GUSTO

Gene expression subtypes of Urothelial cancer:
Stratified Treatment and Oncological outcomes

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Version 3.0 29th January 2024
IRAS 1005487
ISRCTN Number: 17378733
REC Reference: 22/WS/0153
Sponsor Reference: STH20710

Sponsor: **Sheffield Teaching Hospitals NHS Foundation Trust**

Funded by: **National Institute for Health Research (NIHR)**
Efficacy and Mechanism Evaluation (EME) Programme
(Ref: NIHR 128103)

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JOINTLY FUNDED BY
UKRI Medical Research Council
NIHR National Institute for Health and Care Research

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

The University Of Sheffield.

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2. Table of Contents

3. Trial Summary

Title	Gene expression subtypes of Urothelial cancer: Stratified Treatment and Oncological outcomes
Acronym	GUSTO
Background	The current standard of care for organ confined (T2-4a N0 M0) muscle invasive bladder cancer (MIBC), of urothelial cell histology, is radical pelvic treatment (either cystectomy or chemo-radiotherapy) following cisplatin based neoadjuvant chemotherapy (NAC) in those patients fit to receive it. NAC modestly improves survival in unselected MIBC patients with meta-analysis suggesting a 5-6% absolute benefit. However, individual outcomes are mixed: between 20-30% of patients with MIBC who undergo NAC will be found to have a pathological complete response (pCR, defined as pT0), whilst 40% remain locally advanced at cystectomy. Conversely, NAC might potentially be harmful in patients with chemo-resistant tumours by delaying radical treatment. In addition, NAC adds toxicity, cost and treatment complexity, including the potential for some long-term effects (e.g., ototoxicity, neurotoxicity). Single arm phase II neoadjuvant data has shown approximately 40% pCR rates to either monotherapy with atezolizumab or pembrolizumab. Proof of palliative benefit has been demonstrated in advanced disease (where immunotherapy is already a standard treatment option). Of note, all of this prior data is in an unselected patient population. However, as with chemotherapy, immunotherapy appears to benefit only a minority, substantially raising the unmet need for predictive biomarkers for patient selection. MIBC can be sub-classified using gene expression by utilising the Decipher Bladder (Veracyte, USA) commercial gene expression subtyping test for urothelial carcinoma. This test assigns an individual tumour to a molecular subtype using the expression profile of a panel of genes. In this method of classification, the mRNA levels of multiple genes, from ~1000 up to the whole transcriptome (all genes), are measured. The genes which then best delineate cases into discrete groups are then identified and their biological function can be assessed. The resultant gene expression subtypes have shared similarities to those present in other cancers and have biological phenotypes that appear to potentially differ in treatment sensitivity. The basal / squamous class has high PD-L1 and CTLA4 expression with immune cell infiltration consistent with potential for sensitivity to immunotherapy and cisplatin-based NAC. The luminal / luminal papillary class has a better prognosis and appears to respond poorly to NAC. The neuronal class has a poor prognosis and may benefit from combined chemo and immunotherapy. Clinical trial data has shown that the luminal infiltrated subtype may respond best to PD1/PD-L1 targeting immunotherapy. It is hypothesised that the use of gene expression subtyping to select which subsets of MIBC patients should receive NAC and/or immunotherapy, prior to radical cystectomy, is superior to unselected use of NAC for all cancers. It is considered that this can be incorporated within established timelines and processes for NHS care. Ultimately, this would maximise benefit within those patients selected to receive chemotherapy and/or immunotherapy. It would also spare toxicity and cost, and allow rapid transition to cystectomy, in those predicted not to benefit.

Population	Registration: Patients with MIBC (as determined by the local site urology, oncology and pathology teams) who are suitable for NAC with cisplatin and gemcitabine prior to radical cystectomy will be registered into the study. Randomisation: Patients with MIBC (as determined by the local site urology, oncology and pathology teams) who are suitable for NAC with cisplatin and gemcitabine prior to radical cystectomy and are assigned a gene expression subtype using the Decipher Bladder test.
Design	Multi-centre, prospective, open label, individually randomised, controlled, two-arm, parallel-group, multi-stage phase II trial.
Objective	The overarching aim of the GUSTO trial is to obtain information to understand whether this approach could improve outcomes, and therefore to guide the design of a subsequent phase III trial with respect to: • the feasibility of gene expression subtyping within routine NHS care; • the distribution of gene expression subtypes within United Kingdom NHS populations; • the heterogeneity of the intermediate endpoint (pCR as the primary efficacy endpoint in GUSTO) with respect to subtype following standard of care treatment (NAC); • to assess the activity of gene expression subtype guided neoadjuvant treatments for MIBC. Stage 1 objectives: • to assess the feasibility of recruitment • to confirm feasibility of the gene expression subtype process within routine NHS care by examining ▪ the time to return of gene expression subtype allocation ▪ the success rate of gene expression subtype allocation Stage 2 objectives: • to confirm the feasibility of recruitment • to assess assumptions concerning the proportion of each gene expression subtype • to assess the proportion of patients achieving pathologically proved complete response (pCR) in the standard care arm • confirmation / re-estimation of sample size Stage 3 objectives: • to assess the pCR rate in each gene expression subtype in the gene expression subtype-guided arm post-cystectomy
Intervention	Patients will be randomised on a 1:1 basis to receive: • standard neoadjuvant chemotherapy (cisplatin and gemcitabine) prior to radical cystectomy or • to have radical cystectomy with neoadjuvant and adjuvant chemotherapy and/or immunotherapy treatment determined based on the gene expression subtype assigned by the Decipher Bladder Genomic Classifier.

