TRAIN TRANSLATIONAL LAB MANUAL

A Phase III randomised control clinical trial of radiotherapy with radiosensitisation versus intravesical Bacillus Calmette-Guerin therapy for high-risk non-muscle invasive bladder cancer

ISRCTN reference no: 16345179

Ethics reference number: 25/NM/0295

Sponsor reference number: CFTsp223

IRAS number: 1012385

Funder reference number: NIHR163351

SPONSOR: The Christie NHS Foundation Trust

COORDINATING CENTRE: Southampton Clinical Trials Unit (SCTU)

Signatory and Approvals Page

Authorised by:

Name: Professor Ananya Choudhury Role: Chief Investigator

Signature: A Choudhury Date: 21-Jan-2026

A Choudhury (Jan 21, 2026 15:15:47 GMT)

Authorised by:

Name: Daniel Griffiths Role: Senior Trial Manager

(Southampton Clinical Trials Unit)

Signature: D. Griffiths Date: 19-Jan-2026

D. Griffiths (Jan 19, 2026 09:20:09 GMT)

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1. Contacts

Chief Investigator
Professor Ananya Choudhury
Email: ananya.choudhury@nhs.net

Joint-Chief Investigator
Professor Vijay Sangar
Email: vijay.sangar@nhs.net

Senior Trial Manager
Daniel Griffiths
Email: train@soton.ac.uk

Trial Manager
Amber Cole
Email: train@soton.ac.uk

Data Manager
Oli Seymour
Email: train@soton.ac.uk

MCRC Translational Contact
Kim Reeves
Email: train-trans@manchester.ac.uk [central inbox]

Study Sponsor
The Christie NHS Foundation Trust R&I Department
Humera Hussain
Email: humera.hussain3@nhs.net

2. Introduction

TRAIN is a multi-centre phase III randomised controlled clinical trial of radiotherapy with radiosensitisation versus intravesical Bacillus Calmette-Guerin (BCG) therapy for high-risk (G3/cis) non-muscle invasive bladder cancer (HR-NMIBC).

The recruitment target is 328 patients with HR-NMIBC following maximal transurethral resection of bladder tumour (TURBT) and will randomise 1:1 to BCG or radical radiotherapy (55Gy/20#) with radiosensitisation.

This lab manual refers to the associated translational research linked to the TRAIN trial. It details the logistics for sample and data collection, and processing thereof.

The translational study lead for TRAIN is Professor Ananya Choudhury, Chair and Honorary Consultant in Clinical Oncology and Group Leader Translational Radiobiology at The University of Manchester.

3. Purpose

The purpose of this manual is to describe the collection, processing, storage and transportation of the following samples for the TRAIN study:

- **Diagnostic Formalin-fixed, paraffin-embedded (FFPE) tumour samples**: pre-treatment diagnostic biopsies are requested. These will be collected at screening on all patients after consent has been obtained. Alternatively, a pre-trial diagnostic biopsy can be used if it was obtained no more than 3 months prior to consent. Samples to have an associated linked-anonymised pathology report.

- **Blood samples EDTA (n=4) and Streck (n=4))**: These will be taken roughly at the following time points in all patients:
  a) Baseline (*post-randomisation but prior to treatment*)
  b) 12 weeks
  c) 24 weeks
  d) recurrence or end of trial

- **Urine samples (n=4)**: These will be taken roughly at the following time points in all patients:
  a) Baseline (*post-randomisation but prior to treatment*)
  b) 12 weeks
  c) 24 weeks
  d) recurrence or end of trial

Any forms required to record sample processing and preparation are shown as appendices to this document. The latest versions for completion should be obtained directly from the SCTU website (https://www.southampton.ac.uk/ctu/train.page) and printed off as required. Contact train@soton.ac.uk for any issues or passwords.

The Sponsor, Chief/Principal Investigator, site team, and Laboratory staff must ensure that all samples are taken in accordance with Good Clinical Practice (GCP), Guidance on the maintenance of regulatory compliance in laboratories that perform the analysis or evaluation of clinical trial samples, as well as the requirements of the UK Clinical Trials regulations (SI 2004/1031 and SI 2006/1928 as amended), the ICH GCP guidelines and the Declaration of Helsinki.

4. Overview of Train

4.1 Scope

This manual is intended for use by the clinical and the laboratory teams at the participating clinical centres and Manchester Cancer Research Centre (MCRC) at The University of Manchester.
Processing and analysis of samples after they arrive at MCRC laboratory is described in the analysing laboratory internal SOPs.

4.2 Trial Overview

In the UK, 20,000 people develop urothelial type bladder cancer every year, of which 75-80% have non-muscle invasive disease [1]. This may be underestimated as the National Cancer Registration and Analysis Service (and CRUK) does not include all non-muscle invasive bladder cancer (NMIBC) cases. Bladder cancer causes 5,500 deaths each year [2]. NMIBC includes those staged as < T2 N0 M0, meaning the tumour has not spread as far as the muscle layer of the bladder wall or beyond the bladder. Bladder cancer commonly affects older people (70% are over 65 years) [3]. NMIBC is one of the most expensive cancers to treat on a per-patient basis due to the need for long-term surveillance that includes frequent cystoscopies [4].

Standard of care (SoC) for high-risk (HR) NMIBC (T1 grade 3 [T1G3]/CIS) is either by transurethral resection of bladder tumour (TURBT) followed by a course of intravesical Bacillus Calmette-Guérin (BCG), or radical cystectomy. Compared to BCG, data suggest that disease-specific survival is better with cystectomy, but many patients wish to preserve their bladder and avoid a stoma, and at least 25% are not fit for this major surgery.

BCG is given once weekly for six weeks (induction course) followed by maintenance treatments which last for up to 3 years. However, 50% of patients will experience cancer recurrence after BCG, and side effects result in 1 in 4 stopping treatment [5, 6]. These include bacterial (26%) or BCG cystitis (47%), frequency of passing urine (>1/hr) (31%), blood in the urine (34%), fever (15%), malaise (23%), arthralgia (5%), and lung infection (0.2%) [4, 5].

HR-NMIBC (T1G3) can recur despite TURBT and BCG, with a substantial rate of progression to muscle-invasive bladder cancer (44%; MIBC) [7]. Once it progresses to MIBC, bladder cancer becomes a substantial risk to life. In patients who progress to MIBC the outcome is worse than in those presenting with primary MIBC: cancer-specific survival at 3 years is 67% vs 37% in the primary versus progressive setting [7].

Radical cystectomy is reserved for BCG failure and occasionally as a primary treatment. In patients undergoing radical surgery outcomes are good but no trial has been able to randomise patients successfully. The BRAVO study attempted to do so and was unable to randomise enough patients, but in those who did proceed to cystectomy (n = 20) all were free of disease at 12 months [8]. In general terms, radical cystectomy efficacy must be balanced with the significant morbidity of major surgery and bladder removal, and the potential change in the quality of life for patients with a urinary diversion; BRAVO shows that patients often prefer bladder preservation strategies.

