follows:

1) Papillary tumours – tumours highly suspicious for urothelial carcinoma with papillary growth pattern rising from the mucosa
2) Flat tumours – sessile tumours or suspicious tissue confined within the mucosa including those described as velvet-like growth
3) Erythematous lesions – erythematous patches of bladder with no tumour rising from the mucosa
4) Normal cystoscopy – randomly biopsied specimens.

Voided urine cytology results evaluated by a pathologist prior to biopsy or TUR-BT were collected. Voided urine cytology reports included the Papanicolaou classification. (Layfield et al., 2004) The cytology was categorised as negative if normal urothelial cells or benign atypia (class 1–2) were reported, and positive if suspicion of urothelial carcinoma or urothelial carcinoma cells (class 3–5) was reported.

4.2.2 Studies II and III

After linking the register data of the FCR and FNIDR, we identified altogether 162 patients who had both diagnoses of bladder cancer and tuberculosis registered during the study period. There were four patients whose medical charts were not available and 58 patients who had not been treated with BCG and whose tuberculosis was not related to BCG instillations, resulting in 100 patients being included in the comprehensive medical chart review and data collection.

The collected data are presented in **Table 8.** The clinical data included age, gender, smoking history, comorbidities according to the Charlson comorbidity index (CCI), state of immunosuppression, and details of urothelial carcinomas and BCG instillations. The state of immunosuppression during BCG treatment was defined as presence of haematological disorder, history of splenectomy, or systemic prednisolone use for any indication with a daily dose of ≥10 mg.

Table 8. Variables collected in studies II and III.

Variable	**Measure/categorization**
Age	years
Gender	male / Female
Smoking	no smoking / current smoking / ex smoker
Charlson comorbidity index	number
Immunosuppression	Yes / No
Tumor grade	PUNLMP / LG / HG
T-category	Ta / T1 / cis
Date of first BCG instillation	date
Date of last BCG instillation	date
Number of instillations	n
Instillation route	intravesical / upper tract instillations
Onset of symptoms	date
Sampling of mycobacteria	date
Sampling site	urine / sputum / bronchoalveolar lavage / abscess / biopsy
Microbiological verification	positive / negative
Date of radiological diagnosis	date
Infection type	Systemic/Local
Infection Site	Anatomical localization
Initiation of antituberculosis medication	date
End of antituberculosis medication	date
Used antibiotics / antituberculosis medication	name of antimicrobial agents
Surgery due to BCG infection	Type of surgery (e.g. orchiectomy)
Surgery date	date
Outcome	complete resolution / resolution with long term adverse effects / death

BCG infections were categorised according to type and site as follows:

1. Systemic, if the infection focus was found in a non-urogenital system, such as pulmonary infection, or the BCG infection manifested with clinical signs of septicaemia.
2. Local, if the infection focus was in the urogenital tract without systemic signs or symptoms.

We further categorised the two infection types by site according to the anatomical localisation of infection. We categorised the infection type as systemic and the site as pulmonary if radiological pulmonary infiltrates (miliary or single) had developed, supporting the clinical conclusion of pulmonary infection, even though the microbiological verification was based on urine culture only.

We collected the symptoms and relevant time points of the manifestation and treatment of the infection as presented in **Table 8.** The symptoms were collected as reported in the medical charts during visits preceding the diagnosis and initiation of antituberculosis medication. A patient was defined as having no symptoms if this was stated in the medical charts.

The outcome of treatment was categorised as resolution with long-term adverse events if the patient was diagnosed with contracted bladder, benign hydronephrosis, or another chronic dysfunction of the affected organ as judged in medical charts. The outcome was categorised as death if the patient died during antimycobacterial treatment or prior to initiation of treatment of BCG infection.

4.2.3 Study IV

In study IV, we compared the tolerability and oncological efficacy of monthly maintenance and maintenance therapy according to the SWOG protocol, which were both used at Turku University Hospital and Helsinki University Hospital. We identified and retrospectively analysed patients who had been treated with BCG at these hospitals in 2009–2018.

Collected clinical data included age, gender, smoking history, comorbidities according to the CCI, immunosuppression, history of upper tract urothelial carcinoma (UTUC), histopathological details of BC, number of previous bladder resections, data on second resections, and details of BCG instillations. Based on patient age and oncological details, patients were categorised according to the EAU 2021 risk tables into low, intermediate, high, and very high risk groups. (Sylvester et al., 2021) The state of immunosuppression during BCG treatment was defined as presence of haematological disorder, history of splenectomy, or systemic prednisolone use for any indication with a daily dose of ≥10 mg.

Both maintenance schedules—monthly and the SWOG protocol—were used in both institutions during the study period. After a 6-week induction period, maintenance therapy followed a pre-planned schedule as follows: In the monthly maintenance therapy, a single instillation was administered monthly for 1–2 years. In the SWOG protocol, maintenance therapy consisted of 3-week minicourses at 3 and 6 months, whereafter minicourses were applied every 6 months until 1–3 years. There was no strict protocol on the planned length of BCG course in either group, as it was decided individually by the treating urologist according to the EAU guidelines. Follow-up included voided urinary cytology and cystoscopy. Follow-up visits were scheduled according to the EAU guidelines' risk-adopted principles. For imaging there were no strict follow-up protocols.

The dates of all administered and cancelled BCG instillations were collected. The reason for discontinuation of BCG was categorised as follows:

1. BCG failure, if BCG was discontinued due to recurrences or progression.
2. Toxicity, if BCG was discontinued due to local or general symptoms such as dysuria, urgency, haematuria, fever, fatigue, or general malaise.
3. BCG infection, if BCG was discontinued due to BCG infection warranting antituberculosis medication.
4. Compliance, if the patient did not want to proceed with BCG and no side effects or BCG inefficacy were reported.
5. Other, if BCG was discontinued due to comorbidities such as another malignancy, the patient died for reasons unrelated to BCG or BC, or if the reason for discontinuation of BCG was unclear.

Oncological follow-up data including recurrences and progression of BC following BCG instillations were collected. Recurrence was defined as any BC recurrence including LG or HG recurrence, and progression was defined as worsening of T-category or a diagnosis of metastases.

4.2.4 Statistical analysis

Study I

All biopsies and TUR-BT histology samples were analysed as individual events. Urine cytology, cystoscopy appearance of biopsy target, histopathological data, and time point in post-BCG surveillance were taken into account. Data on biopsies with missing cytology were excluded from calculations. Prevalence was defined by the percentage of malignancies detected in each lesion type. Sensitivity, specificity,

negative predictive value (NPV), and positive predictive value (PPV) for cytology to detect malignancy were calculated for each lesion category to identify the prognostic value of the lesion appearance in combination with cytology. The time points of the obtained biopsies or TUR-BT during follow-up revealing either a benign histology, malignant histology in the presence of negative cytology (false negative), or malignant histology in the presence of positive cytology (true positive) were illustrated with bars. Statistical analyses were performed using IBM SPSS Statistics version 24.0.0.1 and JMP Pro 12.2.0.

Studies II and III

In study II, we calculated the incidence proportion of identified BCG infections. To do this, we estimated the number of patients treated with BCG in Finland during the study period, using nationwide data on consumed BCG vials retrieved from Fimea. First, we estimated the rate of discarded BCG vials by retrieving data on discarded BCG doses during the study period at Turku University Hospital and Helsinki University Hospital, which we used for correcting the data retrieved from Fimea. Secondly, we calculated the median number and 95% confidence interval (CI) of per-patient consumption of BCG from individual patient data of the cohort used in study I in the current thesis and another retrospective cohort of BCG-treated patients at Helsinki University hospital (Nummi et al., 2019). The annual number of BCG vials consumed nationwide was then divided by the calculated median and both ends of the 95% CI of per-patient consumption of BCG, which yielded the final estimated number of patients treated with BCG during the study period. The calculation used in the estimation is illustrated in **Figure 3.**

$$\textit{Estimated number of patients treated with BCG in finland during the study period} = \frac{\textit{The number of nationally consumed BCG vials in Finland (Fimea) after correction for discarded BCG vials}}{\textit{Median number of per patient consumption of BCG}}$$

Figure 3. Calculation of the estimated of the number of patients treated with BCG in Finland. The data on the number of nationally consumed BCG vials was retrieved from Fimea and was available for 2000-2016. The median number of per-patient consumption of BCG was calculated using pooled individual patient data from Study I and a retrospective cohort of BCG-treated patients at Helsinki University Hospital. (Nummi et al., 2019).

The annual incidence of BCG infections was defined as the number of BCG courses per year that had subsequently resulted in BCG infection. The incidence proportion was defined as the proportion of incidence of the estimated number of patients treated with BCG. Since data on consumption of BCG vials was only

available for 2000–2016, the analysis of incidence proportion included BCG infections occurring within this time interval.

In study III, we calculated the duration between the last BCG instillation and diagnosis of BCG infection. The date of diagnosis of BCG infection was defined as the date of sampling of mycobacteria or the date of imaging if the diagnosis was based only on radiological findings. The timeline of BCG instillations, latency, and antituberculosis medication for each patient in the cohort was illustrated using a swimmer's plot.

Categorical data were described as percent proportions. The distribution of continuous data was tested for normality, both visually and with Shapiro-Wilk's test. Normally distributed observations were described as means and confidence intervals (CI) and non-normally distributed observations as medians and IQR. Statistical analyses were performed using JMP Pro Version 14.2.0. (SAS Institute Inc., Cary, NC, 1989-2019).

Study IV

In study IV, we compared treatment outcomes of patients who received BCG either with monthly maintenance or according to the SWOG protocol, and the cohort was grouped accordingly. Associations between the groups and collected explanatory variables (age, gender, smoking, CCI, immunosuppression, T-category, grade, number of tumours, size of tumour, number of resections before BCG, performed re-resection, history of UTUC, EORTC 2021 risk groups, disease history at initiation of BCG, number of instillations, duration of BCG, number of discontinued BCG courses and reasons for discontinuation) were summarised with descriptive statistics and studied one by one with the Wilcoxon rank sum test for non-parametric continuous variables, Pearson's chi-squared test for categorical variables, and Fisher's exact test for categorical variables with small sample sizes.

The Kaplan-Meier method was used to estimate the discontinuation of BCG due to toxicity, recurrence, and progression rates, and the survival figures were illustrated according to the applied maintenance schedule. The log-rank test was used to compare groups in terms of cancer RFS and progression-free survival, and the hazard ratios (HR) were calculated using the Cox regression model. The statistical significance level was set at 0.05 in all tests (two-tailed) and 95% CIs were calculated.

Discontinuation of BCG due to toxicity, recurrence, and progression associated with the same explanatory variables (gender, smoking status, CCI, immunosuppression, number of resections before BCG, performed re-resection, history of UTUC, EORTC 2021 risk group, maintenance protocol) to all endpoints were studied using the Cox proportional hazards model. First, explanatory variables

were added to the multivariate model, from which non-significant factors were gradually omitted, and meaningful explanatory variables were combined for all three models.

The analyses were performed using RStudio (version 2022.7.1.554) based on R (version 4.1.0) (RStudio, PBC, Boston, MA, USA).