Sample size	The GUSTO trial aims to randomise 320 patients over a 3-year period (subject to a planned sample size re-estimation at 24 months). Given the estimated gene expression subtype proportions and the anticipated success rate of the gene expression subtype test, it is estimated that 458 patients will be required to be registered.
Follow-up	All patients will be followed up to one-year post-cystectomy as a minimum with a final assessment in all patients when the last patient has been followed up for one-year post-cystectomy.
Primary Endpoint	<u>Stage 1</u> • Recruitment • Time to return of gene expression subtype allocation • Gene expression subtype allocation success rate <u>Stage 2</u> • Recruitment • Gene expression subtype distribution • pCR rate in the standard care arm by gene expression subtype • Confirmation / re-estimation of sample size <u>Stage 3</u> • pCR rate by gene expression subtype in the gene expression subtype-guided arms post-cystectomy
Secondary Endpoints	<u>Stage 3:</u> Gene expression subtype endpoints • RNA quality and mass/yield • Gene expression subtype allocation success rate, re-test rate and repeat assay success rate • Time from patient consent to TURBT sample dispatch • Time to RNA extraction, processing & gene expression subtype allocation Clinical secondary endpoints • Disease-free survival at 12-months post-RC and at the end of trial • Overall survival at 12-months post-RC and at the end of the trial • Metastasis-free survival at 12-months post-RC and at the end of trial • Event-free survival at 12-months post-RC and at the end of trial • Histological outcomes • Quality of life • Patient acceptability to trial procedures • pCR stage by gene expression subtype in the standard and gene expression subtype-guided arms • To evaluate the outcomes from RC including time to RC, safety of RC and pathological completeness • To re-evaluate the stage 1 and 2 feasibility endpoints • Disease-free survival at 12-months post-RC for all consenting patients registered onto the study • Overall survival at 12-months post-RC for all consenting patients registered onto the study

	• Metastasis-free survival at 12-months post-RC for all consenting patients registered onto the study • Event-free survival at 12-months post-RC for all consenting patients registered onto the study Pharmacological secondary endpoints • Toxicity and tolerability • Treatment compliance
Exploratory Endpoints	• PD-L1 status of tumour and infiltrating immune cells in pre-existing diagnostic samples in all patients • Concordance of predicted subtypes for each sample across classifiers. • Assess the prediction of various MIBC classifiers with respect to pathologic complete response and 12 month survival
Translational Research	• Blood and tissue samples will be collected for future research.

Protocol Amendment Summary:

Protocol version	Protocol date	Description of amendment	Sections affected	Reason for amendment
1.0	27/09/2022	Original document	n/a	n/a
2.0	22/03/2023	Change to inclusion and exclusion criteria	Section 9.11	Response to grounds for non-acceptance
		Change to pregnancy testing requirements	Sections 9.12, 12.1, 12.6, 12.8, 12.18	
		Clarification regarding ECG testing	Sections 12.1, 12.3, 12.4, 12.8	
		Change to local laboratory assessments	Sections 12.1, 12.6, 12.8	
		Change to durvalumab and tremelimumab dose modification guidance	Appendix 4	
3.0	29/01/2024	Change to permit the use of adjuvant non-trial treatment in the standard of care arm	Section 6.1, Section 12.10	To accommodate changes in standard of care treatment for this patient population.
		Addition of registration sample size	Section 6	To facilitate the use of registration as record of recruitment.

