Clinical trials registration number: NCT02241122

Status: Patient enrolment ongoing

1 SUMMARY

Bladder cancer (BC) as the most common malignancy arising from the urinary tract continues to be a major health problem. This prospective non-randomized study will enroll 300 patients undergoing magnetic resonance imaging (MRI) at different stages of their diagnostic and therapeutical process. The enrolled patients with suspected BC (BC) based on cystoscopy will have their initial MRI examination before transurethral resection of bladder tumor (TUR-BT) and biomarker collection. After pathology review of the histological specimens, patients will be treated according to standard clinical practice. The second MRI examination will be performed before therapeutic intervention, if TUR-BT alone is not sufficient enough.. Neoadjuvant chemotherapy will be applied in high risk patients having muscle invasison, while intermediate risk patient – T1 high grade or carcinoma in situ patients - will be treated using Bacillus Calmette-Guerin (BCG) instilliations. After the completion of the neoadjuvant chemotherapy or BCG treatment, the patients will undergo the third MRI examination. Low risk patients will be followed by annual with MRI examination.

The aim the study is to determine the applicability of MRI and selected biomarkers for staging and therapy response monitoring of BC. In optimal case we can select patients who don`t need invasive cystoscopy as a monitoring method and can be followed by MRI-investigation only.

2 INTRODUCTION

BC (BC) is the most common malignancy arising from the urinary tract. Endothelial cancers originating from the urothelium present 95 % of BC. Common risk factors include tobacco smoking, and previous radiotherapy of the pelvic region. At the time of diagnosis 70% of patients have non-muscle invasive disease (NMIBC) and the disease is limited to the urothelium or submucosal layers. In 30 % of patients the cancer has already invaded to the detrusor muscle when the diagnosis is established. In 1/3 of patients presenting with muscle invasive disease BC also lymph nodes are involved (Prout Gr Jr et al 1979). Hematuria is most common symptom initiating diagnostic procedures, which include urine sample, urine

cytology and cystoscopy, which still remain the gold standard for visualization and diagnosis of BC. However, cystoscopy as an invasive procedure is uncomfortable and can potentially result in infection or stricture. Ultrasound has only a limited role in the diagnosis of BC (1). Accuracy of computed tomography (CT) depends on tumor size with only a limited accuracy for the early stages of BC (pT1 lesions) (2). In the study by Martingano et al. CT had overall diagnostic accuracy of 90% in 125 patients undergoing cystoscopy due to hematuria. However, the major limitation of CT is the evaluation of depth of possible invasion and metastatic lymph node involvement. Accurate staging and determination of metastatic lymph metastasis is of utmost importance in treatment decision planning and evaluation of therapy response. Standard treatment of muscle invasive BC is radical cystectomy, which in men includes removal of prostate and seminal vesicles and in women removal of the uterus and adnexa (Stenzl A et al 2005). Cystectomy is accompanied with the dissection of regional lymph nodes. Removal of more than 10-15 lymph nodes has been postulated to be both sufficient for the evaluation of the lymph node status as well as being beneficial for overall survival in retrospective studies (Wright JL et al 2008, Fleischmann A et al 2005).

Staging is based on the TNM grading, updated in 2009. T2-T4 tumors are muscle invasive and estimation of stage is dependent on properly performed transurethral resection of bladder tumor (TUR-BT). However there is a large discrepancy in clinical and pathological stage. Shariat et al. reported in their retrospective series pathological upstaging in 42% of patients and downstaging in 22% of patients.

Rapid treatment is mandatory. In a retrospective series of 153 patients a delay of treatment beyond 90 days of primary diagnosis caused a significant increase in extravesical disease (81 vs 52%) (Chang SS et al 2003). Neoadjuvant chemotherapy has been shown to increase overall survival from the disease in 5% of patients, but it is not universally utilized. The main challenge in the decision-making process of perioperative chemotherapy, and in the treatment of invasive or locally advanced BC in general is i) selection of patients to chemotherapy vs. upfront surgery and ii) estimation of the chemotherapy response.