To compound the limitations of the current treatment options, BCG supply has failed globally several times over the last 7 years [9]. A recent study evaluated the clinical and economic impact of BCG shortage on NMIBC patients treated during the period of restricted supply and concluded there was an increased rate of bladder cancer recurrence, and the total cost of care for HR-NMIBC was increased [10].

A European study, involving 3401 individual patient datasets across multiple institutions, found 50% of HR-NMIBC progressed to MIBC. Many patients have side effects from intravesical therapy, with 25% ceasing treatment [6]. BCG therapy causes side effects in up to 75% [5, 6]: both local and systemic. Due to industrial production problems, BCG has had several episodes of low supply over the last 7 years [10]. This has led to national organisations like the British Association of Urological Surgeons (BAUS) implementing different regimens to overcome supply problems. This included reducing the length of BCG treatment, using mitomycin or proceeding directly to cystectomy. However, reducing the length of BCG treatment results in increased recurrence [10-12].

The standard practice for recurrence on BCG therapy (BCG-refractory disease) is to proceed to early radical cystectomy [13-14]. However, cystectomy is associated with mortality (3%), high rates of postoperative complications of nearly 50% at 30 days and often requires a permanent stoma affecting the patient's quality of life [15, 16].

There is, therefore, a pressing need to provide alternative treatment options. These should ideally improve the prevention of cancer recurrence and progression and reduce the need for the life-altering option of cystectomy. We should also remove our current reliance on a repeatedly unpredictable supply of a drug and be cost-effective. The TRAIN trial will attempt to do this through a fundamental change in treatment approach.

Bladder preservation with radiotherapy and radiosensitisers is an equivalent and alternative treatment to radical cystectomy for MIBC [13, 17]. A meta-analysis comparing outcomes for radical cystectomy and bladder preservation showed no difference in overall survival (OS), disease-specific survival or progression-free survival with slightly higher early complications in the surgical group. 5-year OS was estimated at 56.2% with radical cystectomy and 55.0% with bladder preservation [17].

The strongest evidence for using radiosensitisation in MIBC is from the phase III BC2001 [18] and BCON [19] trials. These trials resulted in radiosensitisation becoming the standard of care worldwide. Although the BC2001 [18] trial only included T2 disease or greater, 9.2% in the BCON trial [19] had high-risk T1 disease. Another schedule is weekly gemcitabine combined with hypofractionated radiotherapy (GemX) [20].

Currently, TURBT followed by radiotherapy with a radiosensitiser, also called tri-modality treatment (TMT), is not a standard treatment for NMIBC. A previous UK trial, randomised 210 patients with NMIBC between BCG and radiotherapy alone, showing no significant difference in response between the two arms for the primary endpoint of progression-free interval at 5 years HR 1.07 (95% CI:0.65-1.74 p=0.785) [21]. Importantly, this trial completed recruitment two decades ago, using now outdated techniques and no radiosensitiser.

A systematic review [22] evaluated 13 studies including 746 patients with HR-NMIBC treated with primary TMT. The 5-year rates of recurrence-free survival, cancer-specific survival and overall survival were 54% (95% CI = 38-70%), 86% (95% CI = 80-92%), and 72% (95% CI = 64-79%). Notably, 13% of patients proceeded to salvage radical cystectomy and 9% developed metastatic disease. These results are promising and suggest that TMT is an effective treatment for NMIBC. The systematic review highlighted the need for a prospective clinical trial to clearly define the risks and benefits of this treatment approach [22].

A recent phase II trial, RTOG-0926, investigated the benefit of TMT in recurrent NMIBC following intravesicular treatment. Patients were recruited between 2009-2017 and received chemoradiotherapy (61.2 Gy in 34 fractions) with radio-sensitizing cisplatin or mitomycin/5-FU chemotherapy. Initial data is favourable with

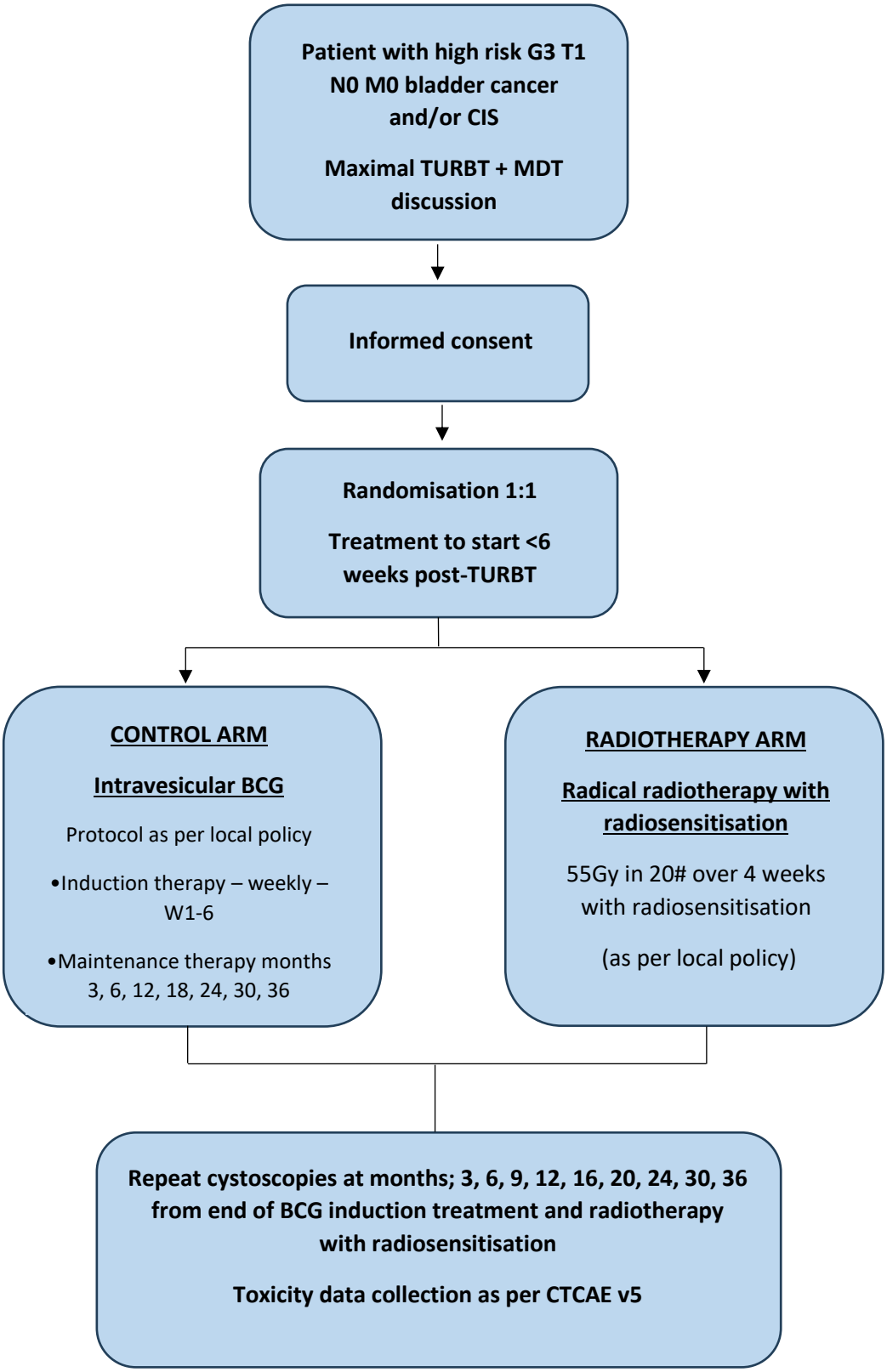
three-year freedom from cystectomy rate of 88% and overall survival at 5 years of 53% (95% CI: 35%, 72%). The rate of distant metastatic disease at 5 years was 20% (95% CI: 8%, 37%) [23].