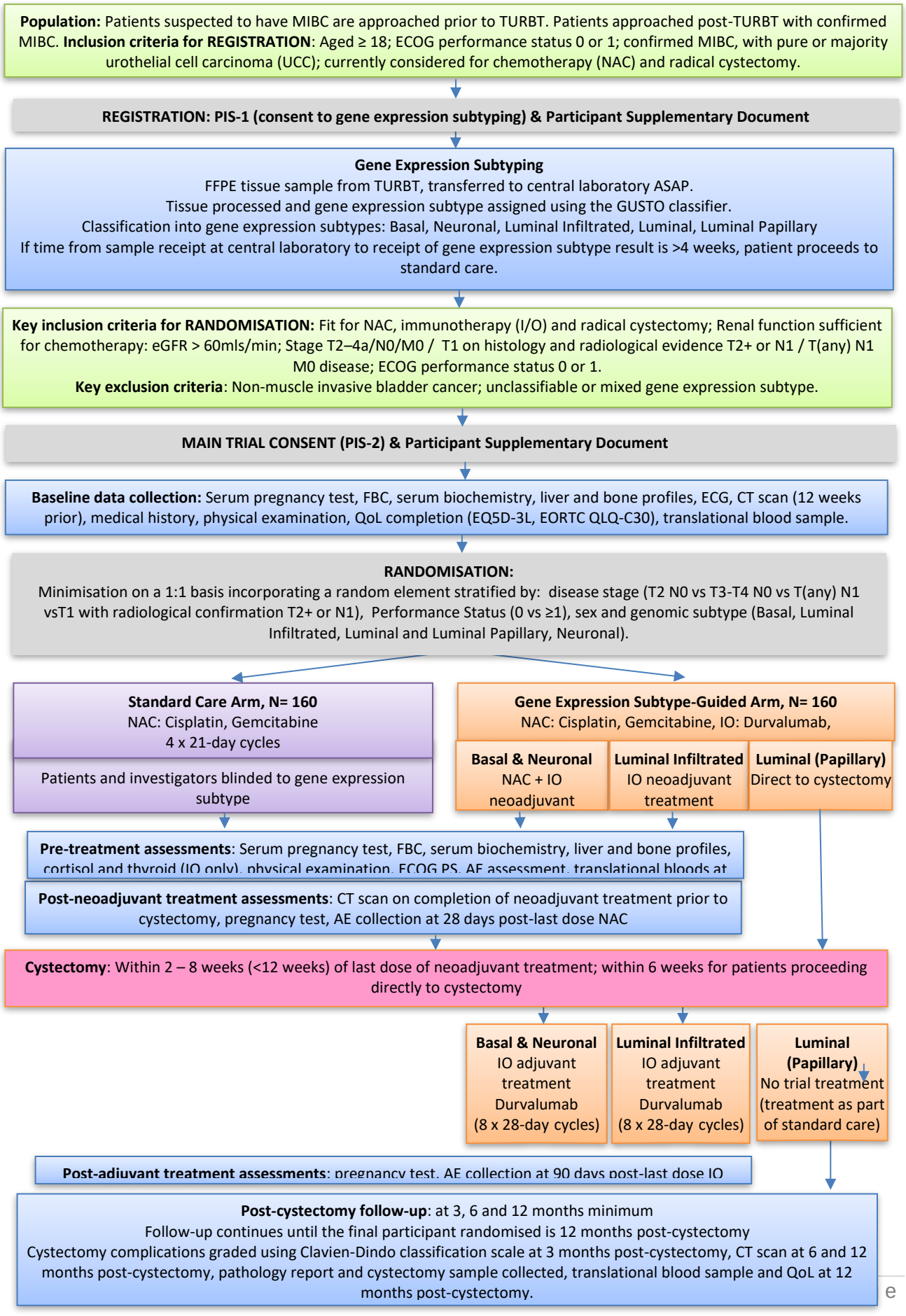
		Addition of ClassIntra intraoperative adverse event classification and assessments	Sections 12.17, 12.18, 13.3, 15.2, 15.4 and Appendix 5	For classification of intra-operative complications.
		Clarification that pregnancy testing must be carried out at 180 days post last dose of tremelimumab or at 90 days post last dose of durvalumab for women of childbearing potential who do not receive a hysterectomy with radical cystectomy (whichever occurs latest)	Section 9.12, 12.1, 12.6, 12.19	Updated in line with manufacturer's requirements.
		Clarification that pregnancy testing is to be carried out at 90 days post last dose of trial treatment for patients in the standard of care arm	Section 12.6	For clarification and consistency between protocol sections
		Clarification that patients who do not receive radical cystectomy should be withdrawn from trial treatment and follow-up	Sections 11.4, 12.14	For clarification
		Addition of Participant Identification Centres	Section 8.1.1	To facilitate patient recruitment from referral sites
		Corrections to Schedule of Events Tables to correct errors	Section 12.1	For correction
		Change to RSI for tremelimumab from tremelimumab IB to tremelimumab SPC	Section 13.2.1	To facilitate referral to the RSI for the combined tremelimumab and durvalumab treatment
		Clarification of post-RC assessments and additional collection of local PD-L1 assessment	Section 12.10	
		Change to registration and randomisation inclusion criteria to include patients with T1 on histology and	Sections 3, 6, 9.6.1, 9.11.1, 9.14.1, 12.2, Figure 1	To permit the inclusion of patients in cases where MIBC is not confirmed histologically due to

		radiological evidence of T2+ or N1 cancer		under sampling at TURBT
		Correct of typographical errors throughout. Addition of REC and ISRCTN numbers.	various	
		Additional contraception guidance	Appendix 3	Updated in line with manufacturer's guidance
		Updated dose modification guidance for immunotherapy	Appendix 4	Updated in line with manufacturer's guidance
		Addition to post-randomisation assessments: an anonymised copy of the CT/MRI report will be collected	Sections 12.3, 12.16, 18.2	For central monitoring of eligibility
		Collection of participant email address at registration	Sections 9.7, 20.0	For administering electronic quality of life questionnaires via the REDCap database
		Clarification of exploratory objectives and endpoints relating to concordance of predicted subtypes across classifiers and prediction of pCR and OS across other classifiers	Sections 3.0, 7.2, 15.3	For clarification
		Addition of coeliac disease controlled by diet alone as a permitted exclusion to exclusion criterion 5.	Section 9.11.2	Updated in line with manufacturer's guidance
		Clarification that the translational analysis of tissue and blood samples will be subject to separate ethical and/or regulatory review and approval	Section 12.24	For clarification
		Change to permit combined chemotherapy and immunotherapy	Section 10.2.3, Section 12.1.2	To accommodate logistical arrangements at

		treatment to be given over 2 days		some participating sites
		Change to permit the 28 day timeline to reset when a repeat TURBT surgery is required as part of local standard practice	Section 9.11.2, Section 11.1	To clarify timelines to accommodate local standard practice
		Addition of further permitted exceptions to the excluded concomitant medications	Section 11.3.2.2	Updated in line with manufacturer's requirements
		Addition of guidance relating to dose delays for logistical reasons and clarification that immunotherapy dose delays are permissible for logistical reasons	Section 11.3.4	Updated to provide guidance to sites
		Clarification that patients with creatinine clearance 40-60 ml/min are eligible only if split dose cisplatin is given on days 1 and 8 of neoadjuvant treatment and that creatinine clearance below 60mL/min must result in the administration of split dose cisplatin	Section 9.11.1, Section 11.3.4.1	Updated to confirm guidance to sites
		Rewording of randomisation inclusion criterion 13 and addition of an exclusion criterion to document that participants in GUSTO are not permitted to participate in in another clinical study with an investigational product during GUSTO trial participation and follow-up.	Section 9.11.2	Updated on request of the manufacturer
		Amendment to the randomisation process which will be carried out by CTRU with information provided by site and the central laboratory.	Section 9.14	To reflect the requirements of the randomisation system while maintaining the blinding of sites to the subtype result