In the current study we investigate the accuracy of MRI in detection and staging of local BC invasion and pelvic lymph node metastasis. Furthermore MRI and biomarkers will be used to identify patients more likely to respond to cisplatin-based neoadjuvant chemotherapy prior to radical cystectomy or to BCG treatment. In addition, we aim to investigate role of MRI for early detection of BC reccurence in low risk patients.

3.1 Anatomical magnetic resonance imaging (MRI)

Anatomical magnetic resonance imaging (MRI) provides better soft tissue contrast compared to CT. On T1-weighted images, the bladder tumor typically has a low-to-intermediate signal intensity similar to the bladder wall. Commonly, bladder tumors are easier to be depicted on T2-weighted images with intermediate signal intensity between that of low signal intensity of bladder wall muscle and higher signal intensity of urine.

3.2 Diffusion weighted imaging (DWI)

To measure diffusion properties of tissue, the Stejskal–Tanner imaging sequence is used (Stejskal and Tanner, 1965). Information about proton diffusion is acquired by applying motion-encoding gradients, which cause phase shifts in moving protons. This phase shift depends on the quantity as well as direction of movement. Several different mathematical models could be applied to quantify DWI signal decay depending on number and range of used b-values.

Due to restricted diffusion, BC has lower apparent diffusion coefficient (ADC) values (using a monoexponential fit) than normal tissue. In large prospective study including 106 patients DWI was significantly more accurate than anatomical MRI in both organ confined disease (69.7% vs. 15.1%) as well as >pT2 disease (92.5% vs. 80.1%) (3). In addition, ADC values could be useful in identifying high grade tumors (4).

Anatomical MRI is more accurate in detecting lymph node metastasis compare to CT (5). The addition of DWI could potentially further increased diagnostic accuracy for lymph node metastasis.

3.3 Dynamic contrast-enhanced MRI (DCE-MRI)

Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) uses intravenously administered gadolinium chelate contrast agent to study tissue vascularization. To obtain reliable pharmacokinetic characteristics of normal and cancerous bladder tissue, sequences with high temporal resolution are desirable. Commonly in bladder imaging, DCE-MRI consists of a series of T1-weighted images covering whole bladder before and after rapid injection of a bolus of a low molecular-weight gadolinium chelate. However, optimization and in particular standardization of DCE-MRI data acquisition and analysis still remains a major challenge

DCE-MRI data can be analyzed in a qualitative, semi-quantitative or quantitative manner. In qualitative analysis, contrast enhancement curves are evaluated visually. BC tissue usually enhances rapidly followed by early wash-out. In semiquantitative analysis parameters such as time-to-peak, or the integral area under the curve could be calculated. The most widely used quantitative model for tracer kinetic evaluation of DCE-MRI is the Tofts model (6). This model extracts the concentration of a contrast medium in the plasma (C_p) derived from the arterial input function, fractional volumes of the extravascular extracellular space (V_e) and plasma (V_p), as well as the permeability of the blood vessels (K^{trans}). It is important to take into account that not every model might be valid for every cancer tissue type. The Tofts model assumes that the contrast medium is not perfusion limited and that signal contributed by the tissue blood volume has a negligible effect on the resulting signal compared with the signal arising from contrast medium in the interstitial space**.** Inappropriate quantitative modeling might lead to overestimation of K^{trans} (7).

Nevertheless, quantitative model DCE-MRI modeling has the potential for assessing response to neo-adjuvant therapy.

3.5 Bladder biomarkers

Biomarker is a patient originated substance, which is in oncology utilized to estimate biological behavior of the cancer tissue. Information may be used e.g. to select patients to various treatments or to estimate risk of disease recurrence. Most common sources for biomarker analyses are blood, urine and tissue. Analyses may include e.g. gene and protein expression analyses.

In BC, pathways of carcinogenesis and biomarkers involved are partially described. The main steps in BC initiation are mutation in FGFR3 or p53 genes. Although many markers, e.g. proliferation index Ki-67, cell cycle indicators (p21, p16) and markers of apoptosis (caspases, survivin, bcl-2) have demonstrated prognostic significance, unfortunately there is no biomarkers in routine clinical use to date. There are several reasons for this but most significantly there has not been properly conducted prospective multicenter studies to validate existing marker data.