Biomarkers could potentially identify relatively radio-responsive T1 disease as opposed to those that would be better treated with either BCG or cystectomy. From the BCON trial, we showed that both necrosis and HIF-1α identify patients who respond to hypoxia modification [24]. Among patients with necrosis, 5-year OS was 34% after radiotherapy alone versus 56% after radiotherapy and CON (p = 0.004) [25]. MRE11, which is involved in DNA-damage signalling, at both the protein and germline levels is associated with better cancer-specific survival [26-28]. Samples will be collected from this trial for biomarker discovery and validation.

We will test if modern hypofractionated radiotherapy with radiosensitisation can lead to superior rates of long-term control and bladder preservation when compared to intravesicular BCG in high-risk and very high-risk NMIBC. If successful, this study will change practice for patients across the UK and worldwide.

Future Endpoints:
Remaining blood, urine and tissue samples will be stored under HTA regulations for future ethically approved studies.

4.3 Trial schema



4.4 Responsibilities

Staff at participating Centres

Staff at the participating clinical centres are responsible for:

- Registering the patient via the Rave Database as per protocol
- Ensuring that all samples are collected, handled, processed and transported to the specified Laboratory, in accordance with the instructions detailed in this manual.
- Notifying MCRC via train@soton.ac.uk and train-trans@manchester.ac.uk on the day that the sample is sent
- Notifying MCRC of the dates to expect sample receipt if possible

Staff at Manchester Cancer Research Centre (MCRC) at The University of Manchester

Staff at the MCRC Lab are responsible for:

- Taking receipt of sample shipment.
- Checking for any inconsistencies between samples received and the accompanying documentation, as described in this manual, and liaising with the relevant teams (e.g. SCTU or Clinical team at site) to resolve any identified inconsistency.
- Processing of FFPE, urine and blood samples appropriately, according to local SOPs.
- Storing FFPE, urine and blood samples appropriately, according to local SOPs.
- Sending laboratory supplies to sites and tracking component expiration dates to ensure sufficient supplies available for patient samples.

Staff at Southampton Clinical Trials Unit

Staff at the SCTU are responsible for:

- Notifying staff at the MCRC Laboratory of a new patient by including them in the registration confirmation email.
- Notifying staff at the MCRC laboratory of new patient consenting via email, so Manchester can send kits out. Kits can be sent within 24 hours if notified before 12pm.
- Coordinating the resolution of any inconsistency flagged by the processing and analysing laboratories.

5. Overview of samples to be collected

Table below provides an overview of the time points and samples to be collected.

Visit All research bloods are taken alongside standard of care bloods (no additional needle pricks required)	**Label Reference**	**B1**- Baseline	**T1** – 12 weeks	**T2** – 24 weeks	**T3** – recurrence or end of trial
EDTA blood (10 ml)	**E**	X	X	X	X
STRECK bloods (10ml)	**CF**	X	X	X	X
Urine (30-50ml)	**U**	X	X	X	X
Diagnostic FFPE block		X			

6. Ethics

The sample & data collection described in this lab manual were included in the main TRAIN trial ethics application 25/NW/0295 approved on 10-NOV-2025 by North West – Greater Manchester South Research Ethics Committee.

7. Patient Consent

The Principal Investigator (PI) retains overall responsibility for the conduct of the trial at their site, including the taking of informed consent from participants. They must ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained, and competent.

Consent to enter the trial must be sought from each participant only after a full explanation has been given, a Participant Information Sheet (PIS) offered, and time allowed for consideration. Informed consent must be obtained prior to the participant undergoing procedures that are specifically for the purpose of the trial.

The right of the participant to refuse to participate without giving reasons must be respected.

After the participant has entered the trial, the clinician remains free to give alternative treatment to that specified in the protocol at any stage if they feel it is in the participant's best interest, but the reasons for doing so should be recorded.

All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment. Participants must be provided with a contact point where they may obtain further information about the trial.

Upon completion of the informed consent form, a copy will be given to the participant, a copy stored in the participant's medical notes, the original filed in the site trial file and a copy of the consent form should be sent to the SCTU via a secure nhs.net account, or using the University of Southampton's SafeSend service, to allow for central monitoring monitorSCTU@soton.ac.uk or using a secure nhs.net email address, SafeSend or encrypted mail to allow for central monitoring. Where a participant is required to re-consent or new information is required to be provided to a participant it is the responsibility of the PI to ensure this is done in a timely manner.

8. Sample Collections

8.1 Sample Collection Kits

An initial batch of sample collection kits will be sent to sites following the site initiation visit, we ask that each site complete a kit checklist to confirm receipt. Additional kits can be requested by emailing train-trans@manchester.ac.uk. Please request additional kits **1-2 weeks before you anticipate using them**.

UN3373 packing regulations – Category B

Human or animal specimens which do not contain pathogens in Category A fall into Category B and must be assigned to UN 3373 "Biological substance, Category B". All UN 3373 substances must be transported in compliance with 49 CFR, Part 173 or IATA Packaging Instruction 650.

Transport Packaging for UN 3373 Substances

Any packaging for biological substances must include tree components:

- A primary receptacle: the tube, vial or other container typically made of glass or rigid plastic (including the stopper, cap or other closure elements) that is in direct contact with the specimen.
- A secondary packing (including cushioning and other materials) that fully encapsulated the primary receptacle.
- An outer packing for shipping or transit.

8.2 Labelling Samples

Sites will be provided with a selection of pre-printed labels for each registered patient, and sites should add patient number in the space provided using a solvent proof marker.