4.2.5 Ethics

All the studies included in this thesis are non-interventional retrospective studies conducted according to good clinical practice and the declaration of Helsinki. For studies I and IV, institutional review board permission was obtained from each study centre. The study protocols for studies II-III were approved by the research ethics board of the hospital district of Southwestern Finland and the review board of THL, who approved access to the registry data (Permission number THL/1139/5.05.00/2017)

5 Results

5.1 Study I

The clinical and pathological characteristics of the study population are outlined in **Table 9**. A total of 206 patients were included in the study, of whom 180 (87%) were men and 27 (13%) women. Median age was 73 (IQR: 64–78) years. Primary NMIBC stage was Ta in 135 (56%), T1 in 49 (24%), and CIS in 22 (11%) patients. All patients fell into intermediate or high risk groups (26% and 74%, respectively) according to the EORTC 2006 risk tables. (Sylvester et al., 2006) Median number of BCG instillations administered was 14 (IQR: 10–18), corresponding to approximately 9–10 months of treatment duration.

Table 9. Characteristics of 206 patients treated with BCG instillation therapy against NMIBC.

Variable		
Gender n (%)		
	Male	180 (87)
	Female	26 (13)
Age	Median, years (IQR)	73 (64–78)
Smoking status n (%)		
	Never smoker	63 (40)
	Ex–smoker	52 (33)
	Active smoker	43 (27)
	N/A	48
ASA-score n (%)		
	1	18 (9)
	2	85 (41)
	≥3	103 (50)
T-category n (%)		
	Ta	135 (65)
	T1	49 (24)
	cis	22 (11)
Number of tumours n (%)		
	1	116 (56)
	2–7	85 (41)
	≥8	5 (3)
Tumour size n (%)		
	<1 cm	14 (7)
	1–3 cm	139 (69)
	>3 cm	49 (24)
	N/A	4
EORTC Risk group n (%)		
	Low risk	0 (0)
	Intermediate risk	53 (26)
	High risk	153 (74)
Number of BCG instillations	Median (IQR)	14 (10–18)

IQR = Interquartile range, ASA = American Society of Anesthesiologists, EORTC = European Organization for Research and Treatment of Cancer, BCG = Bacillus Calmétte-Guerin

After initiation of BCG, 159/206 (76%) patients underwent one or more biopsies or tumour resections during follow-up, resulting in altogether 448 obtained histological specimens. Data on urinary cytology were available for 367 specimens included in the final analyses and are presented in **Table 10**. Erythematous lesion was the most common biopsy target, accounting for 209/367 (57%) biopsies. Of these, 187/209 (90%) had a benign histology representing either nonspecific inflammation or granulomatous inflammation. The cytology was concordant in 156/209 (75%) biopsies

of erythematous lesions. Nine specimens obtained from erythematous lesions revealed a malignant histology with negative cytology, of which six were Ta, two T1, and one CIS. Thirteen specimens were malignant with positive cytology, of which two specimens revealed muscle-invasive tumours, eight CIS, one T1, and two Ta.

Table 10. Frequencies of cytology grading and subsequent biopsy results.

Cystoscopy	Cytology	Biopsy result	
		Benign (n)	Malignant (n)
Papillary tumour n=98	Negative	18	52
	Positive	3	25
Flat tumour n=42	Negative	10	19
	Positive	2	11
Erythema n=209	Negative	143	9
	Positive	44	13
Normal n=18	Negative	10	0
	Positive	6	2

Voided urine cytology was categorized negative with Papanicolaou classes 1-2 and positive with Papanicolaou classes 3-5

The diagnostic accuracy of cytology in detecting malignancy is shown in **Table 11**. Correlated to the histology, the overall sensitivity, specificity, and positive and negative predictive values for cytology to detect malignancy were 39%, 77%, 48%, and 69%, respectively. In case of papillary tumours, the sensitivity, specificity, NPV, and PPV were 32%, 86%, 26%, and 89%, respectively. Flat tumours showed values comparable to papillary tumours with sensitivity, specificity, NPV, and PPV of 37%, 83%, 34%, and 85%, respectively. The cytology was found to be most accurate if only erythema was detected; the sensitivity, specificity, NPV, and PPV were 59%, 76%, 94%, and 23%, respectively. Among random biopsies from normal urothelium, the sensitivity, specificity, NPV, and PPV were 100%, 63%, 100%, and 25%, respectively.

Table 11. Descriptive values of cytology in detecting malignancy in various bladder lesions

Cystoscopy	Prevalence (%)	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
Overall (n=367)	36	39	77	69	48
Papillary Tumour (n=98)	79	32	86	26	89
Flat tumour (n=42)	71	37	83	34	85
Erythema (n=209)	11	59	76	94	23
Normal (n=18)	11	100	63	100	25

Prevalence is the percentage of malignancies diagnosed from each lesion category
NPV = Negative predictive value, PPV = positive predictive value

Figure 4 demonstrates the occurrence of erythematous lesions during follow-up in 3-month periods. Erythematous lesions were most frequently detected during the first 9 months, which correlates with the median duration of the BCG course (around 9–10 months). As a result, after 9 months from the start of BCG treatment the occurrence of erythematous lesions gradually diminished; after 2 years it was significantly lower, although lesions were detected up to 84 months after the first instillation. Malignant histology, however, was detected just as often both before and after 2 years of surveillance (proportion of malignant lesions 10.1% and 10.3%, respectively).

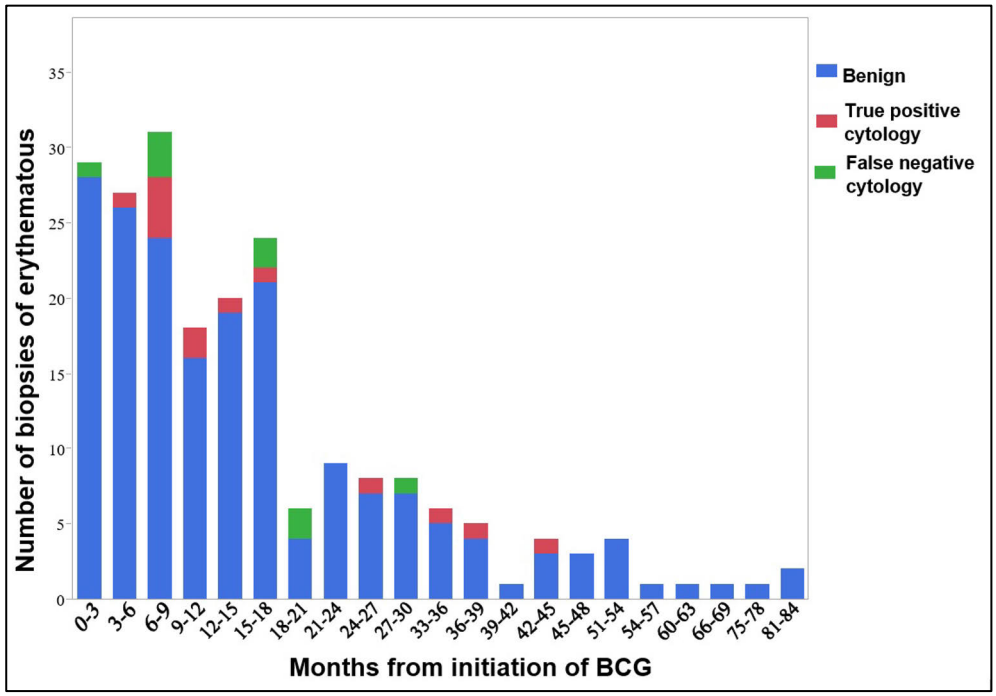

Figure 4. Biopsies or TUR-BT specimens taken from Erythematous lesions during follow up after BCG instillations. Frequencies are presented in 3-month periods. Blue bars indicate the frequency of biopsies with benign histology with any cytology grade. Green bars represent biopsies taken in the presence of negative cytology showing malignant histology (false negative). Red bars represent biopsies taken in the presence of positive cytology showing malignant histology (true positive). Average duration of BCG treatment was 9-10 months.

5.2 Study II

The flowchart of patient identification and exclusion criteria in studies II and III is presented in **Figure 5**. In study II, we wanted to investigate the risk of BCG infection related to BCG instillation therapy and mortality of BCG infections. In 13 patients

the BCG infection resolved without treatment and was considered clinically insignificant; these patients were excluded from the analyses in study II. As a result, 87 patients with BCG infection were included in the analyses.

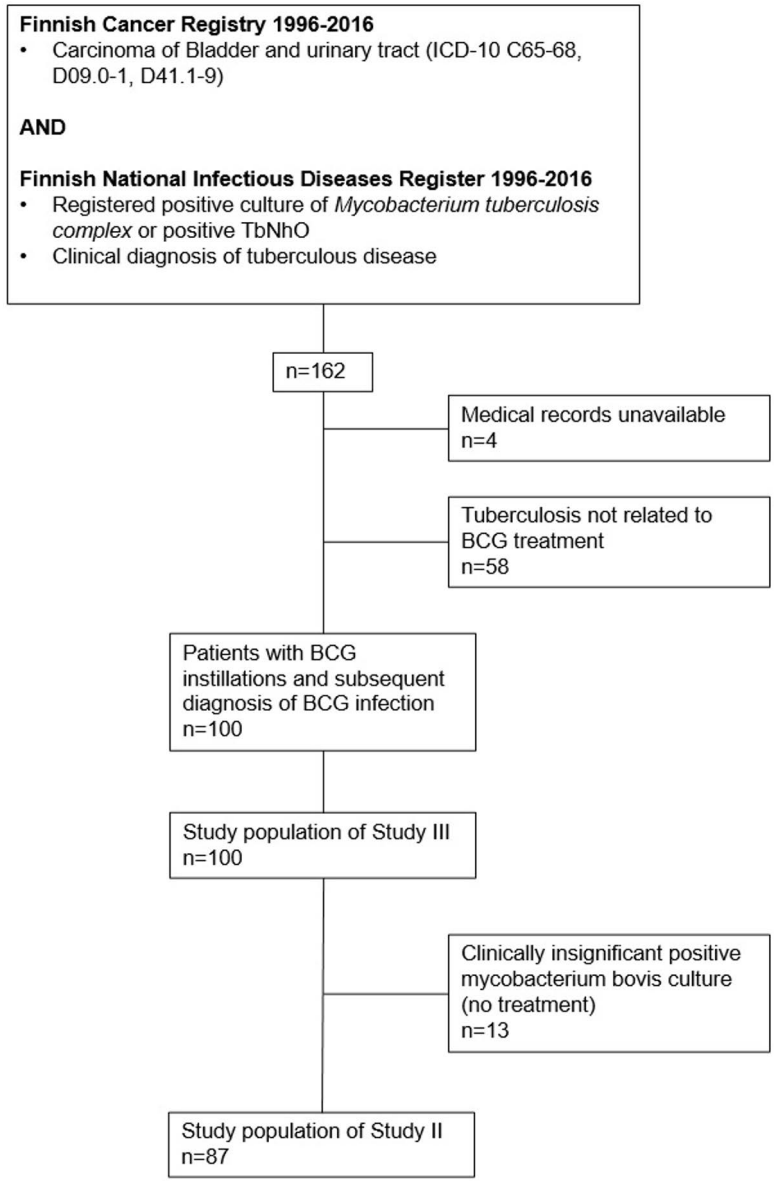

Figure 5. Patient identification and exclusions in studies II and III.