Biomarkers in BC would be extremely important in estimation of tumor aggressiveness to facilitate optimal patient selection to radical surgery and bladder preserving treatment alternatives.

In the current study, biomarkers derived from blood, urine and tumor tissue are investigated to estimate aggressiveness of tumors and also in the estimation of response to neoadjuvant chemotherapy. Methods include state-of-the-art analyses including immunohistochemistry, gene profiling, proteomics, metabolomics and tumor xenografting.

3.6 Study hypothesis

The aim of this prospective study is to assess the applicability of MRI including DWI and DCE-MRI in local and nodal staging of BC and therapy response monitoring. We hypothesize that MRI allows more accurate staging of BC including detection of lymph node metastasis. In addition, MRI could potentially detect those patients who are responding well to neo-adjuvant therapy or BCG and do not need extended surgical operation from those who are non-responders and require more aggressive surgical approach or 2^{nd} line chemotherapy. If the hypothesis is proven, MRI could be used to identify patients who need radical cystectomy after neo-adjuvant therapy.

In addition to imaging, we also investigate the role of tissue, urine and blood based biomarkers in the estimation of tumor biology and chemotherapy response. We hypothesize that methods such as immunohistochemistry, gene and protein profiling allow better patient selection to chemotherapy.

3 OBJECTIVES AND PURPOSE

Specific aims of this project are as follows:

i) To determine the sensitivity, specificity and accuracy of multiparametric MRI, (anatomical MRI, DCE-MRI, DWI) in staging of BC

ii) To assess the effect of neo-adjuvant chemotherapy and BCG treatment using MRI

iii) To develop quantitative models for DWI, DCE-MRI suitable for BC imaging

iv) To identify biomarker profiles which estimate tumor aggressiveness and response to neoadjuvant chemotherapy prior to radical cystectomy

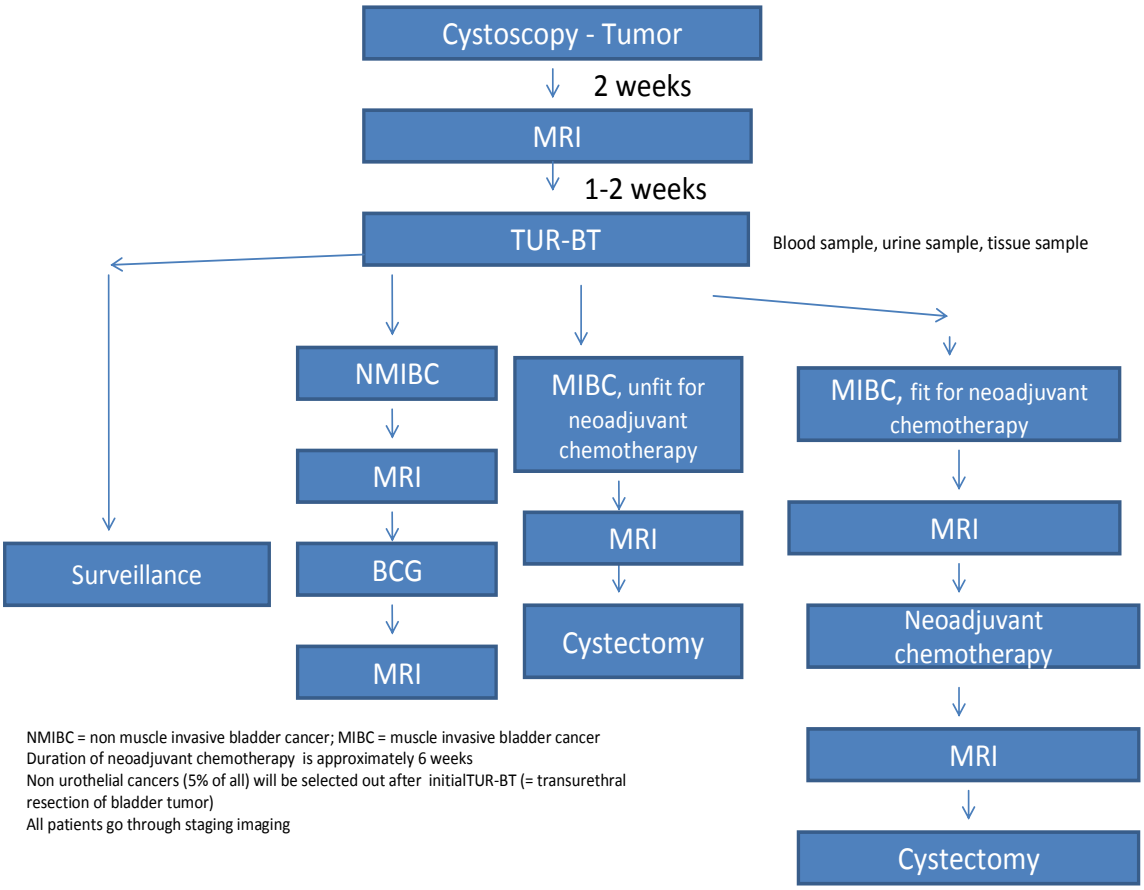
v) Mimic tumor tissues ex vivo (in vitro) in order to recapitulate the mutual interdependence of tumor (epithelial) and stromal counterparts, and to explore mechanisms of action and drug activity versus failure experimentally.

