Imperial Bladder 1 (**IB1**) - Fluorescence **CO**nfocal **M**icroscopy for ra**P**id evaluation of detrusor muscle at primary transurethra**L** rs**E**ct**T**ion of bladd**E**r tumours Short

IB1-LaserCOMPLETE

Statistical Analysis Plan (SAP)

Version 2.0
3rd of November 2025

ISRCTN16114765

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PLAIN ENGLISH SUMMARY

Background and study aim

Bladder cancer is the seventh most commonly diagnosed cancer and it is 3 to 4 times more common in men than in women. Bladder cancer is first diagnosed by tissue obtained from a transurethral resection of bladder tumour (TURBT) operation. This involves passing a telescope into the bladder along the urethra (water pipe) and removing bladder tumours (growths) using diathermy (electrical current) or laser energy. In 7 in every 10 patients diagnosed with bladder cancer, the cancer is present in the “superficial” layer of the bladder and does not grow deeper into the bladder muscle layer. Treatments for this form of superficial cancer such as bladder installations of chemotherapy or immunotherapy have been effective in reducing the chance of the bladder cancer recurring or progressing over time. Therefore, a key determinant of the correct treatment allocation following this operation is whether muscle was obtained to allow a pathologist to report the correct depth of cancer invasion. Unfortunately, performing this operation can be challenging and in up to 30% of patients there will be no muscle present in the tissue obtained to make an accurate assessment. The knowledge of this will become available 1-2 weeks following the operation. In most patients, the treating urologist will ask for the operation to be repeated to obtain this muscle sample. This has a significant impact on patient's health, quality of life, and a large financial burden on our healthcare service.

This study proposes the use of a novel scanner known as “fluorescence confocal microscopy” that could scan and report acquired bladder tissue in the operating theatre “live” to determine if a muscle is present, providing immediate feedback to the operating surgeon. This technology has been used in other urological cancers such as prostate cancer to determine if prostate cancer has spread beyond the gland at the time of prostate removal in real-time. However, it has never been used in this form of bladder cancer operation. If this study proves possible, a larger practice-changing study will be planned to compare this technology against traditional reporting.

METHODS & DESIGN

IB1-LaserCOMPLETE is a single-centre, unblinded, prospective, feasibility study. Bladder cancer specimens from subjects undergoing transurethral resection of bladder tumour (TURBT) operation will be stained with a fluorescent dye (Histolog Dip) and then be scanned on a digital fluorescent confocal microscope (FCM) known as the Histolog Scanner. The specimens will then undergo conventional histopathological analysis. A pathologist will undertake an analysis to evaluate the accuracy of FCM for the evaluation of detrusor status (presence or absence).

Recruitment will take place from one of the UK's largest designated urological cancer units and works in tandem with a dedicated Clinical Trials Unit. Over 150 TURBTs per year are performed by 4 surgeons.

All consecutive patients undergoing primary TURBT and consent to Imperial College Healthcare Tissue Bank (ICHTB) will be included. Where there is radiological or clinical suspicion of muscle invasive bladder cancer (cT2-T4) or a prior diagnosis of NMIBC on prior resection these patients will be excluded.

The study is ex vivo and no deviations from the clinical standard of care will take place during the study.

OBJECTIVES

Primary:

- To determine the feasibility of using fluorescence confocal microscopy to identify the presence of detrusor muscle in primary transurethral resection of bladder tumour (TURBT) specimens.

Secondary:

- Establish a standard operating procedure for scanning fresh bladder tissue from primary TURBTs.

- To assess the agreement of detrusor muscle detection between fluorescence confocal microscopy and standard of care histopathology within a limited number of patients.

- Generate initial pilot data to calculate an appropriately powered sample size for a follow on prospective, comparative, blinded multicentre cohort study to detect detrusor muscle in TURBT specimen against standard of care histopathology reporting

OUTCOME MEASURES

Primary

- Feasibility will be determined by ability to obtain sufficient digital FCM for interpretation of detrusor muscle from TURBT samples.

Secondary

- Agreement of digital FCM with the pathology report for detrusor presence on a specimen at a patient level. Sensitivity, specificity, positive and negative predictive value of digital FCM for detection of detrusor muscle with traditional H&E histopathology as the reference standard, on a per-patient basis.

- Agreement between readers: a) two individual histopathologists b) histopathologist vs urologist (Cohen's kappa coefficient).