The characteristics of the study subjects and details of BCG instillations are shown in **Table 12**. Median follow-up time was 50 (IQR: 27-98) months. There was a strong male predominance, as only two women experienced a BCG infection in Finland throughout the study period. Immunosuppression was present in seven patients of whom five were on concurrent immunosuppressive medication such as methotrexate, hydroxychloroquine, or prednisolone while receiving BCG instillations. Patients with Wegener's granulomatosis and chronic myelomonocytic leukaemia were under surveillance only at the time of BCG instillations and had a history of immunosuppressive medication. Eighty-five patients received intravesical BCG instillations due to intermediate- or high-risk BC. Of these, 26 (30%) were pathologically classified as pTa, 39 (46%) as pT1, and 20 (24%) as CIS. Two patients with CIS of the upper urinary tract received tailored upper-tract BCG instillations. Of these, one patient received the induction course as bladder instillations with a double J stent, but during the maintenance period they received antegrade instillations via nephrostomy tube and developed local BCG cystitis. The other patient was assigned to an induction course of BCG instillations with bilateral retrograde ureteral catheterisations under anaesthesia and developed a generalised infection presenting with high grade fever, elevated liver enzymes, and renal failure.

Table 12. Patient characteristics of 87 NMIBC patients treated with BCG instillations and subsequent BCG infection warranting antituberculosis medication in Finland during 1996–2016 (Study II).

Patient characteristics		
Age, years	Mean (SD)	72 (9)
Gender, n (%)	Male	85 (98)
	Female	2 (2)
Charlson Comorbidity index, n (%)	0 pts	35 (41)
	1 pts	22 (25)
	2 pts	15 (17)
	≥3 pts	15 (17)
Smoking status, n (%)	Never smoker	9 (10)
	Active smoker	11 (13)
	Stopped smoking	38 (44)
	N/A	29 (33)
Immunosuppression, n (%)	No	80 (92)
	Yes	7 (8)
	Rheumatoid arthritis	3 (44)
	Chronic myelomonocytic leukemia	1 (14)
	Wegener´s granulomatosis	1 (14)
	Reticulohistiocytosis	1 (14)
	Chronic bronchitis with systemic corticosteroid treatment	1 (14)
Details of BCG instillations		
Number of instillations	Median (IQR)	11 (7–15)
Administration route, n (%)	Intravesical	85 (98)
	Upper tract	2 (2)
Difficulties with catheterization, n (%)	No	47 (55)
	Traumatic catheterization	21 (25)
	N/A	17 (20)

SD = Standard deviation, IQR = Interquartile range, N/A = Data not available

Table 13 shows the clinical characteristics of the BCG infections. Local and systemic infections were identified in 48 (55%) and 39 (45%) patients, respectively. Microbiological verification of *Mycobacterium bovis* was obtained in 79/87 (91%) of patients. Pulmonary manifestation was the most common systemic infection site (35 patients), of which miliary radiological infection features were the most common subtype. There were three patients whose infection was identified only in the urinary culture but presented with hypotension, acute renal injury, thrombocytopenia, and

one fatal respiratory failure indicating systemic manifestations. Local infections were a slightly more common infection type, presenting mostly as BCG cystitis and epididymitis. Overall, BCG cystitis was the most common local infection type presenting as prolonged symptoms including dysuria, urgency, and haematuria. Of the 17 patients with epididymitis, five presented with cutaneous fistula or abscess warranting surgical exploration.

Table 13. Details of 87 identified BCG infections in Study II.

Type	Site	Details	n (%)
Systemic			39 (45)
	Pulmonary	Miliary	25 (64)
		Pulmonary infiltrate	9 (23)
		Intrapulmonary lymph node infection	1 (3)
	Non-Pulmonary	Spondylodiscitis, psoas abscess and pseudoaneurysm of abdominal aorta	1 (3)
		Septicemia and multiple organ dysfunction	3 (7)
Local			48 (55)
	Urinary Tract	BCG cystitis	28 (58)
		Granulomatous renal abscess	1 (2)
	Genital	Epididymitis	17 (36)
		Granulomatous prostatitis	2 (4)

The treatment outcome of BCG infections is reported in **Table 14**. Nine patients died during antimycobacterial treatment or prior to the initiation of treatment for BCG infection, resulting in 10% overall mortality. Complete resolution was seen in 69 (80%) patients, whereas long-term adverse events developed in nine (10%) patients. Systemic infections showed substantial mortality, with seven (17.5%) deaths of which six resulted from a pulmonary infection and one from a systemic febrile BCG infection with no focal findings other than a positive urine culture of *Mycobacterium bovis*. The median time to initiation of antituberculosis medication from the diagnosis of infection was 3 (range 0–8) days in those who died and 15.5 (range 0–119) days in those who responded to treatment, reflecting the acute presentation of infections deemed to be fatal. Three deaths occurred before any antimycobacterial treatment could be initiated, and a positive culture of *Mycobacterium bovis* either in urine or sputum was confirmed post-mortem. Of these, two patients experienced slowly progressing pulmonary symptoms, loss of weight, and night sweats before acute presentation of symptoms, while one patient presented with a high-grade fever and multi-organ failure following administration of BCG complicated by traumatic catheterisation.

Long-term adverse effects developed in three patients after pulmonary infection, all of whom developed late benign hydronephrosis due to bladder scarring related to BCG infection and multiple bladder resections. Of the patients with local infection, two sudden deaths occurred due to myocardial infarction during antimicrobial treatment for BCG cystitis, resulting in a mortality of 4%. Long-term adverse events developed in six (21%) patients with BCG infection of the urinary tract, including three with contracted bladder, two with benign hydronephrosis, and one with chronic kidney disease and renal failure after treatment for a granulomatous renal abscess. In addition, one patient with BCG cystitis developed concomitant unilateral iritis which persisted until the end of the study period.

Table 14. Outcomes of the treatment of BCG infections in Study II.

	BCG infections n= 87			
Complete resolution (%)	69 (80)			
Resolution with long term adverse effects (%)	9 (10)			
Death (%)	9 (10)			
	Outcome by infection type and site			
	Systemic		**Local**	
	Pulmonary	**Non-Pulmonary**	**Urinary tract**	**Genital**
	n=35	**n=4**	**n=29**	**n=19**
Complete resolution (%)	26 (74)	3 (75)	21 (72)	19 (100)
Resolution with long term adverse effects (%)	3 (9)	0 (0)	6 (21)	0 (0)
Death (%)	6 (17)	1 (25)	2 (7)	0 (0)

Figure 6 shows the estimated nationwide number of patients treated with BCG per year and the number of BCG regimens which subsequently resulted in BCG infection. The data on BCG consumption were available during 2000–2016. The rate of discarded BCG doses was 0.5% and the median number of instillations per patient was 16 (95% CI: 15–17). There was substantial variability on the amount of BCG consumption until 2005, from which the consumption data are more consistent. Based on our estimations, 4100 patients (95% CI: 3859–4373) were treated with BCG during 2000–2016, of whom 79 developed BCG infection after instillations, resulting in a cumulative incidence proportion of 1.9% (95% CI: 1.8–2.0%). However, after 2006 the incidence notably increased, and thereafter the cumulative incidence proportion was 2.5% (95% CI: 2.4–2.6%).

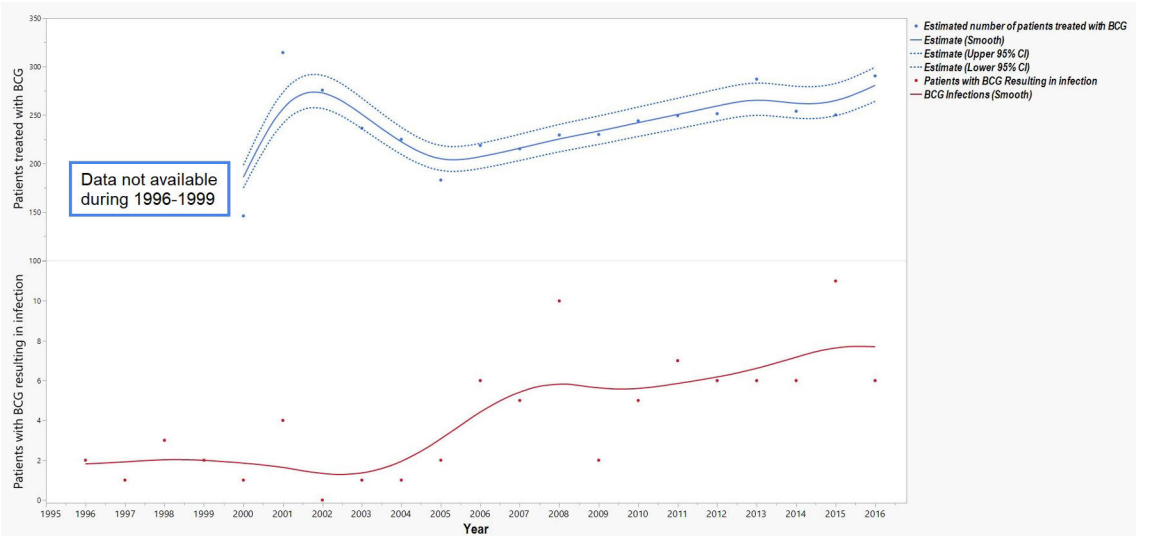

Figure 6. Estimated number of patients treated with BCG and number of BCG regimens subsequently resulting in BCG infection. The blue dots represent the estimated number of patients treated with BCG per year. The red dots represent the number of patients whose BCG resulted in BCG infection, stratified by initiation year of BCG. The smoothened lines (continuous with 95% dotted confidence band) show the spline representing the relationship of consecutive numeric variables. Data on consumption of BCG were not available during 1996–1999.

5.3 Study III

In study III, we aimed to investigate the time intervals between various types of BCG infections following BCG instillations. In this study we also included patients who did not warrant any treatment. In total, 100 patients were included in the analyses.

The clinical characteristics of the patient population are summarised in **Table 15**. Median follow-up time was 50 months (IQR: 25–94). Median age was 72 years (IQR: 67–78), and 98/100 (98%) were male and 2/100 (2%) female.

Table 15. Patient characteristics of 100 NMIBC patients treated with BCG instillations and subsequent BCG infection in Finland during 1996–2016 (Study III).

Patients, n (%)		
Age, years	Median (IQR)	72 (67–78)
Gender, n (%)	Male	98 (98)
	Female	2 (2)
Charlson Comorbidity index, n (%)	0 pts	40 (40)
	1 pts	26 (26)
	2 pts	18 (18)
	≥3 pts	16 (16)
Smoking status, n (%)	Never smoker	11 (11)
	Active Smoker	12 (12)
	Stopped smoking	42 (42)
	N/A	35 (35)
Immunosuppression, n (%)	No	93 (93)
	Yes	7 (7)
	Rheumatoid arthritis	3 (44)
	Chronic Myelomonocytic leukemia	1 (14)
	Wegener´s granulomatosis	1 (14)
	Reticulohistiocytosis	1 (14)
	Chronic bronchitis with systemic corticosteroid treatment	1 (14)

SD = Standard deviation, IQR = Interquartile range, N/A = Data not available

The timing of diagnosis of various types of BCG infections after BCG instillations and number of administered instillations before diagnosis of infection are shown in **Table 16**. Overall, the median number of administered BCG instillations prior to diagnosis of infection was 10 (IQR: 6–14), and most patients developed the infection during the first year of the maintenance period. Although very rare, the most rapidly manifesting infection types were generalised infections, all of which manifested during the induction period with immediate persistent high-grade fever, septicaemia, and multi-organ failure. Of pulmonary infections, miliary infection presenting with a small-nodule pattern on chest imaging manifested after a median of 10 (IQR: 6.5–12) instillations. Pulmonary infections presenting with a solitary pulmonary infiltrate presented with slower manifestation, of which the diagnosis was made after a median of 44 (IQR: 16–522) days after the last instillation. Intrapulmonary lymph node infection was diagnosed with a positive nucleic acid test of *Mycobacterium bovis* BCG in a patient with no symptoms, but with enlarged intrapulmonary lymph nodes found after 3 years of an uncomplicated

full-dose BCG course. Of local infections, BCG cystitis was the most common type, which manifested with gradually worsening voiding symptoms, and no clear manifestation time point of symptoms could be identified. The reasons for obtaining mycobacterial samples of patients with BCG cystitis included general symptoms, such as fatigue or fever or erythematous lesions and granulomas seen on cystoscopy. Epididymitis related to BCG manifested with two distinguishable subtypes: either as an acute swelling and pain of the scrotum (eight patients), diagnosed after a median of 34 (IQR: 16–139) days after the last instillation, or slowly as a tumour-like enlargement of epididymitis (nine patients), diagnosed after a median of 148 (IQR: 26–1277) days after the last instillation.