vi) To get in vitro cultures of BC associated fibroblasts and "normal" bladder fibroblasts.

vii) To isolate BC stem cells, based on the enzymatic degradation of the tissue and the use of cytometric cell sorting after antibody-based labelling of stem cell markers.

4 STUDY DESIGN

This is an open prospective study to obtain information on applicability of MRI for the purpose of staging and the value of MRI and biomarker profiling in therapy monitoring of BC will be assessed. The study principle is presented in Figure 1. Patients with BC will undergo MRI imaging and biomarker collection and analysis. After cystoscopical identification of possible BC, patients will undergo initial MRI examination. A patient is advised to avoid voiding 2 hour before examination, and drin about 500 ml of before followgn voiding. Bladder filling should be 150 +/- 50ml. After first MRI examination, TUR-BT will be performed by an experienced urologist. Neoadjuvant chemotherapy will be applied in high risk patients while intermediate risk patient will be treated using Bacillus Calmette-Guerin (BCG). After the completion of the neoadjuvant chemotherapy or BCG treatment, the patients will undergo the third MRI examination. Low risk patients will be followed by annual with MRI examination. During the operation, blood samples are taken via vein cannula, urine through resectoscope and specimen for biomarker analysis are derived from resected chips. The specimen collection will be undertaken in the operation room at the time of TUR-BT.



5 PATIENT SELECTION

6.1 Source population

Patients with suspected BC based on cystoscopical evaluation are candidates for the study. In addition to Turku University Hospital, patients from Pori central hospital will be included in the study. The total population in these three hospital districts is approximately 2,260 000 (Turku about 460000, Pori about 230000).

6.2 Number of patients

This study will include in total 300 patients with suspected BC based on cystoscopical evaluation.

6.3 Inclusion criteria

- Age: 18 to 85 years old

- Language spoken: Finnish or Swedish
- Suspected BC based on cystoscopical evaluation.
- Mental status: Patients must be able to understand the meaning of the study
- Informed consent: The patient must sign the appropriate Ethical Committee (EC) approved informed consent documents in the presence of the designated staff

6.4 Exclusion criteria

- No history of serious cardiovascular, liver or kidney disease
- Uncontrolled serious infection
- Contraindications for MRI (cardiac pacemaker, intracranial clips etc)
- Patient refusing radical cystectomy or chemotherapy or BCG
- Intravesical Bacillus Calmette-Guerin instillations within 6 months before the first MRI examination

7 MULTIMODALITY IMAGING

7.1 Pre-study evaluation

All patients will first be seen by an urologist who is responsible for initial patient information, blood chemistry, standard blood counts and kidney function tests. If the patient and the urologist agree on referral for TUR-BT, the patient is eligible for the study. After receiving both oral and written information about the study and after signing the Ethics Committee approved consent form the patient will be scheduled for MRI within 14 day from the referral. For the study logistics, please see Appendix 1.