The label should be attached to the appropriate corresponding sample upon collection.

In case of errors, or if samples need to be taken prior to receiving the pre-printed labels (i.e. at baseline), each site will be provided with a supplementary set of labels which can be handwritten in a solvent proof marker using BLOCK CAPITALS. Should handwritten labels be used please mention this in the sample dispatch log and in the notification email to STCU on sample dispatch. Samples requiring handwritten labels should be labelled as follows:

Trial ID: Patient number – Sample type code: Time point code

Trial ID (pre-printed on labels):

- The trial ID for TRAIN is TRN.

Site number (pre-printed on the label): 4 digits. For example 1001

Patient number (to be handwritten onto labels):

- A blank space is provided for a 3-digit patient number generated by the RAVE database. The patient number consist of 3 digits- For example, a patient number could be 001.

Time point codes (to be handwritten onto labels)

- **B1** = Baseline (*pre-randomisation but within 4 weeks of screening*)
- **T1** = 12 weeks
- **T2** = 24 weeks
- **T3** = recurrence or end of trial Sample type codes (pre-printed on labels) are:
- E = EDTA (EDTA sample kit)
- CF = cfDNA (Liquid Biopsy kit)
- U = urine (Urine sample kit)

Example:

The labels below indicate samples collected for TRAIN (TRN), site number (i.e. 1001), patient number (XXX), sample type CF/G/U, time point Baseline #1 (B1)

8.3 Receipt of samples

The members of the staff taking receipt of sample shipments are responsible for checking for and resolving any inconsistencies between samples received and accompanying documentation.

8.4 Day of collection and posting

Samples should be collected at the start of the week wherever possible. They may be taken on a Tuesday or Wednesday if necessary.

Samples should not be posted on a Friday or the day before a bank holiday.

8.5 Diagnostic FFPE Biopsy

Logistics:
Formalin-fixed, paraffin-embedded (FFPE) pre-treatment diagnostic biopsies are requested, at least one block for each patient. Please send additional/ duplicate blocks if these are available.

Blocks should be sent as soon as possible with the TRAIN Tissue Sample Tracking Form (Appendix 11.2) and an anonymised copy of the pathology report. The pathology report should be labelled with trial ID and trial time point code. Patient consent forms should remain at the relevant sites and should not be sent with the samples. No special conditions are required for postage i.e. a courier is not necessary; blocks can be sent at ambient temperature in a padded jiffy bag as per local hospital guidelines.

Please return the completed Tissue Sample Tracking Form, and anonymised pathology report when sending the FFPE block(s) to:

TRAIN study team
Manchester Cancer Research Centre
The University of Manchester
555 Wilmslow Road
Manchester, M20 4GJ

Send a notification via email to train@soton.ac.uk and train-trans@manchester.ac.uk once you have sent the sample. This is to ensure that samples arrive within the expected timeframe.

A copy of the completed TRAIN Translational Samples Dispatch Log (Appendix 11.5) must be retained at site.
Opening hours for sample receipt:

Monday – Friday, 9am – 5pm. Closed on Public Holidays. **Shipments should only be arranged Mon – Thurs to avoid weekend delivery/logistical issues**.

On receipt, pathology reports will be stored in a locked pathology cupboard within the MCRC and blocks will be stored at ambient temperature within the access controlled laboratory at the MCRC.

At the end of the study if material is leftover, blocks will be stored for future study. If you wish to have them returned to the original hospital, please notify the train team via train@soton.ac.uk and ensure details of the return address appear on the pathology report prior to sending.

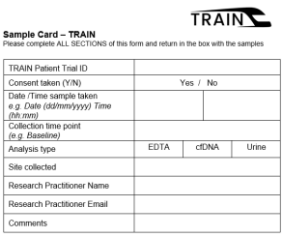
If an urgent return of tissue is required to inform clinical decisions, please contact the TRAIN trial team at train@soton.ac.uk.

8.6 EDTA

Logistics:

- One 10 ml EDTA blood sample will be collected at:
    - **B1** - Baseline
    - **T1** – 12 weeks
    - **T2** - 24 weeks
    - **T3** – recurrence or end of trial

- TRAIN 'EDTA kits' will be provided to each recruitment centre. Each 'EDTA kit' comprises:

Figure	Item	Quantity
	BD Vacutainer® EDTA Tube	X1
	Secondary Mailing container with absorbent liner and screw cap	X1
	Mailing container (outer packing for shipping or transit)	X1
	TRAIN Sample Card	X1
	Pre-paid, Royal Mail 1pm silver bag	X1
	Pre-printed address label	X1

In advance of the patient visit, **check the expiry date** on the BD Vacutainer® EDTA Tube provided to ensure the tube is still in date. If the tube is not in date, or is close to expiry, please contact train-trans@manchester.ac.uk as soon as possible to request additional supplies.

Research Nurse (or phlebotomist) takes blood using the tube provided. Invert the tube 8-10 times to thoroughly mix with the additive. DO NOT SHAKE. Vigorous mixing can cause haemolysis which must be avoided. Blood samples that are overtly haemolysed cannot be analysed. Mark blood tube as per section 8.2 along with the date and time of the sample. **Place the primary blood tube in the secondary mailing container and tighten cap to secure and immobilise blood tube**.

Research Nurse **places the packaged bloods along with the completed Sample Card in rigid box provided**. This packaged rigid box is then placed into a pre-paid royal mail silver bag with a pre-printed address label. It is the responsibility of the research nurse to ensure the blood sample is packaged correctly and put out for collection by Royal Mail that same day.

Opening hours for sample receipt:

Monday – Friday, 9am – 5pm. Closed on Public Holidays. Shipments should only be arranged **Mon – Thurs** to avoid weekend delivery/logistical issues and not posted the day before a Bank Holiday.

A 'TRAIN Translational Samples Dispatch Log' needs to be maintained for each patient. A record of each sample required for the trial is recorded by sites on this log. This is filed in the ISF and updated with each new sample that is taken and sent for the patient.

Send a notification via email to train@soton.ac.uk and train-trans@manchester.ac.uk once you have sent the sample and include details of the samples being shipped. This is to ensure that samples arrive within the expected timeframe.

Recruitment site to detail translational sample collection and shipment on the applicable translational eCRF within the RAVE database.

On receipt, the translational study team will double check the details on the blood tube match the Sample Card and complete the lab eCRF. Receipted bloods will be logged and tracked appropriately using a password protected database.

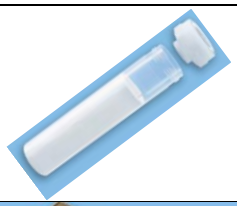
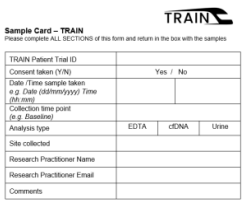
Bloods will be stored in a -80°C freezer at the MCRC, University of Manchester prior to analysis according to local SOPs.