Table 16. Time intervals from the last instillation of BCG, and the number of instillations before developing a BCG infection.

Type	Details	n (%)	Days from the last BCG instillation Median (IQR)	Number of BCG instillations Median (IQR)
Systemic	Miliary	25 (25)	20 (12–25)	10 (6.5–12)
	Pulmonary infiltrate	9 (9)	44 (16–522)	11 (6.5–13.5)
	Intrapulmonary lymph node infection	1 (1)	1157 (N/A)[2]	21 (N/A)
	Spondylodiscitis, psoas abscess and pseudoaneurysm of abdominal aorta	3 (3)	19 (11–27)	2 (1-5)
	Septicemia and multiple organ dysfunction	1 (1)	523 (N/A)	13 (N/A)
Local	BCG cystitis	40 (4)	49 (10-232)	12 (6.25–15.75)
	Granulomatous renal abscess	1 (1)	65 (N/A)	12 (N/A)
	Epididymitis	17 (17)	60 (21-385)	8 (6.25-15)
	Granulomatous prostatitis	3 (3)	9 (8–1369)	9.5 (9-10)

BCG = Bacillus Calmétte-Guerin, IQR = Interquartile range, n/a = Not available

Clinical symptoms prior to diagnosis of systemic and local BCG infections are presented in **Table 17**. The most common symptoms among patients with systemic infections were rising fever up to over 38°C in 30/39 (77%) patients and general malaise in 26/39 (67%) patients. Of the 34 patients with pulmonary infection, 16/34 (47%) did not report any pulmonary symptoms but rather suffering from general malaise, fatigue, night sweats, or high fever only. Among local infections urinary symptoms were more prominent, although 27 (44%) patients also reported fatigue or fever. Nine out of 61 (15%) patients with local infections reported having no symptoms. These included three patients with granulomatous prostatitis, five with

BCG cystitis, and one with granulomatous abscess of the kidney. The reasons for mycobacterial sampling of these patients included rising prostate-specific antigen (PSA), abnormal digital rectal examination, and histological granulomas seen in a cystoprostatectomy specimen among patients with granulomatous prostatitis; persistent pyuria, erythematous bladder lesion, and granulomatous tumour on cystoscopy among patients with BCG cystitis; and biopsy of a renal mass suspicious for malignancy in a patient with granulomatous renal abscess.

Table 17. Symptoms preceding the diagnosis of BCG infection.

Symptom	Details	Systemic n=39 n (%)	Local n=61 n (%)
General	Fever >38 degrees	30 (77)	8 (13)
	Fever < 38 degrees	23 (59)	11 (18)
	General malaise	26 (67)	8 (13)
	Fatigue	18 (46)	10 (16)
	Nausea, loss of appetite	6 (15)	0 (0)
	Weight loss	4 (10)	0 (0)
Non-specific focal	Back pain	4 (10)	1 (2)
	Joint pain	3 (8)	2 (3)
	Abdominal pain	1 (3)	6 (10)
	Skin rash	1 (3)	1 (2)
Pulmonary	Dry couch	12 (30)	1 (2)
	Difficulty in breathing	11 (28)	0 (0)
	Secretion of sputum	7 (18)	0 (0)
	Hemoptysis	1 (1)	0 (0)
Urinary	Dysuria	8 (20)	27 (44)
	Urgency	4 (10)	23 (37)
	Hematuria	2 (5)	10 (16)
	Incontinence	2 (5)	2 (3)
Scrotal	Acute swelling and pain	0 (0)	8 (13)
	Tumor-like enlargement	0 (0)	9 (15)
	Abscess	0 (0)	5 (8)
No symptoms		1 (1)	9 (15)

The time intervals between BCG therapy and initiation of treatment of systemic and local BCG infections are illustrated in **Figure 7**. Among systemic infections, 34/39 (87%) developed rapidly after an instillation of BCG, and the latency consisted

mostly of the duration of mycobacterial cultures and logistical factors. However, five (13%) patients with systemic infections presented after a substantial latency of over 1 year. Of these, three patients had a pulmonary infiltrate, one had an intrapulmonary lymph node infection, and another had a psoas abscess, concomitant spondylodiscitis, and a pseudoaneurysm of the abdominal aorta. Local infections manifested after heterogeneous latency periods, and the division into early and late subtypes was not as clear as among systemic infections. Of the 61 local infections, 12/61 (20%) were diagnosed more than 1 year after the last instillation, including seven patients with cystitis, four with epididymitis, and one with granulomatous prostatitis.

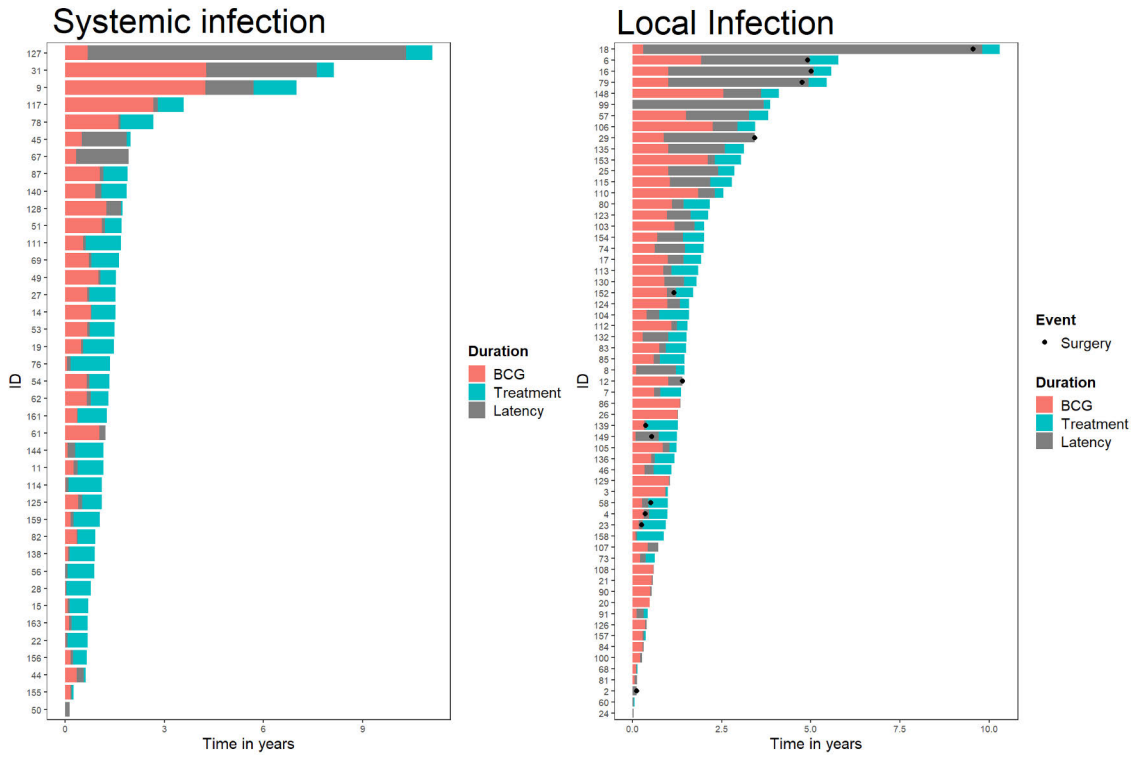

Figure 7. Swimmer's plot illustrating the time intervals of systemic and local BCG infections following a BCG instillation course. The red bar represents the duration of the BCG instillation course. The grey bar represents the time interval between the last instillation and the initiation of antituberculosis medication. The blue bar represents the duration of antituberculosis medication. The black dot indicates the time of surgery.

The treatment of BCG infections in our cohort is outlined in **Table 18.** Overall, 81 (81%) patients received antituberculosis medication alone or in conjunction with surgery. Of these, 78 (96%) received an isoniazid-based regimen, mostly combined

with rifampicin and ethambutol. In addition, there was one patient with epididymitis and two with BCG cystitis, who received only fluoroquinolones. The median duration of antituberculosis medication among systemic and local infections was 9 (IQR 6–10) and 6 (IQR 3–7) months, respectively. Reported side effects associated with antituberculosis medication included elevated liver enzymes in 18 (22%) patients, urticaria in seven (9%), neuropathy in four (5%), and suspected ethambutol-related optic neuropathy in one (1%).

Two patients underwent RC, one of whom had BCG cystitis and contracted bladder, and the other of whom had granulomatous prostatitis 71and was also treated with antituberculosis medication after cystectomy. Of the 17 patients with epididymitis, six underwent orchidectomy, three an incision of an epididymal abscess, and three an extirpation of the epididymis. Finally, 13 patients with BCG cystitis did not receive any antimycobacterial treatment or surgery and were under observation only, as their symptoms resolved spontaneously before commencing any treatment, suggesting that their symptoms represented a rather normal reaction to BCG instillations and not a true infection. The positive mycobacterial sample of these patients was obtained after a median of 8 (IQR: 3–17) days following the last instillation, supporting the fact that in most of these patients the positive urine culture was most likely related to the instillations. Interestingly, there was one patient whose BCG infection was detected in a histological specimen of a resected erythematous granuloma of the bladder 3 months after the last BCG instillation following an uneventful BCG course, and who also showed a positive urinary culture at that time but was not treated due to absolute lack of any symptoms.

Table 18. Treatment of BCG infections according to infection site and type.

Details	Patients	Antituberculosis medication	Medication + Surgery	Surgery only	No treatment
	n	n (%)	n (%)	n (%)	n (%)
Systemic infections					
Miliary	25	25 (100)	0	0	0
Pulmonary infiltrate	9	7 (78)	0	0	2 (22)[1]
Intrapulmonary lymph node	1	1 (100)	0	0	0
Psoas abscess, spondylodiscitis, pseudoaneurysm of abdominal aorta	1	1(100)	0	0	0
Generalized	3	2 (67)	0	0	1 (33)[1]
Local infections					
BCG Cystitis	40	27 (68)	0	1 (2)	12 (30)[2]
Granulomatous renal abscess	1	1 (100)	0	0	0
Epididymitis	17	5 (29)	10 (59)	2 (12)	0
Granulomatous prostatitis	3	1 (33)	1 (33)	0	1 (33)[2]

[1] Patient died before initiation of antituberculosis medication (patient ID 27,89,143)
[2] BCG Infection resolved without any treatment

5.4 Study IV

In study IV, we identified a total of 802 consecutive patients (196 at Turku University Hospital and 606 at Helsinki University hospital) who had been treated with BCG against BC. Since the objective of this study was to compare the tolerability and oncological efficacy of two maintenance schedules, we excluded 76 patients who did not receive maintenance therapy. We further excluded three patients whose pathological stage was ultimately classified as pT2 despite treatment with TUR-BT and BCG. In all, 723 patients were included in the final analyses. Of these, 545 (75%) received monthly maintenance and 178 (25%) maintenance according to the SWOG protocol.