8.3 MRI

MR imaging of the prostate will be performed on using a 1.5T or 3T Siemens system (Magnetom Aera 1.5T or Verio 3T, Erlangen, Germany) or 3T Philips system (Philips Ingenia, Best, Netherlands) or 3T Philips PET/MRI system (Philips Ingenuity, Best, Netherlands). No PET imaging will be performed. The body matrix coil in combination with a spine coil will be used for image data acquisition. Glucagon (0.2 mg - 0.5 mg, GlucaGen, Novo Nordisk A/S) will be injected subcutaneously into lower abdomen immediately prior to beginning the MR imaging examination to reduce peristalsis as a part of the normal clinical routine. T2-weighted anatomic imaging will be performed in axial and sagittal plane. Single-shot spin-echo echo-planar imaging sequence will be used for DWI. Axial dynamic contrast

enhanced images (DCE-MRI) will be acquired before, during and after injection of contrast agent. Contrast agent (0.1 mmol/kg Dotarem [Gueber, France] at institution A) will injected 30 s after beginning of the sequence through a peripheral vein at a speed rate of 2 ml/s via a mechanical injector (Spectris, Medrad, Indianola, USA). In total, 60 time points at temporal resolution of 6.9 seconds will be acquired using three-dimensional VIBE sequence (8). The total scan time will be approximately 25 minutes for each MRI examination.

8 CLINICAL TREATMENT COURSE AND FOLLOW UP

After the pathology review of TUR-BT specimens, patients will be treated according to standard clinical practices (low risk NMIBC – surveillance, high risk NMIBC – BCG, and muscle invasive BC – cystectomy with or without neoadjuvant chemotherapy according if the patient tolerates cisplatin based combination chemotherapy). MRI is repeated before and after BCG instillations. Instillation therapy consists of indiuction series; one instillation every week six weeks – and booster series – one weekly instillation 3 weeks every third month. Neoadjuvant chemotherapy consists of three cycles of cisplatin 75mg / m^2 and gemcitabine 1000mg in intravenous infusions. Patients who are selected to neoadjuvant group will go to MRI before and after the neoadjuvant chemotherapy . After chemotherapy radical surgery is performed. The surgery will include removal of bladder and in males removal of prostate and seminal vesicles and in females removal of uterus and adnexae. Extended pelvic lymph node dissection will be performed. The node dissection will include nodal tissue from common iliac, external iliac, obturator, internal iliac and presacral areas. Any suspicious nodes detected in MRI will be removed separately for pathological analysis. Blood, urine and tumor tissue will be collected for biomarker analysis at the time of TUR-BT and radical cystectomy.

After cystectomy, adjuvant therapy can be offered to patients presenting nodal infiltration or proven metastasis. Follow up schedule includes outpatient clinic visits every 3 months for the first year and semiannually thereafter. In addition to history and physical examination, follow-up visits include blood chemistry, renal function tests and imaging studies (CT of the abdomen and pelvis and chest x-ray). In addition, patients with symptoms of metastatic disease will undergo imaging as decided by the responsible urologist.

9 BIOMARKER STUDIES

9.1 Histopathology and immunohistochemistry

Histopathology and immunohistochemistry is carried out at Turku Center for Disease Modeling (TCDM), holding all expertise needed, with the help of clinical pathologist involved in the consortium. Tissues will be collected according to the needs of the techniques. For morphological analysis, formalin-fixed paraffin-embedded sections (or alternatively, tissue fixed in PaxGene, Qiagen) will be used. The sections will be stained with H&E. In addition, a tissue microarray (TMA) of each tumor type will be constructed. Tumor material will also be collected and stored for gene and protein expression analyses at the Turku Centre for Biotechnology (Finnish Microarray and Sequencing Centre (FMSC) and Translational Proteomics Research and Facility (TPRF). Last not least, small cores of tumor material will be transferred for temporary, short term ex vivo culture, of cancer-associated fibroblasts (CAF) and carcinoma cells of the bladder. Conditions for cell cultures of primary tumor materials are currently being optimized at VTT.