8.7 Liquid Biopsy

Logistics:

- One 10 ml CELL-FREE DNA BCT® blood tube (manufactured by Streck) blood sample will be collected at:
    - **B1** - Baseline
    - **T1** – 12 weeks
    - **T2** - 24 weeks
    - **T3** – recurrence or end of trial

- TRAIN 'Liquid Biopsy kits' will be provided to each recruitment centre. Each 'Liquid Biopsy kit' comprises:

Figure	Item	Quantity
	CELL-FREE DNA BCT® blood tube	X1
	Secondary Mailing container with absorbent liner and screw cap	X1
	Mailing container (outer packing for shipping or transit)	X1
	TRAIN Sample Card	X1
	Pre-paid, Royal Mail 1pm silver bag	X1
	Pre-printed address label	X1

Procedure for sample collection

- Blood tubes should always be stored at ambient temperature (15°C to 30°C) prior to use.
- In advance of the patient visit, **check the expiry dates on the Streck Cell-Free DNA BCT® Tubes** provided to ensure the tubes are still in date. If the tubes are not in date, or are close to expiry, please contact train-trans@manchester.ac.uk as soon as possible to request additional supplies.
- Since Streck tubes contain chemical additives, it is important to avoid possible backflow from the tube. To guard against backflow, the following precautions are recommended:
    - It is recommended to use tubing at least 5" in length equipped with a multi-sample Luer adapter (MSLA) to eliminate the possibility of a back-flush.
    - Keep the individual's arm in a downward position during the collection procedure.
    - Hold the tube vertically below the donor's arm, with the stopper in the uppermost position so that the tube contents do not touch the stopper or the end of the needle during sample collection.
    - Release tourniquet once blood starts to flow in the tube, or within two minutes of application.
    - Allow at least 10 seconds for a complete blood draw to take place.
    - Ensure blood flow has stopped flowing into tube before removing tube from holder.
- Tubes should be fastened securely, and **gently inverted 8-10 times immediately after blood collection**. **DO NOT SHAKE**. Vigorous mixing can cause haemolysis which must be avoided. Blood samples that are overtly haemolysed cannot be analysed.

Sample storage

- Samples taken in Streck tubes must always be stored at ambient temperature prior to shipment / processing.

'Liquid Biopsy' bloods do not require processing on site.

Research Nurse (or phlebotomist) takes blood using the tubes provided following the instructions in Sample Collection above. Mark each blood tube as per section 8.2. Place the primary blood tubes in the secondary mailing containers and tighten cap to secure and immobilise blood tubes.

Research Nurse places the packaged blood along with the completed Sample Card in the rigid box provided. This packaged rigid box is then placed into a pre-paid Royal Mail silver bag with a pre-printed address label It is the responsibility of the research nurse to ensure the blood sample is packaged correctly and taken to the post room for shipment using a Royal Mail 1pm silver bag that same day. Samples should not be sent on a Friday or the day before a bank holiday. The address for shipment is:

TRAIN study team
Manchester Cancer Research Centre
The University of Manchester
555 Wilmslow Road
Manchester, M20 4GJ

A 'TRAIN Translational Samples Dispatch Log' needs to be maintained for each patient. A record of each sample required for the trial is recorded by sites on this log. This is filed in the ISF and updated with each new sample that is taken and sent for the patient. Send a notification via email to the following email addresses: train@soton.ac.uk, and train-trans@manchester.ac.uk.

On receipt, the translational study team will double check the details on the blood tube match the Sample Card and complete the lab eCRF. Receipted bloods will be logged and tracked appropriately using a password protected database. Streck bloods will be processed at The MCRC following internal standard operating

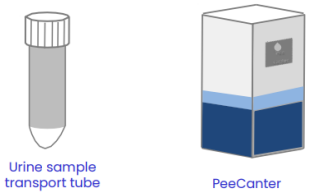
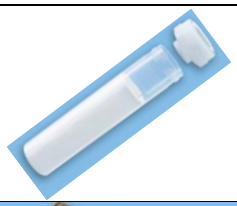
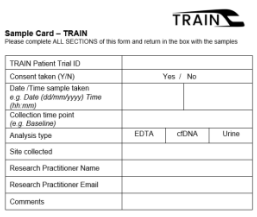
procedures (SOPs). The extracted cfDNA will be stored in a -80°C freezer at MCRC, University of Manchester ready for future analyses.

8.8 Urine

Logistics:

- One 50ml Nonacus sample will be collected at:
    - **B1** - Baseline
    - **T1** – 12 weeks
    - **T2** - 24 weeks
    - **T3** – recurrence or end of trial

- TRAIN 'Urine kits' will be provided to each recruitment centre. Please check the expiry dates of the components within the kit. Each 'Urine kit' comprises:

Figure	Item	Quantity
	Nonacus tube	X1
	Secondary Mailing container with absorbent liner and screw cap	X1
	Mailing container (outer packing for shipping or transit)	X1
	TRAIN Sample Card	X1
	Pre-paid, Royal Mail 1pm silver bag	X1
	Pre-printed address label	X1

TRAIN TRAIN study Manchester Cancer Research Centre The University of Manchester 555 Wilmslow Road Manchester, M20 4GJ		

Urine processing

- In advance of the patient visit, **check the expiry dates of the Nonacus and falcon tube**
- Urine samples will be collected prior to any routine procedures by the patients. Self-collected first void urine samples will be collected first, using a 50ml Nonacus collection tube. Ideally, we would prefer to collect the patient's first urine void of the day as evidence would suggest that these samples may contain more cells and DNA than subsequent voids. **However, this is not essential – collecting any urine sample is better than not collecting a sample at all!**
- Invert the tube until the preservative has completely dissolved in the urine sample. Instructions are provided with the kit.

The sample should then be posted to the MCRC via the pre-paid Royal Mail silver bag with a pre-printed address label. It is the responsibility of the research nurse to ensure the blood sample is packaged correctly and taken to the post room for shipment using a Royal Mail 1pm silver bag that same day. Samples should not be sent on a Friday or the day before a bank holiday. The address for shipment is:

TRAIN study team
Manchester Cancer Research Centre
The University of Manchester
555 Wilmslow Road
Manchester, M20 4GJ

Opening hours for sample receipt:
Monday – Friday, 9am – 5pm. Closed on Public Holidays.

A 'TRAIN Translational Samples Dispatch Log' needs to be maintained for each patient. A record of each sample required for the trial is recorded by sites on this log. This is filed in the ISF and updated with each new sample that is taken and sent for the patient. Send a notification via email to the following email addresses: train@soton.ac.uk, and train-trans@manchester.ac.uk.