		Clarification of neoadjuvant pre-treatment assessments	Section 12.4	Separation of day 8 assessments to make this clearer
		Change to SAE reporting procedure to clarify that the main route of reporting is via the MACRO Remote Data Entry Database	Section 1; Section 13.4.5	For correction
		Addition of data collection at the end of the trial for participants who have progressed	Section 12.14	To capture outcome information for this patient population
		Clarification that GCP training is requires for staff responsible for trial activities as appropriate	Section 8.3	For clarification
		Clarification of the timeframe for readmission post radical cystectomy	Section 15.2; Section 15.4	For clarification
		Clarification that the PD-L1 analysis may involve a third party service provider	Section 22.1	Updated information

Figure 1: Trial Schema



4. Abbreviations

Abbreviation	Definition
AChE	Acetylcholinesterase
ACTH	Adrenocorticotropic hormone
AE	Adverse Event
AR	Adverse Reaction
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
ASCO	American Society of Clinical Oncology
AST	Aspartate aminotransferase
BC	Bladder cancer
(T)Bili	(Total) Bilirubin
BNP	B-type natriuretic peptide
BUN	Blood urea nitrogen
CI	Confidence Interval
CHI	Community Health Index Number
CRF	Case Report Form
CRP	C-reactive protein
CT	Computerised Tomography
CTC	Common Toxicity Criteria
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Cytotoxic T-lymphocyte antigen-4
DFS	Disease-free survival
DMEC	Data monitoring and ethics committee
DSUR	Development safety update report
DVT	Deep vein thrombosis
ECG	Electrocardiogram
ECHO	Echocardiogram
ECOG	Eastern Co-operative Oncology Group
EGFR	Epidermal growth factor receptor
ESMO	European Society of Medical Oncology
EORTC QLQ-C30	European Organisation for Research and Treatment of Cancer Core Quality of Life questionnaire
eCRF	Electronic case report form
EQ-5D-3L	Standardised instrument for use as a measure of health status
FBC	Full blood count
FFPE	Formalin-fixed paraffin-embedded
GCP	Good clinical practice
G-CSF	Granulocyte colony stimulating factor
GDPR	UK General Data Protection Regulations
GI	Gastrointestinal
GP	General Practitioner

HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDU	High dependency unit
HgA1c	Hemoglobin A1C
HR	Hazard Ratio
HRA	Health Research Authority
HRQOL	Health-related Quality of Life
IB	Investigator Brochure
ICD	International Statistical Classification of Diseases and Related Health Problems
ICI(s)	Immune checkpoint inhibitor(s)
IFN-γ	Interferon-γ
ILD	Interstitial lung disease
imAE(s)	immune-mediated adverse event(s)
IMP	Investigational medicinal product
IRAS	Integrated Research Application System
ISF	Investigator Site File (This forms part of the TMF)
ISRCTN	International Standard Randomised Controlled Trials Number
ITU	Intensive care unit
ITT	Intention-to-treat
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system
IV	intravenous
IVDD	In vitro diagnostic device
kg	Kilogram
LDH	Lactate dehydrogenase
LFTs	Liver function tests
LLN	Lower limit of normal
MDT	Multi-disciplinary team
MedDRA	Medical Dictionary for Regulating Activities
mg	Milligram
MHRA	Medicines and Healthcare and products Regulatory Authority
MIBC	Muscle invasive bladder cancer
ml	Millilitre
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NAC	Neoadjuvant cisplatin
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NMIBC	Non-muscle invasive bladder cancer
OS	Overall survival
pCR	Pathological complete response
PD1	Programmed cell death 1

PD-L1	Programmed cell death ligand 1
PFS	Progression-free survival
PIC	Participant Identification Centre
PJP	Pneumocystis jerovicii pneumonia
PO	By mouth
PS	Performance status
PSF	Pharmacy site file
RC	Radical Cystectomy
REC	Research ethics committee
RNA	Ribonucleic acid
RSI	Reference safety information
RUT	Reconstruction of the upper urinary tract
SAE	Serious adverse event
SAP	Statistical analysis plan
SAR	Serious adverse reaction
SC	Serious complications
SCAR	Severe cutaenous adverse reaction
SITC	Society for Immunotherapy of Cancer
SITraN	Sheffield Institute for Translation Neuroscience
SJS	Stephen Johnson Syndrome
SmPC	Summary of Product Characteristics
SUSAR	Suspected unexpected serious adverse reaction
TCR	T-cell receptor
TCGA	The Cancer Genome Atlas
T1DM	Type 1 diabetes mellitus
T3	Triiodothyronine
T4	Thyroxine
TEN	Toxic epidermal necrolysis
TKI	Tyrosine kinase inhibitors
TMG	Trial management group
TMN	Tumour-Node-Metastasis
TNF	Tumour necrosis factor
TSC	Trial steering committee
TSH	Thyroid stimulating hormone
TURBT	Transurethral resection of a bladder tumour
UCC	Urothelial cell carcinoma
ULN	Upper limit of normal
USC	Unexpected serious complications
RC	Radical Cystectomy
IC	Ileal Conduit
WOCBP	Woman of child-bearing potential

5. Background

5.1. Bladder cancer

Bladder cancer (BC) is a common disease with approximately 10,000 new cases and 5,500 deaths each year, and 69,000 people living with the malignancy in the UK (1). Across Europe, we spend nearly 5 billion Euros on this cancer annually (2, 3) and globally it is the seventh most prevalent cancer (4). However, bladder cancer patients have poor patient experiences (5-7) and survival rates from MIBC are not improving (8-11).

Bladder cancer is best stratified according to pathological grade. Low grade cancers account for around 60% of new cases and are characterised by frequent local recurrence within the bladder, but very little progression and few distant metastases. Consequently, disease specific mortality rates are low and overall survival rates approach those of the general population. In contrast, high grade bladder cancer is an aggressive disease. It may be detected before or after the onset of muscle invasion.