Immunohistochemical (IHC) staining will be performed with various diagnostic markers used for cancer diagnostics. Specific relevance will be given to the utility of the immunohistochemical markers in subtyping and response prediction of tumors. These include antibodies against different receptors used in BC. The same stainings have already been optimized for the use of ex vivo cultured tissue slices (after paraffin embedding), and are also available for short-term in vitro cultures of tumor cells and tumor-associated fibroblasts.

Virtual Microscopy resource: The slides will be digitized and stored in a database. If needed, the slides can be annotated (*e.g.* areas of specific morphological or immunohistochemical features marked). All participants can view the stored virtual slide images on a computer screen via the internet using the WebMicroscope software.

9.2 Gene profiling, bioinformatics and biostatistics

Molecular profiling will be carried out by gene expression micro arrays and discovery & targeted proteomics. Omics profiling is also expected to be of key importance to identify novel biomarkers for various stages of the disease, and to identify potential mechanisms of progression of the BC. All steps of the microarray analysis will be carried out at the FMSC using the Sentrix Human Illumina 6 V2 Expression BeadChips (Illumina, San Diego, CA). Normalization of the microarray data will be performed using the statistical software R package limma (http://www.R-project.org). Gene expression profiles can also be generated

from ex vivo cultured cells, such as primary short-term cultures of cancer-associate fibroblasts and bladder epithelial or urothelial carcinoma cells.

Several advanced bioinformatics methods will be utilized to combine the measured parameters from the models applied with the relevant information sources from public domain. The high-dimensional datasets from the genome-scale molecular profiling studies will be analyzed using novel data mining methods, which enable identification of multiple sets of molecular markers that effectively characterize distinct disease subtypes. Such resampling-based statistical procedures have shown to greatly improve both the sensitivity and specificity of the markers, as well as to allow identification of disease subtype-specific genes and proteins that have remained undetected in conventional statistical analyses (Hiissa et al., 2009). The identified panels of markers will be mapped into predictive machine learning models. Furthermore, we have ongoing collaboration with Laxman Lekuri, VTT, Finland, who analyses the correlations of steroid profiles with gene expression profiles. Validation of gene expression studies, e.g. by realtime RT-PCR, Northern – and Western blotting, or other standard methods in molecular biology, can be performed at VTT-MBT.

9.3 Protein profiling

Discovery proteomics will employ a novel iterative MS discovery method that achieves the highest attainable proteome coverage by first indexing measureable content, and subsequently guiding the MS to select those peptides that normally are undetected in standard MS analyses. This approach is required as cancer tissues vary greatly between patients, and consequently so will the proteome content. Virtual proteome maps of tissue content will be qualitatively and quantitatively compared, and analyzed for their similarities and differences.

Targeted analyses follows discovery analysis, with *a priori* knowledge of which proteins are analysed using highly parallel SRM measurements on up to several 100 proteins. Absolute quantitation can be performed on any number of patients. In addition to findings from discovery proteomics, targeted analysis can be performed on those proteins for which IHC analyse is performed and on discoveries from gene expression measurements. The SRM analyses will be performed for tissue, urine and serum samples

9.4 Tumor xenografts

"Patient-derived xenografts" (PDX) of BC will be developed for investigation developing or testing drug compounds for "personalized" therapies of BC. Small pieces of

tumor tissue will be implanted a) under renal capsule of Nod/Scid mice for serial reimplantation and propagation of tumor tissue mass, which can then be used for morphological and molecular analysis or for orthotopic implantation in immune deficient mice, short-term tissue cultures, which allow mechanistic studies and f.i. testing drug efficacies. b) orthotopically in bladder of immune deficient mice without preceding implantation under renal capsule. The group has a long-term experience on subcutaneous, orthotopic and intratibial xenograft models