The baseline characteristics of the patients are listed in **Table 19** and the oncological details in **Table 20**. Patients in the monthly maintenance group were older than in the SWOG group, with a median age of 73 (IQR: 67–79) years and 71 (IQR: 65–77) years, respectively (p=0.019). Additionally, there were fewer HG tumours in the monthly maintenance group than in the SWOG group, 93% and 96%

respectively (p=0.040). However, there was no difference in the EORTC 2021 risk group classification between the groups (p=0.700). Median follow-up time was 66 (IQR: 45–99) months.

Table 19. Baseline characteristics and oncological details of the patients treated with Bacillus Calmette-Guerin (BCG) therapy using either monthly maintenance or the maintenance schedule of the Southwest Oncology Group (SWOG).

		Overall n (%)	**Monthly n (%)**	**SWOG n (%)**	**p-value[1]**
Number of patients		723	545 (75)	178 (25)	
Age	Median (IQR)	73 (66–79)	73 (67–79)	71 (65–77)	0.019
Gender					0.200
	Male	607 (84)	452 (83)	155 (87)	
	Female	116 (16)	93 (17)	23 (13)	
Smoking					0.500
	Never	210 (33)	155 (32)	55 (35)	
	Ex-smoker	256 (40)	190 (40)	66 (42)	
	Active smoker	170 (27)	134 (28)	36 (23)	
	N/A	87	66	21	
Charlson Comorbidity Index					0.500
	2	355 (49)	261 (48)	94 (53)	
	3	179 (25)	139 (26)	40 (22)	
	≥4	189 (26)	145 (27)	44 (25)	
Immunosuppression					0.900
	No	696 (96%)	52 (96%)	171 (96%)	
	Yes	27 (4)	20 (4)	7 (4)	

[1] Wilcoxon Rank Sum test; Chi-Square test; Fisher's exact test
IQR = Interquartile range

Table 20. Oncological characteristics of the 723 patients treated with Bacillus Calmette-Guerin (BCG) therapy using either a monthly maintenance or the maintenance schedule by the Southwest Oncology Group (SWOG).

		Overall n (%)	Monthly n (%)	SWOG n (%)	p-value[1]
T-category					0.500
	Ta	237 (33)	173 (32)	64 (36)	
	T1	368 (51)	280 (51)	88 (49)	
	cis	118 (16)	92 (17)	26 (15)	
Grade					0.040
	High Grade	669 (94)	504 (93)	165 (96)	
	Low Grade	40 (6)	35 (7)	5 (3)	
	PUNLMP	3 (0.4)	1 (0.2)	2 (1)	
	N/A	11	5	6	
Number of tumors					0.130
	1	355 (50)	276 (51)	79 (45)	
	2-7	339 (47)	251 (47)	88 (50)	
	≥8	20 (3)	12 (2)	8 (5)	
	N/A	9	6	3	
Size of the largest tumor					0.140
	<1 cm	80 (11)	68 (12)	12 (7)	
	1-3 cm	400 (56)	299 (55)	101 (60)	
	>3 cm	231 (32)	177 (33)	54 (32)	
	N/A	12	1	11	
Number or resections prior BCG	Median (IQR)	2 (2- 3)	2 (2-3)	2 (2-2)	0.600
Re-resection performed					0.200
	No	302 (43)	234 (44)	68 (38)	
	Yes	407 (57)	297 (56)	110 (62)	
	N/A	14	14	0	
History of UTUC					0.900
	No	668 (92)	504 (92)	164 (92)	
	Yes	55 (8)	41 (8)	14 (8)	
EORTC risk group					0.700
	Low risk	1 (0.1)	1 (0.2)	0 (0)	
	Intermediate risk	99 (14)	79 (15)	20 (11)	
	High risk	479 (67)	359 (66)	120 (68)	
	Very High risk	141 (20)	104 (19)	37 (21)	
	N/A	3	2	1	

[1] Wilcoxon Rank Sum test; Chi-Square test; Fisher's exact test

IQR = Interquartile range, PUNLMP = Papillary urothelial neoplasm of low malignant potential, N/A = Not available, UTUC = Upper tract urothelial carcinoma, EORTC = European Organization for Research and Treatment of Cancer

Details of BCG instillations and data on unplanned discontinuation of BCG in the two groups are shown in **Table 21**. Patients in the monthly maintenance and SWOG groups received a median of 17 (IQR: 14–22) and 18 (IQR: 13–21) instillations, respectively (p=0.200). However, the instillations were administered during a shorter period in the monthly maintenance group, with a median of 13 (IQR: 11–20) months and 17 (IQR: 8–25) months in the SWOG group (p=0.036). Overall, there were no differences in the rate of unplanned discontinuation of BCG between the groups, with 241/545 (44%) in the monthly maintenance group and 79/178 (44%) in the SWOG group (p=>0.9).

Table 21. Details of Bacillius Calmette-Guérin (BCG) treatment in 723 patients undergoing monthly maintenance or maintenance according to the Southwest oncology group (SWOG).

Variable	**Details**		**Group**		
		Overall n=723 n (%)	Monthly n=545 n (%)	SWOG n=178 n (%)	p-value[1]
Disease history at initiation of BCG					0.200
	Primary tumour	523 (72)	387 (71)	136 (77)	
	Recurrent disease	200 (28)	158 (29)	42 (23)	
Number of instillations, median (IQR)		17 (14–21)	17 (14–22)	18 (13–21)	0.200
Duration of BCG (Months), median (IQR)		13 (10–22)	13 (10–20)	17 (8–25)	0.036
Completion of BCG					>0.900
	Finished	400 (56)	301 (56)	99 (56)	
	Discontinued	320 (44)	241 (44)	79 (44)	
	N/A	3	3	0	
Reasons for discontinuation					0.300
	BCG failure	70 (22)	56 (24)	14 (18)	
	Toxicity	164 (51)	116 (48)	48 (61)	
	BCG infection	17 (5)	15 (6)	2 (2)	
	Compliance	19 (6)	16 (7)	3 (4)	
	Other	49 (15)	37 (15)	12 (15)	

[1] Wilcoxon Rank Sum test; Chi-Square test; Fisher's exact test
N/A=Not available (moved to another hospital district), IQR = Interquartile range

The Kaplan-Meier plot estimating the unplanned discontinuation of BCG due to toxicity or BCG infection is illustrated in **Figure 8**. In the SWOG group, the rate of discontinued BCG courses was slightly higher during the first half of the maintenance period and lower in the latter half, with curves crossing at 18 months. As a result, no significant difference was observed in a univariate Cox regression analysis (HR 1.01, 95% CI: 0.73–1.41, p=0.940).

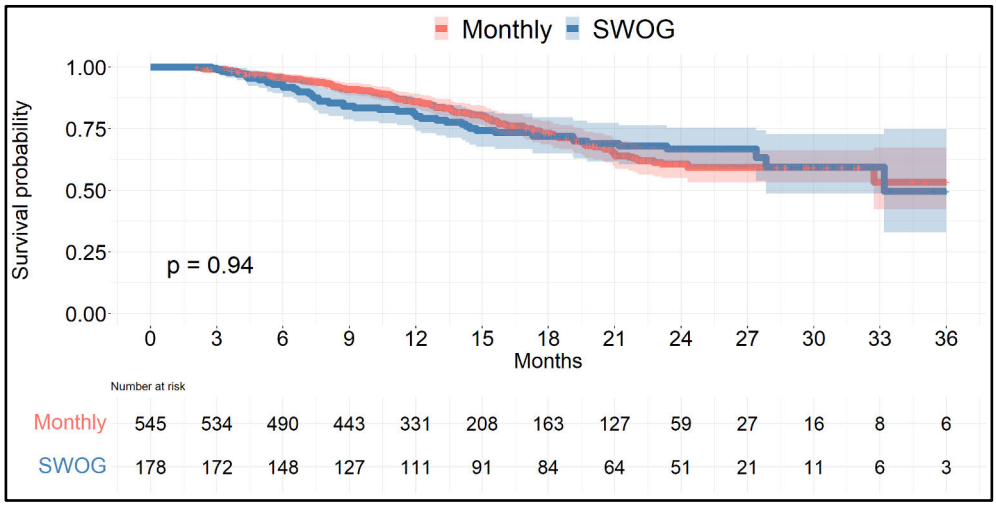

Figure 8. Kaplan-Meier curves of unplanned discontinuation of Bacillus Calmette-Guérin (BCG) course due to toxicity or suspected BCG infection among 723 patients receiving BCG maintenance therapy with monthly instillations (red curve) or according to the maintenance protocol of the Southwest Oncology Group (SWOG) (blue curve). Censored values (+) indicate events such as discontinuation due to BCG failure, poor compliance, other reason, or death unrelated to BCG.

The Kaplan-Meier plot estimating the survival for recurrences (A) and progression (B) are illustrated in **Figure 9**. In the whole cohort, disease recurrence was observed in 253/723 (35%) patients, of whom 198/545 (36%) were in the monthly maintenance group and 55/178 (31%) in the SWOG group. In a univariate Cox regression analysis, no significant difference in recurrence rate was observed (HR 0.87, 95% CI: 0.65–1.18, p=0.370). The 5-year RFS in the monthly and the SWOG group was 65% (95% CI: 61–69%) and 71% (95% CI: 64–79%), respectively (p=0.370). Disease progression was observed in 86/723 (12%) patients of whom 70/545 (13%) were in the monthly maintenance group and 16/178 (9%) in the SWOG group. In a univariate Cox regression analysis, there was no significant difference in progression rate (HR 0.72, 95% CI: 0.42–1.24, p=0.240). The 5-year progression-free survival in the monthly and the SWOG group was 89% (95% CI: 86–92%) and 91% (95% CI: 86–96%), respectively, (p=0.240).