Mortality rates approach 50% at 5 years despite radical local and systemic therapies. Given this natural history of high-grade invasive bladder cancer, patients who are suitable and fit enough, should receive radical treatment, in the form of either surgery (radical cystectomy) or radiotherapy. Radical cystectomy involves the removal of the bladder, local lymph nodes and adjacent organs. This includes the prostate and seminal vesicles in men, and the ovaries, uterus and fallopian tubes in females (12). Urinary drainage is then reconstructed through either a stoma or neobladder.

Treatment failure after radical cystectomy can be common and varies according to tumour stage, lymph node status and surgical margins (13) Improvements in outcomes can be achieved by combining surgery with systemic treatment.

5.2. Treatment of Muscle Invasive Bladder cancer

5.2.1. Standard treatment for muscle invasive bladder cancer

The current standard of care for organ confined (T2-4a N0 M0) muscle invasive bladder cancer (MIBC), of urothelial cell histology, is radical pelvic treatment (either cystectomy or radiotherapy) following cisplatin based neoadjuvant chemotherapy (NAC) in those patients fit to receive it (‘cisplatin eligible’) (14). There is less data to guide treatment decisions for patients staged with T(any) N1 M0 disease, but most investigators would favour a radical approach to treatment, supported by guidelines (14). NAC modestly improves survival in unselected MIBC patients with meta-analysis suggesting a 5-6% absolute benefit (15-17). However, individual outcomes are mixed: between 20-30% of patients with MIBC who undergo NAC will be found to have a pathological complete response (pCR), defined as pT0 (18, 19)), whilst 40% remain locally advanced at cystectomy. Achieving a pCR at cystectomy is a surrogate marker for subsequent long-term disease-free status (17, 20). Conversely, NAC might potentially be harmful in patients with chemo-resistant tumours by delaying radical treatment. In addition, NAC adds toxicity, cost and treatment complexity, including the potential for some long-term effects (e.g., ototoxicity, neurotoxicity).

5.2.2. Immunotherapy

Multiple unselected peri-operative phase III immunotherapy trials are in progress for this disease (e.g. NCT03732677, NCT03661320, NCT03472274, NCT02736266). These are based on single arm phase II neoadjuvant data showing approximately 40% pCR rates to either monotherapy with atezolizumab or pembrolizumab (21, 22), encouraging data from dual PD1/CTLA4 blockade (23, 24) and proof of palliative benefit in advanced disease (25-29) (where immunotherapy is already a standard treatment option). Of note, all of this prior data is in an unselected patient population. However, as with chemotherapy, immunotherapy appears to benefit only a minority, substantially raising the unmet need for predictive biomarkers for patient selection.

5.2.2.1. Durvalumab

Durvalumab is a human monoclonal antibody of the immunoglobulin G1 kappa subclass that blocks the interaction of programmed cell death ligand-1 (PD-L1) (but not programmed cell death ligand-2) with programmed death-1 (PD-1) on T cells and CD80 (B7.1) on immune cells. The proposed mechanism of action for durvalumab is interference in the interaction of PD-L1 with PD-1 and CD80 (B7.1). Blockade of PD-L1/PD-1 and PD-L1/CD80 interactions releases the inhibition of immune responses, including those that may result in tumour elimination. In vitro studies demonstrate that durvalumab antagonises the inhibitory effect of PD-L1 on primary human T cells resulting in the restored proliferation of interferon-γ (IFN-γ) (30). In vivo studies have shown that durvalumab inhibits tumour growth in xenograft models via a T cell dependent mechanism (30). Based on these data, durvalumab is expected to stimulate the participant's antitumour immune response by binding to PD L1 and shifting the balance toward an antitumour response. Durvalumab has been engineered to reduce antibody dependent cellular cytotoxicity and complement-dependent cytotoxicity. As of October 2021, durvalumab has been given to more than 12874 participants as part of ongoing studies either as monotherapy or in combination with other anticancer agents.

Durvalumab is approved in the UK and Europe for treatment of non-small cell lung cancer as monotherapy or in combination with chemotherapy (31). It is also licenced by the FDA for patients with locally advanced or metastatic urothelial carcinoma who either have disease progression during or following platinum-containing chemotherapy, or have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy (32).

5.2.2.2. Tremelimumab

Tremelimumab is a human immunoglobulin G2 monoclonal antibody that is directed against CTLA-4; CD152, a cell surface receptor that is expressed primarily on activated T cells and acts to inhibit their activation. Tremelimumab completely blocks the interaction of human CTLA-4 with CD80 and CD86, resulting in increased release of cytokines (interleukin-2 and IFN-γ) from human T cells, peripheral blood mononuclear cells and whole blood (33).

Tremelimumab is under investigation for use in the treatment of cancer.

5.2.2.3. Combination PD-1/PD-L1 and CTLA4 inhibition for urothelial carcinoma

The mechanisms of action of CTLA-4 and PD-1 are non-redundant, targeting both PD-1 and CTLA-4 pathways. Therefore, they may have additive or synergistic activity (34); and the use of durvalumab and tremelimumab in combination therapy for the treatment of cancer is being

investigated. To date, more than 3000 participants have received the combination using a number of doses and dosing schedules.

Two phase II trials have evaluated combined PD-1/PD-L1 and CTLA4 blockade in a neoadjuvant setting for urothelial carcinoma prior to radical cystectomy (23, 24). In both trials, the toxicity profiles were consistent with that seen within the now extensive experience with these, and similar, agents.