9.5 Validation of personalized 3D co-cultures

As a part of this study we will collect tissue samples from surgically removed bladders to develop personalized 3D cell culture model of BC. The validation of personalized 3D co-cultures compared with the original tissues will be based on e.g. local invasiveness, expression and localization of biomarkers such as AMACR, E-cadherin, vimentin, αSMA, p63, matrix metalloproteinases, as well as proliferation markers (Ki67, PCNA, BrdU-incorporation), apoptosis related markers (TUNEL, caspase-3, survivin), activation of signaling pathways such as PI3K-pathway, and drug resistance. Small libraries that contain primarily FDA-approved anti-cancer drugs are currently available in the host laboratory at VTT. All of these characteristics can be assessed both in organotypic models utilizing in vitro experimentation and in histological tissue sections with additional clinical data linked to the patient material.

9.6 Interplay between BC cells and tumor stroma

Immediately (as soon as possible) after the bladder tissue has been evacuated from the patient small pieces of tissue should be cut out both from the tumor and a corresponding "healthy" bladder. Small tissue pieces will be used to culture fibroblasts and/or to isolate bladder stem cells. For successful cell culture it is critical to avoid the contamination by microbes.

10 ADVERSE EVENTS AND RADIATION EXPOSURE

Since MRI does not deliver radiation, the risk for adverse events in properly selected patients is associated only with administration of paramagnetic contrast agents which may occasionally cause renal problems, particulate in patients with a renal disease (9). Only patients with normal kidney function tests will be included. The presence of claustrophobia

will be evaluated in the screening phase and patients with serious symptoms will be excluded from study.

Biomarker specimen collection will not cause any additional risks for patients as the specimens are collected in the operation room at the time of surgery (TUR-BT and cystectomy). Blood samples are taken via vein cannula which is inserted for anesthesiological purposes and urine is collected through resectoscope at the time of TUR-BT or from catheter, which is inserted at the time of cystectomy.

11 ETHICS

11.1 Ethical considerations

The study will be conducted in compliance with the current revision of Declaration of Helsinki guiding physicians and medical research involving human subjects (59nd World Medical Association General Assembly, Soul, Korea, 2008).

11.2 Ethical Review

Prior to commencement of this investigation, the study protocol, patient information sheet and informed consent form will be submitted for approval to ethics committee of the Hospital District of Southwest Finland. The principal investigator is responsible for obtaining approval of the ethics committeefor the study protocol including its appendices. The Principal Investigator shall file all correspondence with the ethics committee in the Investigator`s Study File.

11.3 Potential risks and benefits to study subjects

The risks for the patient inflicted by participation in study are deemed minimal. Participating patients potentially benefit of a more exact diagnosis. It is also possible that MRI may disclose metastatic disease unseen with conventional staging. Clearly benefits of participation outweigh risks for patients eligible for study. No additional risks are caused for patients from the biomarker specimen collection.

12 DATA ANALYSIS

12.2 Quantitative analysis of DCE data

K_{trans} maps will be automatically generated from up-sloop part of DCE curve using a dedicated oftware package. Dynamic contrast enhanced imaging data sets and data for T_{10} calculation were analyzed using Osirix DCE plugin (http://kyungs.bol.ucla.edu/software/DCE_tool/DCE_tool.html).

12.3 Quantitative analysis of DWI data

Apparent diffusion coefficient map will be generated using monoexponential fit. Several b-values will be used in calculation to achieve highest accuracy of ADC values.

Monoexponential calculation of apparent diffusion coefficient (ADC) can be described by following equation (eq.1):

$$ADC = -\ln\left(\frac{SI(b_2)}{SI(b_1)}\right) \qquad \text{(eq. 1)}$$

where $SI(b_2)$ and $SI(b_1)$ denotes the signal intensity at higher (b_2) and lower b-value (b_1).

To exclude intra-voxel incoherent movements sufficiently low b-value, in addition to b-value of 0, will be used.