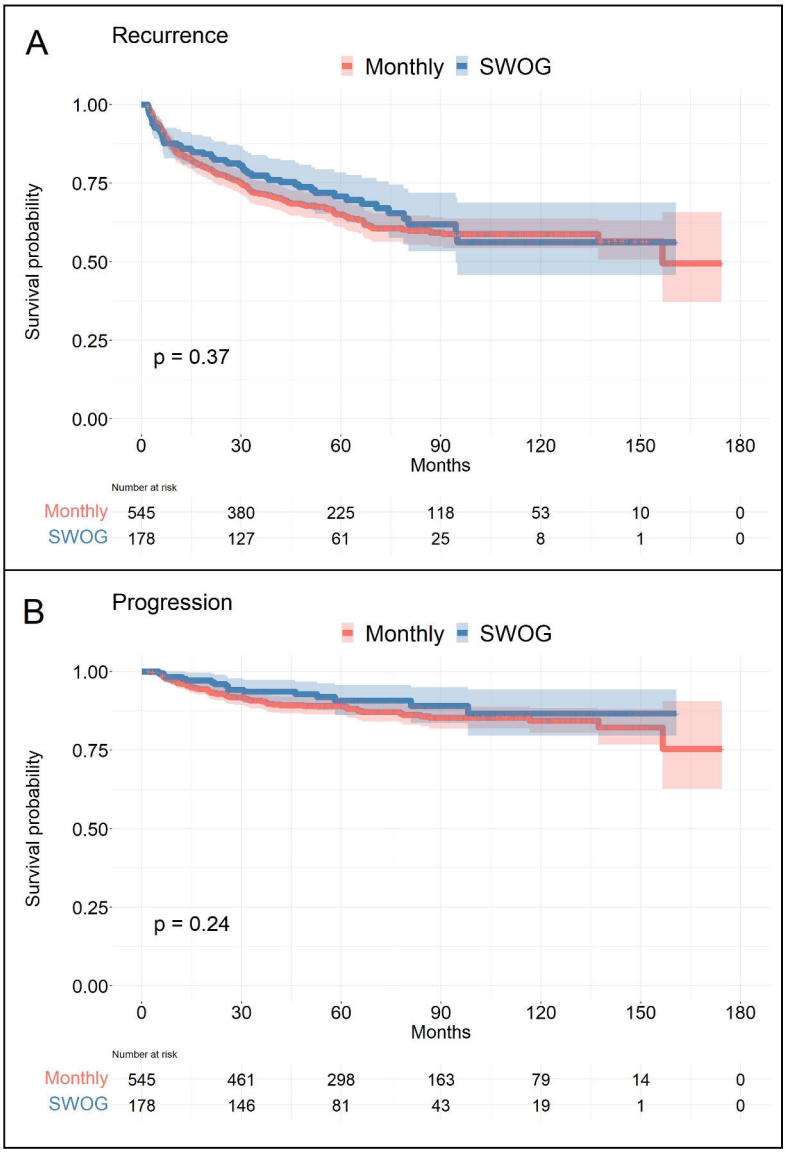

Figure 9. Kaplan-Meier curves for recurrences (A) and progression (B) of non-muscle-invasive bladder cancer (NMIBC) following Bacillus Calmette-Guérin (BCG) therapy among 723 patients receiving BCG maintenance therapy with monthly instillations (red curve) or according to the maintenance protocol of the Southwest Oncology Group (SWOG) (blue curve). Median follow-up time was 66 (IQR: 45-99) months. Censored values (+) indicate events such as lost to follow-up or death unrelated to urothelial cancer.

The Cox proportional hazards model assessing risk factors for discontinuation of BCG due to toxicity or BCG infection, recurrences, and progression is shown in **Table 22**. In the multivariable model, neither the maintenance schedule nor other independent

risk factors affected the risk of discontinuation of BCG due to toxicity. Independent risk factors for recurrences included a history of UTUC (HR 1.77, 95% CI: 1.15–2.73) and very high EORTC risk group (HR 1.91, 95% CI: 1.15–3.18), while risk factors for progression included a history of UTUC (HR 2.53, 95% CI: 1.24–5.19), >4 CCI (HR 1.90, 95% CI: 1.11–3.24), and high (HR 4.09, 95% CI: 1.23–13.60) or very high (HR 7.26, 95% CI: 2.01–26.17) EORTC risk group. On the contrary, performing re-resection appeared to be a significantly protective factor for both recurrence (HR 0.61, 95% CI: 0.45–0.84) and progression (HR 0.51, 95% CI: 0.29–0.91).

Table 22. Assessment of risk factors for discontinuation of Bacillus Calmette-Guérin (BCG) therapy due to toxicity or BCG infection, recurrence and progression using Cox proportional hazards model among 723 patients with non-muscle invasive bladder cancer.

	Discontinuation of BCG due to toxicity or BCG infection	**Recurrence**	**Progression**
Variable	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Gender			
Female	ref	ref	ref
Male	1.01 (0.64–1.60)	0.95 (0.65–1.37)	0.83 (0.44–1.55)
Smoking status			
No smoking	ref	ref	ref
Ex-Smoker	1.23 (0.84–1.80)	1.01 (0.73–1.38)	1.03 (0.58–1.84)
Current smoking	0.89 (0.57–1.38)	0.72 (0.49–1.05)	1.11 (0.59–2.07)
Charlson Comorbidity Index			
2	ref	ref	ref
3	1.02 (0.69–1.50)	1.03 (0.73–1.45)	0.59 (0.29–1.22)
>4	0.94 (0.63–1.39)	1.26 (0.91–1.75)	1.90 (1.11–3.24)
Immunosupression			
No	ref	ref	ref
Yes	0.74 (0.27–2.02)	1.06 (0.55–2.03)	0.29 (0.04–2.13)
Number of resections before BCG	0.98 (0.75–1.29)	1.16 (0.95–1.41)	0.98 (0.66–1.46)
Re-resection performed			
No	ref	ref	ref
Yes	0.76 (0.52–1.13)	0.61 (0.45–0.84)	0.51 (0.29–0.91)
History of UTUC			
No	ref	ref	ref
Yes	0.43 (0.17–1.05)	1.77 (1.15–2.73)	2.53 (1.24–5.19)
EORTC 2021 Risk group			
Low-Intermediate risk	ref	ref	ref
High Risk	0.94 (0.53–1.66)	1.19 (0.78–1.81)	4.09 (1.23–13.60)
Very High risk	1.20 (0.62–2.30)	1.91 (1.15–3.18)	7.26 (2.01–26.17)
Maintenance protocol			
Monthly	ref	ref	ref
SWOG	0.93 (0.65–1.34)	0.88 (0.64–1.21)	0.76 (0.42–1.38)

IQR = Interquartile range, HR = Hazard ratio, CI = Confidence interval, ref = Reference variable, UTUC = Upper tract urothelial carcinoma, EORTC = European organization for research and treatment of cancer

6 Discussion

6.1 Main results and discussion of the sub-studies

6.1.1 Summary

Intravesical BCG has been used for over 40 years in the treatment of NMIBC and its efficacy is well documented. However, there are several challenges related to the follow-up of BCG-treated patients due to the inflammatory mechanism of BCG. Adverse effects are common, and there is also a risk of serious complications and mortality. NMIBC is a burdensome and costly disease due to the need for regular invasive follow-up, which is further intensified when adjuvant BCG therapy is assigned along with multiple instillation visits and related side effects. Therefore, the number of unnecessary invasive procedures should be sought to minimise during follow-up. Although BCG is known to be associated with a risk of developing serious and even life-threatening BCG infections, estimations of definitive risk and clinical manifestations of BCG infections are based mostly on cohort studies with limited numbers of patients and show inconsistent results. Furthermore, given that definitions of BCG infection, diagnosis, and treatment are still poorly described, infections pose a clinical challenge when rarely encountered. Finally, different administration schedules of BCG are based on empirical experience, and a limited number of comparative studies are available for estimating differences between various treatment schedules in terms of toxicity and oncological efficacy.

In study I, we investigated the histological nature of bladder lesions biopsied for cystoscopic follow-up of BCG-treated patients. We found that more than half of the lesions were erythematous, leading to a suspicion of disease recurrence during follow-up, most of them being inflammatory manifestations of a BCG mechanism. We found that biopsies of erythematous lesions can be performed safely when guided by urinary cytology.

In studies II and III, we investigated the incidence, mortality, and clinical presentation of BCG infections related to BCG instillation therapy. In these studies, we reported a higher nationwide incidence of BCG infections than previously reported. In addition, BCG infections were associated with substantial mortality, emphasising the severity of infections and the importance of recognition of these

rare complications. We also investigated the timing of manifestation of infections and found that BCG infections may manifest even several years after completion of a BCG course.

In study IV, we compared BCG maintenance therapy applied with a monthly instillation schedule and the SWOG protocol in relation to toxicity and oncological efficacy. We found that the toxicity profile of the two instillation schedules was different, but eventually no difference in discontinued BCG courses or oncological efficacy was observed. Rather than the chosen maintenance schedule, patient characteristics and surgical treatment were associated with risk of recurrences and progression.

6.1.2 Discussion of study I

In study I, we conducted a retrospective analysis of all biopsied or resected bladder lesions during follow-up of 206 NMIBC patients treated with BCG instillation therapy. We also investigated the accuracy of urinary cytology in detecting malignancy in various types of bladder lesions.

In this study, we found that cytology adds limited value to the evaluation of papillary or flat tumours, as such morphology is highly suggestive of tumour recurrence and resection should be performed regardless of urinary cytology. However, more than half of the biopsied bladder lesions presented as erythematous lesions, with 90% revealing a benign histology, demonstrating the frequent occurrence of benign erythematous lesions. It is unclear how these lesions should be managed. Diagnostic options include 1) TUR-BT or biopsy under general anaesthesia, 2) biopsy in an outpatient clinic, and 3) follow-up. Cytology had a PPV of 23% and NPV of 94% among patients with erythematous lesions, suggesting that biopsy or TUR-BT is indicated when the cytology is positive but is not routinely needed if it is negative. However, erythematous bladder lesions should not be categorically overlooked, since 10% may harbour significant malignancies including muscle-invasive tumours and T1 tumours. Therefore, a proper TUR-BT under general anaesthesia should be undertaken if the biopsy shows malignancy.

Several studies have investigated the need for routine bladder biopsy after an induction course of BCG to confirm the treatment response before proceeding to maintenance therapy. (Dalbagni et al., 1999; Guy et al., 2006; Highshaw et al., 2003; Skemp & Fernandes, 2002) These studies have shown that erythematous lesions are of a benign nature in the presence of negative cytology after an induction course of BCG. In fact, bladder inflammation and granuloma formation after an induction period of BCG have been associated with an improved BCG response, underlining the pertinent nature of these lesions related to an inflammatory mechanism of BCG. (Jallad et al., 2014) A retrospective study and cumulative analysis by Swietek et al.

estimated the predictive value of individual lesion type in cystoscopy performed after an induction course of BCG. (Swietek et al., 2012) The authors found that the risk of malignancy of erythema was 56–59% with positive and 9% with negative cytology, concluding that overall, a routine biopsy is not needed after an induction course of BCG and is optional if only erythema is found while the cytology is negative.

In our study, we analysed all biopsies taken throughout follow-up after BCG with a median follow-up time of 3 years, and found that erythematous lesions are frequently encountered during BCG treatment and around 1 year after completion of BCG. However, benign erythematous lesions were encountered even several years after BCG treatment. Our results showed that the proportion of malignancies found in erythematous lesions is equal during the BCG course and later during follow-up, suggesting that the diagnostic strategy, as concluded by Swietek and co-workers, is safe also later during follow-up. (Swietek et al., 2012)

The limitations of this study include the retrospective design, which may have affected our results. The appearance of each lesion, which was the most essential study variable, was categorised depending on the initial assessment described on medical charts. Since it was not possible to re-review the cystoscopic findings, mis-categorisation of lesion types may have occurred. In addition, as there were no standard protocols for systematic biopsies, each being decided individually by the treating urologist, we did not have data on lesions that were not biopsied. This may have caused overestimation of the percentage of malignancies found among erythematous lesions.

6.1.3 Discussion of studies II and III

In study II, we found that the cumulative nationwide incidence proportion of infectious complications in Finland during 2000–2016 was 1.9% among patients treated with BCG instillations and increased during the study period to 2.5%. The mortality of systemic BCG infections is high, up to 17.5%, reflecting the severity of the disseminated manifestation of the disease. Local infections were mostly initially resolved with a 96% survival rate, but a significant proportion (21%) of patients with local BCG infections developed long-term adverse effects such as benign hydronephrosis or contracted bladder later during follow-up, demonstrating the significance of these infections as well. In study III, we found that although most systemic BCG infections develop during the weeks after instillation and mostly during the first year of maintenance therapy, 13% of systemic infections presented after a latency of more than 1 year after the last instillation, the longest being 9.6 years from instillation therapy to pulmonary BCG infection. Among local infections, an early and late infection type may be identified mostly in the epididymitis, which

may present either as subacute swelling of the epididymis or as a tumour-like enlargement even several years after instillation therapy. However, the time point of presentation of BCG cystitis is more heterogeneous, and no clear patterns could be identified among them.