Gao et. al., evaluated durvalumab and tremelimumab in a two-arm trial testing different dosing schedules. Patients were cisplatin ineligible (a group with a less favourable prognosis to the GUSTO trial) with UC suitable for treatment with radical intent but having high-risk features defined as bulky tumours, variant histology, lymphovascular invasion, hydronephrosis or high-grade upper tract disease (23). Cohort 1 (n=28) has been reported and received durvalumab 1500 mg plus tremelimumab 75 mg every 4 weeks for two cycles. (Cohort 2 (n=17) received durvalumab 1500 mg plus tremelimumab 300 mg x 1 dose and then only durvalumab at 1500 mg 4 weeks later). The primary endpoint was safety. Six patients (21%) experienced CTCAE grade ≥3 immune-related adverse events. These were asymptomatic laboratory abnormalities (n = 4), hepatitis and colitis (n = 2). Complete pathological response was seen in 37.5% of the participants with down staging to ≤pT1 in 58% of patients who underwent surgery (n = 24).

The NABUCCO study treated 24 patients in a single arm study to evaluate two doses of neoadjuvant nivolumab (1 mg/kg, weeks 1 and 4) and ipilimumab (3 mg/kg, weeks 4 and 7) (24). The authors studied the ability to complete neoadjuvant immunotherapy within a 12 week window prior to surgery, in patients ineligible or refusing chemotherapy. All but one patient received surgery on time, including 46% who had a complete pathological response with 14 (58%) having no remaining invasive disease (pCR or pTisN0/pTaN0). Grade ≥3 immune-related adverse events occurred in 55% of patients (41% after excluding clinically insignificant laboratory abnormalities).

In the first line metastatic setting, the DANUBE trial has reported outcomes from 1032 patients randomised to durvalumab (n=346), durvalumab plus tremelimumab (n=342) or chemotherapy (n=344) as a first-line treatment for metastatic urothelial carcinoma (29). Median follow-up for survival was 41.2 months (IQR 37.9–43.2) for all patients. With respect to the co-primary endpoints in the intention-to-treat population:

In a sub-population with high PD-L1 expressing cancers, median overall survival (OS) was 14.4 months (95% CI 10.4–17.3) in the durvalumab monotherapy group (n=209) versus 12.1 months (10.4–15.0) in the chemotherapy control group (n=207; hazard ratio 0.89, 95% CI 0.71–1.11; p=0.30).

In unselected patients, median overall survival was 15.1 months (13.1–18.0) in the durvalumab plus tremelimumab group versus 12.1 months (10.9–14.0) in the chemotherapy group (0.85, 95% CI 0.72–1.02; p=0.075).

Whilst the trial failed to meet either primary outcome, secondary analysis suggested that durvalumab plus tremelimumab was superior to chemotherapy in those with PD-L1 high expressing cancers (HR 0.74; 95% CI 0.59–0.93). This trial therefore supports a hypothesis that combination immunotherapy will be an effective intervention in some patients but that selection strategies are required to optimise treatment strategies.

In addition, in the peri-operative setting, two further trials have presented data for adjuvant immunotherapy in unselected patient groups. CheckMate 274 demonstrated an improvement in median disease free survival (DFS) from 20.8 months (95% CI, 16.5 to 27.6) with nivolumab compared to 10.8 months (95% CI, 8.3 to 13.9) with placebo (hazard ratio 0.70; 98.22% CI, 0.55 to 0.90; P<0.001) (35). The IMvigor010 trial did not result in an improvement in DFS with the use of adjuvant atezolizumab (median DFS 19.4 months (95% CI 15.9–24.8) with atezolizumab versus 16.6 months (11.2–24.8; hazard ratio 0.89 (95% CI 0.74–1.08); p=0.24) (36). These data are supportive of a strategy to identify subsets for which use of immunotherapy is justified in the peri-operative setting.

Full information the safety profile, chemistry, pharmacology, mechanism of action and efficacy of durvalumab and tremelimumab combination therapy is included in the current editions of the durvalumab Summary of Product Characteristics and tremelimumab Investigator Brochure.

5.2.2.4. Tremelimumab and durvalumab in combination with cisplatin and gemcitabine

Tremelimumab and durvalumab have been evaluated in combination with platinum doublet chemotherapy, including cisplatin and gemcitabine, in advanced solid tumours, in the IND226 trial (37). This large multi-centre phase Ib trial established a recommended phase II dose for durvalumab, with or without tremelimumab, with various chemotherapy combinations using a dose escalation design. 136 patients were enrolled. Most drug-related adverse events were ≤ grade 2 and attributable to chemotherapy. Toxicity considered related to immunotherapy was mainly ≤ grade 2 and with the most frequent relating to colitis/diarrhoea, skin rash and thyroid dysfunction. Durvalumab and tremelimumab exposures did not appear affected by chemotherapy. Anti-tumour activity was observed across cancer and PD-L1 expression subtypes. Durvalumab at 1500 mg q3w with tremelimumab 75 mg q3w could be safely combined with platinum-doublet chemotherapy, including with gemcitabine at 1250 mg/m^2 day 1 and 8 combined with cisplatin 70 mg/m^2 both q3w.

5.2.2.5. Rationale for fixed dosing

A population pharmacokinetic model was developed for durvalumab using monotherapy data Study 1108 (N = 292; doses = 0.1 to 10 mg/kg q2w or 15 mg/kg q3w; solid tumours). Population pharmacokinetic analysis indicated only minor impact of body weight on the pharmacokinetics of durvalumab (coefficient of ≤ 0.5). The impact of body weight-based (10 mg/kg q2w) and fixed dosing (750 mg q2w) of durvalumab was evaluated by comparing predicted steady state PK concentrations (5th, median and 95th percentiles) using the population pharmacokinetic model. A fixed dose of 750 mg was selected to approximate 10 mg/kg (based on median body weight of ~75 kg). A total of 1000 participants were simulated using body weight distribution of 40 to 120 kg. Simulation results demonstrate that body weight-based and fixed dosing regimens yield similar median steady state PK concentrations with slightly less overall between-participant variability with fixed dosing regimen.