13 SAMPLE SIZE

This prospective feasibility study which assesses utility of MRI in staging and therapy response monitoring of BC will enrol 300 patients. An interim analysis will be made after 30 patients with emphasis on imaging characteristics and the study may then be interrupted at the discretion of principal investigator after consulting other chief investigators if the imaging findings are found to be of no use to the patients.

14 QUALITY ASSURANCE

14.1 Training and information of study personnel

The technical and other supporting personnel of Department of Diagnostic Radiology, Turku University Hospital and Department of Radiology, Pori Central Hospital are well experienced in performing MRI studies. Likewise, the staffs of Departments of Oncology and

Radiotherapy, Turku University Hospital and Department of Surgery, Division of Urology, Turku University Hospital routinely apply neo-adjuvant chemotherapy, radical cystectomy and BCG in treatment of BC. In the beginning of the study all involved personals will be informed on the practical implementation of the protocol in a separate institutional meeting. They will be informed on the rationale of the study and possible clinical implications as well.

14.2 Protocol amendments

According to Finnish national regulations, protocol amendments can be made if all investigators agree. They are presented in a written form and dated as applicable. They include the original chapter of the study protocol and the amended chapter, with an explanation to this change. Important protocol amendments are reviewed by the local ethics committee.

15 STUDY SCHEDULE

The study will start in February 2013 pending all mandatory authorizations have been obtained. All MRI studies are expected to be performed within three years. Analysis and modelling of the MRI data is feasible once 7-10 patients have been imaged. Preliminary analysis of results will be available in late 2014 and first reports are expected to be written 2015.

16 FINANCING

The study will be financed in part by Finnish Governmental Special Funding (In Finnish: ’Erityisvaltionosuus, EVO’). Additional funding is sought through national non-profit organizations such as Sigrid Juselius Foundation and Cancer Foundations of Finland.

17 INSURANCE

The study patients are insured during the MRI procedure by the “Insurance against medicine-related injuries” (In Finnish: “Lääkevahinkovakuutus”) under regulations currently in effect in Turku University Hospital.

18 STUDY REPORT AND PUBLICATION(S)

Any formal presentation or publication of data collected within this research protocol will be considered as a joint publication by the investigator(s) and other appropriate persons deemed to have a significant academic output in the implementation of the study. Full reports

of this study will be submitted to peer-reviewed journals in concerned fields (mainly nuclear medicine and radiology).

19 ARCHIVING

The PI retains a list of all patients and their identifying codes for at least 15 years after completion or discontinuation of the study. All patient files, including ethics committee approvals and amendments, all source documents and case report copies, PET raw data, and patient informed consent forms are kept in a locked room at the Turku PET Centre of Turku University Hospital for a minimum of 15 years. All MRI studies including reconstructed images are stored up on PACS system at the Medical Imaging Centre of Southwest Finland as other routine clinical imaging data.

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21 APPENDICES

Appendix 1 Sequence of events

SEQUENCE OF EVENTS

Event

Cystoscopy;
Inform consent by patient

↓

1st MRI examination

↓

TUR-BT, diagnosis confirmed

↓

Surveillance, BCG, radical surgery or neoadjuvant chemotherapy

↓

MRI repeated before second therapeutical event

↓

Completion of neo-adjuvant therapy or BCG

↓

3rdMRI examination for neoadjuvant or BCG -patients

↓

Radical cystectomy including extended lymph node dissection (for MIBC patients)

↓

Imaging and pathology correlation

Appendix 2 Dosing of neoadjuvant therapy

Standard regimen day 1: cisplatin 75mg / m² i.v. + gemsitabine 1000mg / m² i.v.
day 8 gemsitabine 1000mg i.v.
day 22 cisplatin 75mg / m² i.v. + gemsitabine 1000mg /m² i.v.
day 29 gemsitabine 1000mg i.v.
day 43 cisplatin 75mg /m²i.v. + gemsitabine 1000mg / m² i.v.

Appendix 3 BCG scheme
day 1: instillation
day 8: instillation
day 15: instillation
day 22: instillation
day 29: instillation
day 36: instillation

Booster series every three month 1-3 years
day 1: instillation
day 8: instillation
day 15 instillation