POPULATION

Patients undergoing initial or first transurethral resection of bladder tumour (TURBT) for suspected bladder cancer.

ELIGIBILITY

Key Inclusion Criteria

• Patients undergoing initial or first transurethral resection of bladder tumour (TURBT) for suspected bladder cancer.

Key Exclusion Criteria

- Radiological or clinical suspicion of muscle invasive bladder cancer (cT2-T4)
- Prior diagnosis of NMIBC or MIBC on prior resection
- Patients who do not consent for ex vivo tissue research through Imperial College Healthcare Tissue Bank (ICHTB).
- Patients enrolled in concurrent clinical trials requiring ex vivo tissue for research.

SAMPLE SIZE

A sample size of 35 tissues is expected to be collected and analyzed during a 6-months recruitment period. Given the pilot nature of the study, this was not calculated to power any specific hypothesis, but only to provide feasibility data to be able to conduct a more definitive trial.

Thirty-five patients would allow us to encounter substandard processes with a high probability (97.5%) when there is a 10% true chance of any occurring. (Viechtbauer et al. 2015) It will also provide us with a range of plausible values for nuisance parameters, such as the prevalence of muscle invasiveness in this population, to be able to carefully plan a larger study.

The first five consecutive tissues will comprise a lead-in phase to develop and/or optimize the standard operating procedures.

ANALYSIS

The primary feasibility objective will be assessed with the progression criteria below using a traffic light or Red Amber Green (RAG) system. RAG predefines criteria for concluding feasibility and progressing to a more definitive trial, using clinically meaningful and pragmatic thresholds. They are quantitative and are provided as guidance rather than rules, to highlight problems that might be faced in a larger trial. (Eldridge et al. 2016, Mellor et al. 2023)

Feasibility objective: Obtaining sufficient FCM images to visualize detrusor muscle in a TURBT specimen, i.e. Out of all specimens obtained, how many FCM images were successfully acquired?

- Green — proceed with the larger trial: ≥90%
- Amber — proceed with changes: 70 to 89%
- Red — do not proceed unless changes are possible: <70%

Additionally, the study aims to explore the diagnostic accuracy of FCM in determining muscle invasiveness against the reference standard histopathological evaluation, with a paired design. Measures of sensitivity, specificity, and predictive values will be estimated from a 2×2 contingency table. The 95% confidence intervals (CI) will be computed with the Wilson (score) method.

Inter-reader agreement will be primarily quantified with Cohen's kappa statistic (κ) to account for chance agreement and will be interpreted following Altman (1990)'s recommendations, as follows: Poor: < 0.20; Fair: 0.21–0.40; Moderate: 0.41–0.60; Good: 0.61–0.80; Very good: 0.81–1.00. The observed kappa will be tested against a null kappa (κ_0) = 0, denoting no agreement. In addition, the simple matching coefficient, defined as the proportion of tissues for which both raters agree, and the proportion of positive agreements, will be measured. (Vanbelle et al. 2025)

REFERENCES

Altman, D.G. (1990). Practical Statistics for Medical Research (1st ed.). Chapman and Hall/CRC. https://doi.org/10.1201/9780429258589

Eldridge, S. M., Chan, C. L., Campbell, M. J., Bond, C. M., Hopewell, S., Thabane, L., Lancaster, G. A., & PAFS consensus group (2016). CONSORT 2010 statement: extension to randomised pilot and feasibility trials. *BMJ (Clinical research ed.)*, *355*, i5239. https://doi.org/10.1136/bmj.i5239

Mellor, K., Albury, C., Dutton, S.J. *et al.* Recommendations for progression criteria during external randomised pilot trial design, conduct, analysis and reporting. *Pilot Feasibility Stud* **9**, 59 (2023). https://doi.org/10.1186/s40814-023-01291-5

Vanbelle S, Engelhart CH, Blix E. Measuring Agreement in Diagnostics: A Practical Guide for Researchers. Stat Med. 2025;44(23-24):e70299. doi:10.1002/sim.70299

Viechtbauer, W., Smits, L., Kotz, D., Budé, L., Spigt, M., Serroyen, J., & Crutzen, R. (2015). A simple formula for the calculation of sample size in pilot studies. *Journal of clinical epidemiology*, *68*(11), 1375–1379. https://doi.org/10.1016/j.jclinepi.2015.04.014