Similarly, a population pharmacokinetic model was developed for tremelimumab using data from phase 1 through phase 3 (N = 654; doses = 0.01 to 15 mg/kg q4w or every 90 days; metastatic melanoma) (38). Population pharmacokinetic modelling indicated a minor impact of body weight on the pharmacokinetics of tremelimumab (coefficient of ≤ 0.5). The weight-based (1 mg/kg q4w) and fixed dosing (75 mg/kg q4w; based on median body weight of ~75 kg) regimens were compared using predicted PK concentrations (5th, median and 95th

percentiles) using population pharmacokinetic model in a simulated population of 1000 participants with body weight distribution of 40 to 120 kg. Similar to durvalumab, simulations indicated that both body weight-based and fixed dosing regimens of tremelimumab yield similar median steady state pharmacokinetic concentrations with slightly less between-participant variability with fixed dosing regimen.

Similar findings have been reported by others (39-42). Wang and colleagues investigated 12 mAbs and found that fixed and body size-based dosing perform similarly, with fixed dosing being better for 7 of 12 antibodies (41). In addition, they investigated 18 therapeutic proteins and peptides and showed that fixed dosing performed better for 12 of 18 in terms of reducing the between-participant variability in pharmacokinetic/pharmacodynamic parameters (42).

A fixed dosing approach is preferred by the prescribing community due to ease of use and reduced dosing errors. Given the expectation of similar pharmacokinetic exposure and variability, it was considered it feasible to switch to fixed dosing regimens.

In those patients allocated to receive immunotherapy (either alone or in combination with NAC), fixed doses will be used as follows:

- Neoadjuvant
    - Durvalumab: 1500 mg on day 1, every 21 days, for 4 cycles
    - Tremelimumab: 75 mg on day 1, of cycles 1 and 3 only
- Adjuvant
    - Durvalumab: 1500 mg on day 1, every 28 days, for 8 cycles

5.3. Gene expression subtype guided care in muscle invasive bladder cancer

Most cancers are currently classified histologically according to their microscopic features. In addition, information about their biological behaviour may be added through techniques such as immunohistochemistry which allows semi-quantitative measurement of individual protein abundance within a tissue sample. More recently, high-throughput technologies have entered clinical use to better stratify patients for existing or novel therapy: RAS oncogene mutations in metastatic colorectal cancer are used to stratify patients for targeted therapies. In breast cancer the Prosigna and Oncotype DX gene expression profiling tests are used to stratify patients for post-operative chemotherapy (43). Both tests are performed on formalin-fixed paraffin-embedded (FFPE) tissue removed as part of biopsy or resection.

Histologically similar MIBC can also be sub-classified using gene expression signatures (44, 45). In this method of classification, the mRNA levels of multiple genes, from ~1000 up to the whole transcriptome (all genes), are measured. The genes which then best delineate cases into discrete groups are then identified and their biological function can be assessed. In MIBC the names given to gene expression subtypes reflect the phenotype of normal urothelium that the subtype most resembles. For example, MIBC classified as ‘luminal’ expresses genes associated with the most differentiated / mature layer of the urothelium – luminal cells. By contrast, basal / squamous tumours express genes found in the basal (closest to the underlying muscle) cells of the urothelium. Gene expression subtypes can also incorporate information about tumour immune infiltration. Tumour associated lymphocytes express specific genes which can be factored into a classification system.

The resultant gene expression subtypes have shared similarities to those present in other cancers (46) and have biological phenotypes that appear to potentially differ in treatment sensitivity (45, 47-49). The basal / squamous class has high PD-L1 and CTLA4 expression with immune cell infiltration consistent with potential for sensitivity to immunotherapy and cisplatin-based NAC (45). The Luminal / Luminal Papillary class has a better prognosis and appears to respond poorly to NAC. The Neuronal class has a poor prognosis and may benefit from combined chemo and immunotherapy. Clinical trial data has furthered knowledge by identifying that the luminal infiltrated subtype may respond best to PD1/PD-L1 targeting immunotherapy (28, 50, 51). These proposed associations between gene expression subtype and systemic chemo- and immuno-therapy response are enticing as a means for a precision use of peri-operative therapy. To date, no prospective randomised trial has tested this.

5.3.1. Gene expression classifiers

Gene expression classifiers for MIBC have been described by multiple groups (44). These classifiers have been used to predict overall survival, disease-free survival (DFS) and response to chemotherapy in retrospective cohorts. A gene expression classifier labels samples as belonging to a specific subtype, often using a linear combination of gene expression values, or a non-linear combination in the case of some machine learning algorithms. Each classifier labels using a different nomenclature for subtype names, and the number of subtype varies between classifiers. In addition, there are differences in the choice of genes in each classifier and each gene's relative weight in assigning a patient's tumour to a given molecular subtype. As a classifier is essentially a collection of genes and describes how the expression level of those genes should be used to label samples, a classifier can be applied to any gene expression dataset produced from the same protocols as those used to initially train the classifier. To this end, Kamoun and colleagues (44) collated a consensus classification of MIBC subtypes by comparing multiple classifiers.

Whilst there is agreement regarding the existence of MIBC molecular gene expression subtypes, the optimal classifier methodology is currently unclear (52). This paradigm was seen in other cancers (53). For MIBC, Seiler et al. have reported outcomes, in a retrospective non-randomised analysis, with or without NAC, stratified using the Decipher platform (54). The basal subtype represented one third of the cohort and had lower survival rates without NAC (e.g. mortality HR 2.2 (95% CI, 1.3-3.7) than luminal cancers). Survival was improved in those patients that had received NAC, supporting prior data that the basal subtype is chemo-sensitive. Conversely, survival in luminal and other gene expression subtypes did not appear to be improved in those patients that had received NAC, suggesting chemo-resistance and potentially a benefit from immediate radical treatment or addition of neoadjuvant immunotherapy. A follow up analysis compared subtypes in MIBC sampled before and after NAC and identified subtype switching in around half, potentially reflective of clonal selection for chemo-resistant cells (55).