Incidence of BCG infections

The cumulative incidence proportion of BCG infections during 2000–2016 was 1.9%, with a rising annual incidence of up to 2.5% during the latter half of the study period calculated between 2006 and 2016. Previous estimations of incidence of BCG infections have varied by 1.3–4.3% in retrospective single-centre cohort analyses (Gonzalez-Del Vecchio et al., 2016; Nummi et al., 2019; Perez-Jacoiste Asin et al., 2014; Steg et al., 1989) and by around 1% in the Danish register-based nationwide study. (Larsen et al., 2019) Our result thus falls between these previous estimations. However, compared to the Danish nationwide study with the most similar study design, the incidence proportion reported in our study is substantially higher, especially during the latter half of the study period during which we observed a 2.5% incidence proportion. Unfortunately, as the Danish study did not report the annual incidence, the result of rising incidence cannot be compared.

The underlying reason for the rising incidence proportion in Finland is unclear. One possible explanation is that the national practice for registration of tuberculosis changed after 2006, when it became mandatory to register tuberculosis treated with antituberculosis medication even without microbiological verification. This change may also have increased the reporting of clinically diagnosed BCG infections.

Another explanation may be increased knowledge of BCG infections and more active testing of mycobacterial cultures in patients suffering from symptoms suggestive of BCG infections. One downside of our study is that we could not analyse any risk factors for BCG infections, since we lacked clinical data on patients who were treated with BCG but did not develop a BCG infection during the study period.

The effect of used strain on infections remains unclear, since the instilled strain was not indicated on medical charts, nor did we have that information for patients who did not develop BCG infections. During 1996–2016 there were two strains available in Finland: BCG TICE during the earlier part of the study period and BCG RIVM during the latter. The shift from BCG TICE to BCG RIVM occurred during 2002–2006, when sales approval was granted for BCG RIVM. Looking at the availability of TICE and RIVM strains in Finland, the incidence of BCG infections was lower during the former and higher during the latter. However, the true causality cannot be assessed, as it may be affected by several cofounding factors that we did not have access to. In addition, during 2015, around 20–30% of BCG doses delivered

to Finland were BCG TICE because of a shortage of BCG RIVM, and during that time the incidence was the highest of the entire study period, with 11 infections. Previous studies have not found any evidence of differences either in toxicity or efficacy between different strains in intravesical use (Boehm et al., 2017), although one retrospective study has suggested that TICE was associated with a lower risk of moderate to severe complications compared to BCG RIVM and BCG Moreau. (Krajewski et al., 2018) In the study by Larsen et al., the strains used in the study population included the Danish strain and BCG RIVM, but also that study could not address the effect of strain on BCG infections.

Interestingly, a Finnish nationwide register study investigating the risk of BCG vaccination-induced osteomyelitis among Finnish infants in 1960–1988 showed that the incidence of tuberculous osteomyelitis after vaccination varied substantially, between 2.7 and 72 infections per 100 000 vaccinated infants with a mean incidence of 6.2/100 000 infants during the latter part of the study period. (Kröger et al., 1994) In this study, a time association with the availability of various BCG strains and varying incidence of BCG osteomyelitis was observed, and the authors concluded that this incidence depended on the different strains of BCG. These results also imply that the overall risk of BCG osteomyelitis after BCG vaccination has been substantially higher in Finland compared the Japanese population, where an incidence of 0.3 BCG osteomyelitis cases/100 000 vaccinated infants has been reported. (Okuno et al., 2022) The disparity corresponds to a roughly 20-times higher incidence in Finland. Inspired by this difference, a Finnish case-control study investigated single nucleotide polymorphisms (SNPs) in the Toll-like receptor (TLR) subfamily among patients treated for BCG osteomyelitis after BCG vaccination. They found that compared to the control group of the healthy Finnish population, SNPs in the TLR subfamily were associated with a risk of BCG osteitis. (Poyhonen et al., 2015) TLRs are pattern-recognition proteins that play a key role in activating innate immunity responses, and it can be hypothesised that polymorphisms in genes regulating the transcription of these proteins might affect the immunological response after intravesical BCG instillation. One Tunisian prospective study sought to investigate this hypothesis but failed to identify the polymorphisms of interest in the TLR2 subfamily in the recruited study population. (Ajili et al., 2010) However, other polymorphisms of the genes regulating innate host response mechanisms have been connected to BCG vaccination safety and to efficacy of intravesical BCG against BC. (Lima et al., 2015; Messina et al., 2020) As the prevalence of these polymorphisms vary between populations, they may also partly account for the differences seen in national BCG infection rates.

However, BCG infections may also be linked to BCG administration-related factors affecting the natural barrier of the urothelium, such as traumatic catheterisation, urinary tract infections, and bladder procedures. Indeed, in our study

population, catheterisation difficulties were identified in 21 (25%) patients, which probably contributed more to their developing a BCG infection than did their genetic makeup. This may also partly explain the substantial male predominance (98%) of the study population, as catheterisation difficulties are very rare among female patients. Although BC prevalence is higher among men, it cannot explain the significant disparity of the risk of BCG infections between male and female patients observed in our study.

Mortality of BCG infections

In the cohort of 87 patients, nine patients died after being diagnosed with BCG infection, resulting in an overall mortality of 10%. Seven patients died after systemic infection, resulting in 17.5% mortality in systemic infections. Among these, three deaths occurred before antimycobacterial treatment could be initiated, reflecting the diagnostic challenge of identifying systemic BCG infection as being related to BCG instillations and the importance of prompt initiation of antimycobacterial treatment during these events. The two cardiac deaths among patients with local infections, however, were unlikely to be related to the BCG infection, even though they did occur during the antituberculosis regimen of these patients. Our study adds important knowledge on the mortality of BCG infections, since previous knowledge on this has been based on systematic reviews of published case reports and patient series, representing a highly selected patient population. (Cabas et al., 2021; Perez-Jacoiste Asin et al., 2014) Among these studies, Perez-Jacoiste et al. reported an overall mortality of 5.4%, and 9.9% mortality in disseminated infections, thus showing a notably lower mortality rate compared to our results. Identified risk factors for mortality included patient age >65 years, disseminated infection, and vascular involvement. Cabas et al. identified 16 deaths among 303 reported cases of BCG infections, yielding a 5.3% overall mortality, and further identified vascular involvement as the most severe complication, with 15.7% mortality among these patients. A systematic review investigating the case reports of vascular BCG infections found a 10% mortality risk. (Ho et al., 2022) However, vascular involvement was a very rare manifestation in nationwide cohorts, as only one and two cases were identified in our and the Danish cohort, respectively. This reflects the selection of cases in pooled analyses involving data from case reports, as more severe cases are more likely to be published. Nonetheless, the overall reported mortality in our study population was twice as high (10% vs. 5.3-5.4%) and among systemic infections also higher (17.5% vs 10%) compared to previously reported mortality rates.

Clinical presentation of BCG infections

Given the mortality risk associated with BCG instillations, it is imperative to identify a developing BCG infection in a patient treated with BCG to promptly initiate antituberculosis treatment. However, identifying BCG infections is not always evident due to their rarity and varied clinical presentation. One of the varying characteristics of BCG infections is the manifestation time point of infection in relation to the administered BCG instillations. Gonzalez et al. described an early and late presentation of BCG infections, where 43% of them may present with a latency of 1 year or more after initiation of BCG, most (60%) of the late infections being local. (Gonzalez et al., 2003) The latency of BCG infections was studied by Cabas et al. in a larger systematic review including 307 patients, in which the authors found that penile lesions, pulmonary infections, and granulomatous hepatitis were the most common sites for early infections and testicular, vascular, and muscular infections were more common among late infections. (Cabas et al., 2021) We studied the latency calculated from the last BCG instillation and found that 13% and 20% of systemic and local infections, respectively, presented more than 1 year after the last BCG instillation, demonstrating the rare manifestation of post-instillation BCG infections. Indeed, these late infections may not necessarily be recognised as being related to the patient's history of BCG instillations if the patient suffers from fever, fatigue, and general malaise after several years following BCG therapy. However, it should be emphasised that these late BCG infections were observed only in five patients with systemic infections and 12 patients with local infections over a 20-year study period in Finland. Thus this represents a rare presentation type, most BCG infections being diagnosed during the first year of the maintenance period. In our study, the median (IQR) number of instillations before development of infection was 10 (6–14), which is consistent with previously published studies (Cabas et al., 2021; Gonzalez et al., 2003; Larsen et al., 2019)

Treatment of BCG infections

Treatment of BCG infections relies on a combination of isoniazid, rifampicin, and ethambutol. It should be noted that *Mycobacterium bovis* is resistant to pyrazinamide and should be avoided. (Raynaud et al., 1999) In our study cohort, the treatment of systemic infections involved prompt initiation of an isoniazid-based regimen as soon as evidence or a suspicion of systemic BCG infection arose. However, the diagnosis and definition of local BCG infections is not as clear. Thirteen patients with a positive urinary culture of *Mycobacterium bovis* from a sample taken due to transient symptoms of cystitis were not treated, since their symptoms resolved spontaneously before any antituberculosis medication was initiated. These patients did not develop any later complications and some of them continued BCG instillations. Most likely

these symptoms were normal side effects of BCG that should not be considered BCG infections. In study II, we defined these as clinically insignificant and excluded them from the analyses, since we wanted to estimate the incidence proportion of BCG infections with significant treatment implications. The good outcomes of these patients also demonstrated that a positive sample of *Mycobacterium bovis* in urine should not be automatically interpreted as a BCG infection and treated with an isoniazid-based regimen. Given the low mortality risk associated with local BCG infections, patients with symptoms suggestive of BCG cystitis should first be given non-steroidal anti-inflammatory medication (NSAID) or fluoroquinolones, or should be assigned to intravesical instillations of an anaesthetic agent before proceeding with isoniazid-based treatment. (Palou, Rodríguez-Villamil, et al., 2001; Witjes et al., 2008) However, BCG cystitis cannot be overlooked, since there is a risk of contracted bladder associated with it that may warrant cystectomy, although the role of isoniazid, rifampicin, or ethambutol in reducing the risk of contracted bladder is unknown. (Medina-González et al., 2022)

Antituberculosis medication consisting of isoniazid, rifampicin, and ethambutol is a potentially toxic regimen. In our patient population, the reported rates of side effects such as elevated liver enzymes, urticaria, neuropathy, and ethambutol-related optic neuropathy are consistent with previously reported incidences of adverse effects from anti-tuberculosis drugs. (H. Choi et al., 2022; Tostmann et al., 2008) Therefore, antituberculosis medication should be reserved only for patients who have developed a clinically significant infection. Among patients with systemic infections the indication for treatment is obvious, but given the substantially large proportion of BCG cystitis in our cohort compared to previous cohort studies, and variable practices among patients suffering from it, indications for isoniazid-based treatment of local BCG infections need further clarification.