On receipt, the translational study team will double check the details on the urine sample match the Sample Card and complete the lab eCRF. Receipted urine samples will be logged and tracked appropriately using a password protected database. Urine samples will be processed at The MCRC following internal standard operating procedures (SOPs).

Urine will be stored in a -80°C freezer at the MCRC, University of Manchester.

9 Data/Specimen Management

9.1 Storage and Access

Translational research data and samples will be stored for at least 20 years beyond the end of the study. Consent will be sought from patients for samples and data to be stored for future medical research on this or

a related project to be carried out at academic institutions, hospitals or by commercial companies involved in cancer research worldwide.

New research projects can be proposed at any time via submission of a Concept Form. Following open discussion and review by the TRAIN Trial Management Group, a decision will be made by consensus as to whether the project should be taken forward. All new research work planning to make use of samples and/or translational research data must ensure appropriate ethics approvals are in place. A material and/or data transfer agreement is also required.

Professor Ananya Choudhury at The University of Manchester is custodian of the samples.

9.2 Plans for identifiers

A unique code on the sample will be used to identify the participant but will not, by itself, reveal who the participant is. Researchers on this study working with patient samples will not hold the link to be able to identify who the participant is. The TRAIN trial ID will be used to identify each sample.

10 References

1) ABC UK. The Facts About Bladder Cancer. [cited 2022 December]; Available from: https://actionbladdercanceruk.org/the-facts-about-bladder-cancer/.
2) CRUK. Bladder cancer statistics. [cited 2022 December]; Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bladder-cancer.
3) Mossanen, M., et al., Evaluating the cost of surveillance for non-muscle-invasive bladder cancer: an analysis based on risk categories. World J Urol, 2019. 37(10): p. 2059-2065.
4) Sylvester, R.J., et al., The side effects of Bacillus Calmette-Guerin in the treatment of Ta T1 bladder cancer do not predict its efficacy: results from a European Organisation for Research and Treatment of Cancer Genito-Urinary Group Phase III Trial. Eur Urol, 2003. 44(4): p. 423-8.
5) van der Meijden, A.P., et al., Maintenance Bacillus Calmette-Guerin for Ta T1 bladder tumors is not associated with increased toxicity: results from a European Organisation for Research and Treatment of Cancer Genito-Urinary Group Phase III Trial. Eur Urol, 2003. 44(4): p. 429-34.
6) Sylvester, R.J., et al., European Association of Urology (EAU) Prognostic Factor Risk Groups for Non-muscle-invasive Bladder Cancer (NMIBC) Incorporating the WHO 2004/2016 and WHO 1973 Classification Systems for Grade: An Update from the EAU NMIBC Guidelines Panel. Eur Urol, 2021. 79(4): p. 480-488.
7) Schrier, B.P., et al., Prognosis of muscle-invasive bladder cancer: difference between primary and progressive tumours and implications for therapy. Eur Urol, 2004. 45(3): p. 292-6.
8) Catto, J.W.F., et al., Radical Cystectomy Against Intravesical BCG for High-Risk High-Grade Nonmuscle Invasive Bladder Cancer: Results From the Randomized Controlled BRAVO-Feasibility Study. J Clin Oncol, 2021. 39(3): p. 202-214.
9) Ourfali, S., et al., Recurrence Rate and Cost Consequence of the Shortage of Bacillus Calmette-Guerin Connaught Strain for Bladder Cancer Patients. Eur Urol Focus, 2021. 7(1): p. 111-116.
10) Oddens, J., et al., Final results of an EORTC-GU cancers group randomized study of maintenance bacillus Calmette-Guerin in intermediate- and high-risk Ta, T1 papillary carcinoma of the urinary bladder: one-third dose versus full dose and 1 year versus 3 years of maintenance. Eur Urol, 2013. 63(3): p. 462-72.

11) Moon, Y.J., et al., Effects of intravesical BCG maintenance therapy duration on recurrence rate in high-risk non-muscle invasive bladder cancer (NMIBC): Systematic review and network meta-analysis according to EAU COVID-19 recommendations. PLoS One, 2022. 17(9): p. e0273733.
12) Babjuk, M., et al., European Association of Urology Guidelines on Non-muscle-invasive Bladder Cancer (Ta, T1, and Carcinoma in Situ). Eur Urol, 2022. 81(1): p. 75-94.
13) Tang, D.H. and S.S. Chang, Management of carcinoma in situ of the bladder: best practice and recent developments. Ther Adv Urol, 2015. 7(6): p. 351-64.
14) Novara, G., et al., Systematic review and cumulative analysis of perioperative outcomes and complications after robot-assisted radical cystectomy. Eur Urol, 2015. 67(3): p. 376-401.
15) Ramani, V.A., S.J. Bromage, and N.W. Clarke, A contemporary standard for morbidity and outcome after radical cystectomy. BJU Int, 2009. 104(5): p. 628-32.
16) Management, N.I.f.H.a.C.E.G.B.C.D.a. Published February 2015.
17) Vashistha, V., et al., Radical Cystectomy Compared to Combined Modality Treatment for Muscle-Invasive Bladder Cancer: A Systematic Review and Meta-Analysis. Int J Radiat Oncol Biol Phys, 2017. 97(5): p. 1002-1020.
18) Huddart, R.A., et al., Randomized noninferiority trial of reduced high-dose volume versus standard volume radiation therapy for muscle-invasive bladder cancer: results of the BC2001 trial (CRUK/01/004). Int J Radiat Oncol Biol Phys, 2013. 87(2): p. 261-9.
19) Hoskin, P.J., et al., Radiotherapy with concurrent carbogen and nicotinamide in bladder carcinoma. J Clin Oncol, 2010. 28(33): p. 4912-8.
20) Choudhury, A., et al., Phase II study of conformal hypofractionated radiotherapy with concurrent gemcitabine in muscle-invasive bladder cancer. J Clin Oncol, 2011. 29(6): p. 733-8
21) Harland, S.J., et al., A randomized trial of radical radiotherapy for the management of pT1G3 NXM0 transitional cell carcinoma of the bladder. J Urol, 2007. 178(3 Pt 1): p. 807-13; discussion 813.
22) Rodrigues Pessoa, R., et al., Systematic review and meta-analysis of radiation therapy for high-risk non muscle invasive bladder cancer. Urol Oncol, 2021. 39(11): p. 786 e1-786 e8.
23) Weiss, C., et al., Radiochemotherapy after transurethral resection for high-risk T1 bladder cancer: an alternative to intravesical therapy or early cystectomy? J Clin Oncol, 2006. 24(15): p. 2318-24.
24) Eustace, A., et al., Necrosis predicts benefit from hypoxia-modifying therapy in patients with high risk bladder cancer enrolled in a phase III randomised trial. Radiother Oncol, 2013. 108(1): p. 40-7.
25) Hunter, B.A., et al., Expression of hypoxia-inducible factor-1alpha predicts benefit from hypoxia modification in invasive bladder cancer. Br J Cancer, 2014. 111(3): p. 437-43.
26) Choudhury, A., et al., MRE11 expression is predictive of cause-specific survival following radical radiotherapy for muscle-invasive bladder cancer. Cancer Res, 2010. 70(18): p. 7017-26.
27) Teo, M.T.W., et al., Next-generation sequencing identifies germline MRE11A variants as markers of radiotherapy outcomes in muscle-invasive bladder cancer. Ann Oncol, 2014. 25(4): p. 877-883.
28) Laurberg, J.R., et al., Expression of TIP60 (tat-interactive protein) and MRE11 (meiotic recombination 11homolog) predict treatment-specific outcome of localised invasive bladder cancer. BJU Int, 2012. 110(11 Pt C): p. E1228-36.