Strengths and limitations of studies II and III

The strength of our study is the nationwide nature and comprehensive retrieval of microbiologically verified BCG infections in Finland over the last 20 years. The main limitation of study II is that BCG infections without microbiological verification may not be comprehensively registered in Finland. For this reason, our study most likely underestimates the incidence of BCG infections. In addition, as we did not identify all patients with clinically diagnosed BCG infections, the analysis of mortality may be compromised. Another limitation of study II is potential inaccuracy in estimating the number of patients treated with BCG, as no register is being held of all BCG instillations in Finland. Indeed, throughout 2000–2005 the data on BCG vial consumption varied substantially, creating inaccuracies in determining incidence for the earlier part of the study period. However, comparing the estimated

annual number of patients treated with BCG in 2006–2016 with the national incidence of BC retrieved from the FCR, our estimate suggests that 30% of all patients with BC would have been treated with BCG, consistent with the natural history of BC and current guidelines. (Babjuk et al., 2022; Pasin et al., 2008) In view of these limitations, the results on incidence and mortality should be interpreted with some degree of caution, and emphasised more than is discussed in the original publication of study II.

Other limitations of study II include the retrospective design and lack of clinical data on patients who received BCG and did not develop BCG infection. Thus our study cannot assess any risk factors for BCG infections. However, given the low incidence of BCG infections a retrospective design is justified, as it allows to estimate the scale of a disease entity in a large population over a long time period. The greatest limitation of study III is also related to the retrospective design, which limits characterisation of the spectrum of symptoms and biological manifestations of BCG infection. Thus, the latency reported in the study overestimates the true latency of infection, as the biological development of infection had occurred earlier than was noted on medical charts. However, as we used definitive dates of instillation, sampling of mycobacteria, and initiation of antituberculosis medication, our study accurately reflects the practical timeline of events during the diagnostic and therapeutic path of these patients.

6.1.4 Discussion of study IV

In study IV, we showed that there is no difference in the rate of discontinuation of BCG courses due to toxicity when the maintenance treatment is applied with monthly instillations or according to the SWOG protocol. Fewer recurrences and progressions were observed in the SWOG group, but the difference was not significant in univariate or multivariate analysis. Rather than the chosen maintenance schedule, patient characteristics and oncological factors were shown to significantly affect recurrence and progression rate, such as history of UTUC, EORTC risk group, and CCI, which were all independent risk factors for progression. In contrast, re-resection appeared protective for both recurrences and progression, highlighting the importance of meticulous surgical eradication of bladder tumours before proceeding with any BCG instillation regimen.

Our study demonstrated a 5-year RFS in the monthly and SWOG group of 65% (95% CI: 61–69%) and 71% (95% CI: 64–79%), respectively, with no significant difference between the groups (p=0.370). Our results are thus consistent with the results of randomised trials by the SWOG and Finnbladder groups. (Järvinen et al., 2009; Järvinen et al., 2015; Lamm et al., 2000; Marttila et al., 2016) Two earlier studies directly comparing monthly instillations and the SWOG protocol each had a

small number of patients and observed events. (Gupta et al., 2020; Lashay et al., 2021) Gupta et al. reported the results of a recent prospective randomised trial assigning 90 patients to receive maintenance therapy either according to the SWOG protocol for 3 years or monthly instillations for 1 year. The authors observed six recurrences among patients allocated to the SWOG arm, five recurrences in the monthly instillation arm, and one progression of disease in both arms. Two patients in each arm discontinued treatment due to toxicity, showing no difference in terms of efficacy or toxicity. (Gupta et al., 2020) Lashay et al. reported similar results in a retrospective study of 80 patients with 18% and 25% recurrence rate in the monthly and SWOG arms, respectively (p=0.340). (Lashay et al., 2021) Our study reports far more events, with 253 recurrences and 86 progressions observed, and confirms the conclusion of these previous reports that in terms of efficacy and tolerability, it makes no difference which maintenance schedule is used.

In a prospective randomised trial (NIMBUS) by Grimm et al., the authors compared a reduced number of induction and maintenance instillations during a 1-year maintenance therapy with the standard 1-year SWOG protocol but found that reducing the number of instillations negatively affected efficacy. (Grimm et al., 2020) In addition, Nummi et al. demonstrated in a retrospective study that patients who discontinued BCG due to toxicity were at greater risk of recurrence and progression, with most BCG courses being interrupted during the first year. (Nummi et al., 2019) These results suggest that completion of the first year is the most important to maintaining the therapeutic effect of BCG. However, despite more instillations being administered during the first year in the monthly maintenance group, the difference did not translate into better oncological results in our study. Therefore, the nine maintenance instillations of the SWOG protocol during the first year seems sufficient to maintain the potential efficacy of BCG. (Lamm et al., 2000) It is also worth noting that in the SWOG protocol the BCG consumption is divided over a longer period, hence more BCG courses may be simultaneously initiated during times of BCG shortage.

The main limitation of this study is the retrospective design, which precluded us from investigating the effect of maintenance on the quality of life and irritative symptoms not leading to treatment discontinuation. For this reason, we used a definitive endpoint of unplanned discontinuation of BCG due to toxicity or BCG infection, which was clearly identifiable from medical charts. However, multiple factors may have affected the decision to discontinue, especially among elderly patients and those with comorbidities, possibly creating bias. Furthermore, patients in the monthly maintenance group were older than those in the SWOG group (p=0.019), possibly affecting the results, as it has earlier been shown that the oncological efficacy of BCG therapy is poorer among elderly patients. (Inoue et al., 2022; Oddens et al., 2014) However, age does not seem to affect the risk of

discontinuation of BCG due to toxicity. (Krajewski et al., 2020; Oddens et al., 2016) As we compared the groups using the EORTC 2021 risk tables, which apply multiple factors including age and grade, the baseline risk of progression in the study groups was similar (p=0.700). (Sylvester et al., 2021) Nevertheless, the difference in age between groups is a limitation of our study, and the analysis of efficacy needs to be interpreted with some degree of caution. Finally, we did not have the individual data on used BCG strains; therefore the distribution of RIVM and TICE between study groups cannot be estimated. However, BCG TICE represents less than 5% of BCG instillations in this cohort; therefore, the effect of BCG strain in this study is likely to be minimal.

6.1.5 Implications and future perspectives

BCG treatment has been the first-line adjuvant treatment option for intermediate and high risk NMIBC for over 40 years.

In this thesis, we described a common occurrence of erythematous bladder lesions during follow-up, which are often biopsied because of uncertainty in the cystoscopic findings after BCG instillations. Although urinary cytology yielded a fairly good NPV, there is still room for improvement given the importance of detecting BCG-unresponsive disease. As novel biomarkers are sought to reduce the need for cystoscopies during follow-up of NMIBC, they may also provide a better predictive value in evaluating the risk of malignancy in erythematous bladder lesions and thus reduce the need for repeated biopsies and unnecessary TUR-BTs in the future. (Laukhtina et al., 2021) The accuracy of novel biomarkers should thus be validated meticulously among BCG-treated patients.

In the second part of this thesis, we clarified the incidence and mortality of BCG infections. Although BCG infections are rare complications, it is important to recognise that BCG treatment itself carries a risk of dying. In addition, isoniazid-based treatment of BCG infections is a long and burdensome regimen and is linked to substantial side effects. (H. Choi et al., 2022; Tostmann et al., 2008) These risks should be considered when balancing between the harms and benefits of BCG at individual level. We emphasise that BCG treatment should be indicated primarily for patients who are at risk of progression, since progression is a path to metastatic disease and BC-related mortality. Indeed, it has been well established that BCG applied with maintenance therapy is the most effective available treatment modality in reducing the risk of progression of NMIBC and is the gold standard treatment for high-risk patients. (Bohle & Bock, 2004; Sylvester et al., 2002) However, a 1-year regimen of BCG is currently indicated as the first-line treatment also for intermediate-risk NMIBC, which carries primarily a high risk of recurrence but also a low risk of progression, especially among patients with recurrent and multiple LG

tumours. (Babjuk et al., 2022) Since these patients are at low risk of BC-related death, the risk-harm balance of BCG among these patients is debatable. Although there is some controversy in the results of meta-analyses comparing intravesical chemotherapy and BCG in terms of recurrences, it has been suggested that intravesical chemotherapy has a comparable efficacy in reducing recurrences in intermediate-risk NMIBC. (Böhle et al., 2003; Laukhtina et al., 2022; Shelley et al., 2004) In addition, since intravesical chemotherapy does not harbour a risk of instillation-related mortality like BCG, its role should be highlighted more clearly in guideline recommendations regarding treatment of intermediate-risk NMIBC. Interesting evidence is also emerging on the use of sequential gemcitabine and docetaxel among patients with BCG-unresponsive disease, which may provide a safe and effective alternative for selected BCG-naïve NMIBC patients as well. (Kawada et al., 2022; McElree et al., 2023) As stated in the guidelines, BCG should not be offered to patients with low-risk NMIBC. (Babjuk et al., 2022)

Since BCG infections are rare, awareness of the diagnosis and treatment of local BCG infections was variable in our cohort. In the latest updates of the EAU guidelines, recommendations on management of BCG infections have emerged that both benefit patients and clarify the treatment of BCG infections. It is important to recognise that these guideline recommendations did not exist during our study period, thus the treatment of local BCG infections varied. Recommendations on the treatment of BCG infections in the EAU guidelines are largely based on expert opinions and a low level of evidence; information on how they were treated in Finland in 1996–2016 may aid in updating these recommendations.

In the third part of the thesis, we compared two widely utilised treatment schedules of BCG. Although there were no statistically significant differences between the groups, it should be noted that the SWOG protocol resulted in a slightly sparer consumption of BCG, while a trend towards better efficacy was observed with the SWOG protocol. BCG shortage is a global crisis for NMIBC patients; therefore, our results suggest that although monthly maintenance is an acceptable option, the SWOG protocol should be considered as the standard schedule of maintenance BCG. (Lamm et al., 2000)

While new agents are emerging in the treatment of BCG-unresponsive disease, some of these drugs could possibly replace BCG in treatment-naïve high-risk NMIBC as well. Since many of these promising novel agents such as pembrolizumab and adenovirus vectors are immune-oncological agents, similarities in the challenges related to the follow-up of patients after these treatments may be expected. (Balar et al., 2021; Boorjian et al., 2021) Thus, it is imperative to seek evidence that the new drug aiming to replace BCG can demonstrate a beneficial effect on progression and survival especially, should the adjuvant treatment itself harbour a risk of dying.

7 Summary/Conclusions

Study I described the histopathological natural history of post-BCG lesions. The study showed that papillary and flat tumours should be always removed by TUR-BT regardless of cytology grade. Erythematous lesions are mostly benign in 90% of cases throughout the post-BCG surveillance, although we emphasise their clinical significance since some may harbour significant malignancies. Hence, positive cytology warrants further examination including a biopsy of erythematous lesions whenever detected. Most unnecessary biopsies of erythematous lesions can be avoided with the guidance of urine cytology, and routine biopsy is not recommended if erythema is detected with negative cytology.

Study II demonstrated that the incidence proportion of BCG infections nationwide in Finland is 1.9% among patients with bladder cancer treated with BCG. BCG infections are associated with significant mortality, and it is important for clinicians to recognise this rare complication to achieve a favourable clinical outcome.

Study III confirmed the concept of early and late BCG infections, particularly amongst systemic infections. Despite the rarity, it is important to remember the possibility of BCG infection if the patient suffers from fatigue, fever, or pulmonary symptoms, even if BCG was administered several years earlier.

Study IV demonstrated that BCG therapy applied either with a monthly maintenance or the SWOG protocol resulted in a similar number of discontinued BCG courses, and no significant difference in efficacy could be observed. The status of the SWOG protocol as the standard BCG schedule need not be questioned, but monthly maintenance may be considered a valid option.

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