11 Appendix

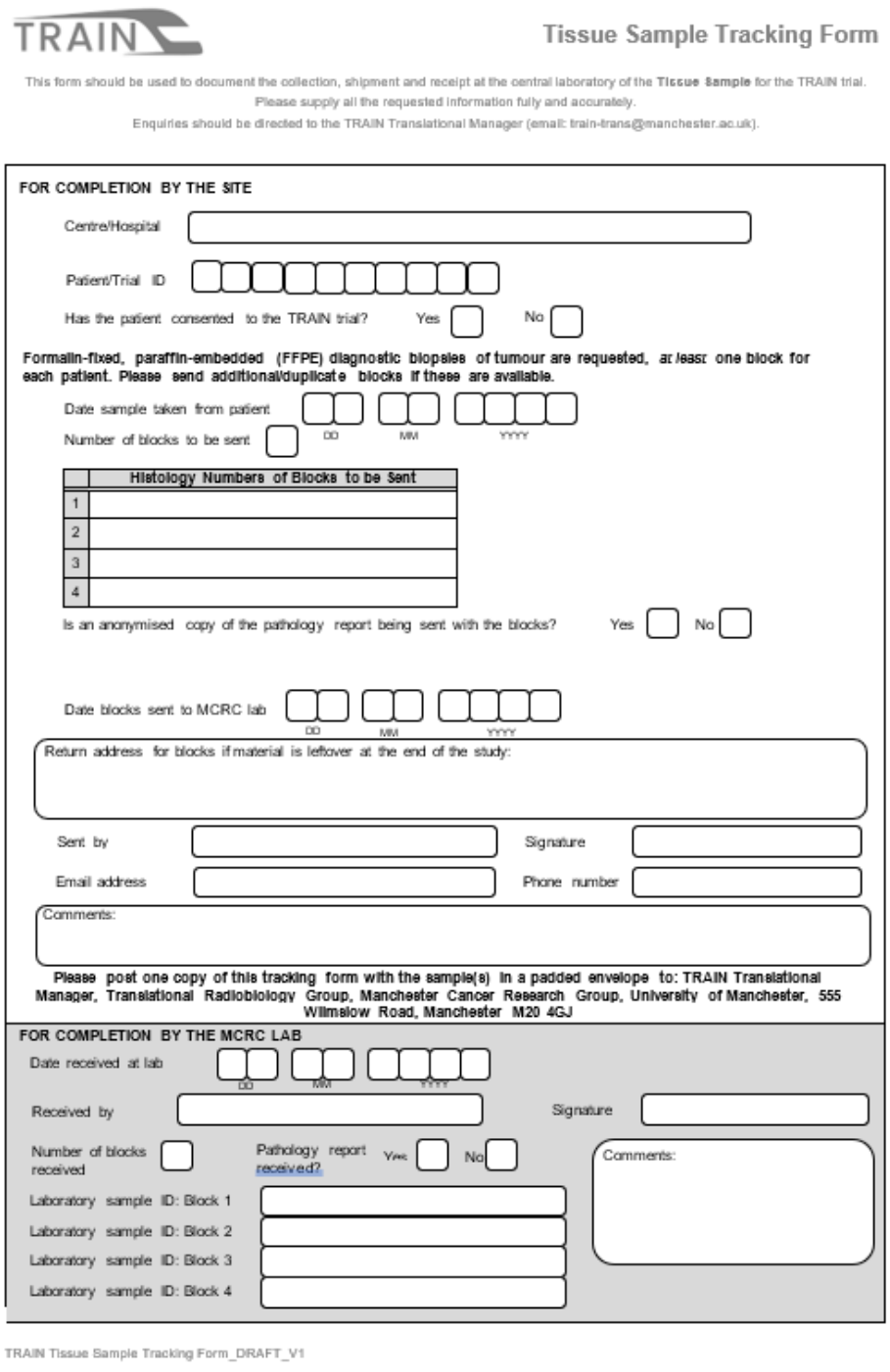
11.1 Train Sample Card

Sample Card – TRAIN

Please complete ALL SECTIONS of this form and return in the box with the samples

TRAIN Patient Trial ID			
Consent taken (Y/N)	Yes / No		
Date /Time sample taken *e.g. Date (dd/mm/yyyy) Time (hh:mm)*			
Collection time point *(e.g. Baseline)*			
Analysis type	EDTA	cfDNA	Urine
Site collected			
Research Practitioner Name			
Research Practitioner Email			
Comments			

11.2 Train Tissue Tracking Form



TRAIN

Tissue Sample Tracking Form

This form should be used to document the collection, shipment and receipt at the central laboratory of the **Tissue Sample** for the TRAIN trial.
Please supply all the requested information fully and accurately.
Enquiries should be directed to the TRAIN Translational Manager (email: train-trans@manchester.ac.uk).

FOR COMPLETION BY THE SITE

Centre/Hospital

Patient/Trial ID

Has the patient consented to the TRAIN trial? Yes No

Formalin-fixed, paraffin-embedded (FFPE) diagnostic biopsies of tumour are requested, at least one block for each patient. Please send additional/duplicate blocks if these are available.

Date sample taken from patient DD MM YYYY

Number of blocks to be sent

	Histology Numbers of Blocks to be Sent
1	
2	
3	
4	

Is an anonymised copy of the pathology report being sent with the blocks? Yes No

Date blocks sent to MCRC lab DD MM YYYY

Return address for blocks if material is leftover at the end of the study:

Sent by Signature

Email address Phone number

Comments:

Please post one copy of this tracking form with the sample(s) in a padded envelope to: TRAIN Translational Manager, Translational Radiobiology Group, Manchester Cancer Research Group, University of Manchester, 555 Wilmslow Road, Manchester M20 4GJ

FOR COMPLETION BY THE MCRC LAB

Date received at lab DD MM YYYY

Received by Signature

Number of blocks received

Pathology report received? Yes No

Comments:

Laboratory sample ID: Block 1

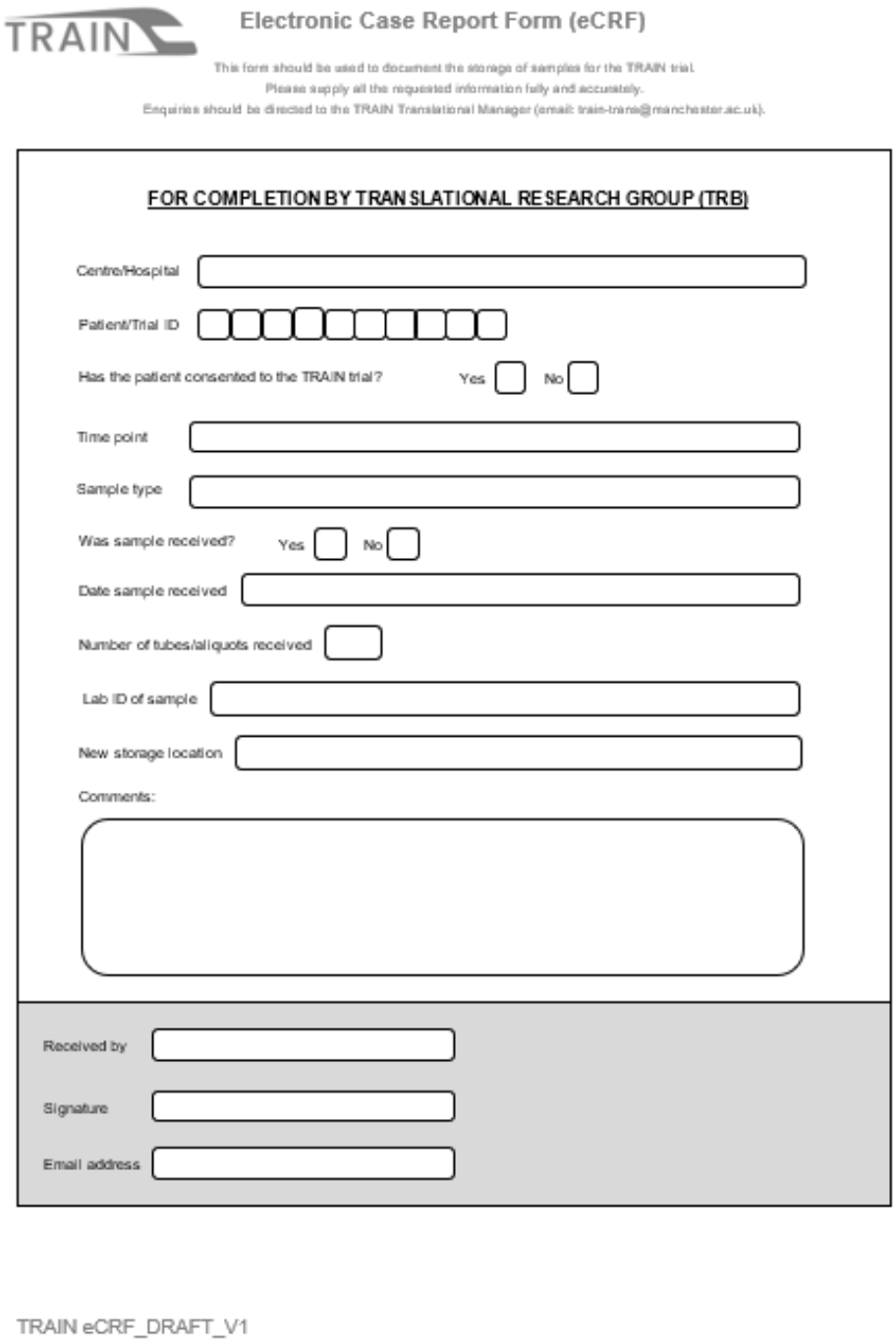
Laboratory sample ID: Block 2

Laboratory sample ID: Block 3

Laboratory sample ID: Block 4

TRAIN Tissue Sample Tracking Form_DRAFT_V1

11.3 TRAIN Electronic Case Report Form (eCRF)



TRAIN

Electronic Case Report Form (eCRF)

This form should be used to document the storage of samples for the TRAIN trial.
Please supply all the requested information fully and accurately.
Enquiries should be directed to the TRAIN Translational Manager (email: train-trans@manchester.ac.uk).

FOR COMPLETION BY TRANSLATIONAL RESEARCH GROUP (TRG)

Centre/Hospital

Patient/Trial ID

Has the patient consented to the TRAIN trial? Yes No

Time point

Sample type

Was sample received? Yes No

Date sample received

Number of tubes/aliquots received

Lab ID of sample

New storage location

Comments:

Received by

Signature

Email address

TRAIN eCRF_DRAFT_V1

11.4 TRAIN Translational Samples Dispatch Log

TRAIN

TRANSLATIONAL SAMPLES DISPATCH LOG – One log per patient			
Study short title:	TRAIN	**REC number:**	25/NM/0295
Site name:		**IRAS number:**	1012385
PI name:		**Sponsor:**	The Christie NHS Foundation Trust

Patient Initials		**Patient ID**	

Time Point		**Sample Type (Label Reference)**	**Quantity Expected**	**Quantity Sent**	**Date sample collected (DD-MMM-YYYY)**	**Date sent to MCRC Lab (DD-MMM-YYYY)**	**Royal Mail Tracking Number**	**Sent By Full Name and Signature**
Baseline	B1	Diagnostic FFPE block	1 or more (if applicable)					
	B1	EDTA (E)	1 x 10ml					
	B1	STRECK bloods (CF)	1 x 10ml					
	B1	Urine (U)	1 x 50ml					
12 weeks	T1	EDTA (E)	1 x 10ml					
	T1	STRECK bloods (CF)	1 x 10ml					
	T1	Urine (U)	1 x 50ml					
24 weeks	T2	EDTA (E)	1 x 10ml					
	T2	STRECK bloods (CF)	1 x 10ml					
	T2	Urine (U)	1 x 50ml					
Recurrence or end of trial	T3	EDTA (E)	1 x 10ml					
	T3	STRECK bloods (CF)	1 x 10ml					
	T3	Urine (U)	1 x 50ml					

TRAIN study samples to be sent to: TRAIN Study Team, Manchester Cancer Research Centre, The University of Manchester, 555 Wilmslow Road, Manchester, M20 4GJ
Diagnostic FFPE blocks should be sent with the TRAIN Tissue Sample Tracking Form and an anonymised copy of the pathology report (+Trial ID). eCRF and Sample Card should accompany blood and urine samples (Time point and Trial ID should be stated).
Please send a notification via email to train@soton.ac.uk and train-trans@manchester.ac.uk once samples have been sent Mon-Thurs and include sample details.