Four studies have retrospectively assessed how gene expression subtypes predict response to neoadjuvant chemotherapy (NAC)(49, 54, 56, 57). A key result from these studies is that neoadjuvant chemotherapy improves overall survival in patients with basal-like tumours. In contrast, patients with luminal-type tumours see no such benefit from NAC. Three of these studies used the Decipher bladder gene expression platform and classifier. This is currently the only commercially available assay that can predict response to NAC. Importantly, this assay can produce subtypes described by The Cancer Genome Atlas (TCGA) trained classifiers, which most closely aligns with the consensus classification described above. The

TCGA classifier was developed from the largest cohort of MIBC cases to date and was validated for use in predicting response to NAC in a separate cohort of 343 cases (54).

5.4. Decipher bladder platform

Decipher Bladder (Veracyte, USA) is a commercial gene expression subtyping test for urothelial carcinoma. This test assigns an individual tumour to a molecular subtype using the expression profile of a panel of genes, as determined by the GeneChip® Human Exon 1.0 ST Array (Affymetrix) microarray. To date, Decipher Bladder has been studied in various retrospective cohorts with differing objectives and primary outcomes. As stated above, Seiler et al. found Decipher could identify a cohort of tumours with a poor prognosis that respond to chemotherapy, compared to others without an apparent improvement in survival with chemotherapy (54).

In a follow up study, the same group reported that Decipher Bladder identified a shift in gene expression subtype following chemotherapy, suggesting that some tumours are characterised by heterogenous cell populations and chemotherapy can select resistant clones (57). Within the radical cystectomy context, Lotan et al. reported Decipher genotyping identified luminal and luminal papillary tumours that were less likely to be upstaged (final stage after radical cystectomy compared to pre-operative clinical TURBT stage) than non-luminal tumours (58). Decipher Bladder also appears to have a prognostic ability with radiotherapy. For example, Efstathiou et al. reported genotyping patterns in 136 invasive bladder tumours receiving Bladder-sparing trimodality therapy (59). The authors found stromal expression profiles were associated with lower disease specific survival, and that inflammatory gene expression subtypes (T cell activation and interferon gamma signalling) were associated with improved survival. Most recently, Necchi et al. used the Decipher Bladder platform to gene expression subtype the tumours in patients receiving neoadjuvant immunotherapy with pembrolizumab (60). They found higher rates of complete response (CR) in tumours with a basal and luminal infiltrated gene expression subtypes with pembrolizumab (20/30 cases). Currently, the Decipher assay is used within the USA (CAP, CLIA and various state licences), although there has been no prospective validation to test its predictive performance.

5.5. Hypothesis and Trial Rationale

The over-arching hypothesis for the GUSTO trial is that use of gene expression subtyping to select which MIBC patients should receive NAC and/or immunotherapy (using a durvalumab / tremelimumab combination), prior to radical cystectomy, will be superior to unselected use of NAC for all cancers. We hypothesise that this can be incorporated within established timelines and processes for NHS care and does not affect the ability to perform radical surgery. Ultimately, this would maximise benefit within those patients selected to receive chemotherapy and/or immunotherapy. It would also spare toxicity and cost, and allow rapid transition to cystectomy, in those predicted not to benefit.

6. Trial Design

GUSTO is a multicentre, prospective, open-label, individually randomised, controlled, parallel-group, multi-stage phase II trial of patients with T2-4a N0 M0 MIBC, T(any) N1 M0 MIBC or T1 on histology with radiological evidence of T2+ or N1 who are suitable for NAC with cisplatin and gemcitabine prior to radical cystectomy. The trial will assess whether the use of gene expression subtype stratified care using the GUSTO Classifier (Decipher Bladder platform and TCGA 2 classification system), to assign patients to whether they receive NAC and/or

immunotherapy (durvalumab / tremelimumab), demonstrates sufficiently improved treatment activity to warrant a phase III trial.

Stage 1 will assess the feasibility of both recruitment and the embedding of gene expression subtype stratification into the clinical pathway; stage 2 will confirm feasibility of recruitment and assess the assumptions for the sample size calculation and stage 3 will involve an assessment of treatment outcomes using this stratified approach. Movement between trial stages will be dependent upon the meeting of pre-defined progression criteria.

The trial plans to randomise 320 participants over a 3-year period (this is estimated to require the registration of 458 patients). The total randomised sample size will be confirmed or re-estimated at the end of stage 2 (see Section 17.3.2). The final randomised sample size will depend on whether the frequencies of gene expression subtype and pCR rate in the standard care arm are as assumed based on available evidence, and whether the trial continues to stage 3. The sample size proposed (320) is based on a single-arm assessment within the gene expression subtype-guided arm, where the randomisation to the standard care arm will enable the correct design parameters to be estimated and a secondary unpowered randomised comparison to be carried out. Conducting a larger phase II study with powered randomised comparisons is considered infeasible in a reasonable timeframe.

Eligible participants will be randomised via minimisation in a 1:1 ratio to receive either neoadjuvant chemotherapy (cisplatin and gemcitabine) prior to radical cystectomy (standard treatment) or to have radical cystectomy with neoadjuvant and adjuvant chemo/immunotherapy treatment (cisplatin, gemcitabine, durvalumab and tremelimumab) determined based on the gene expression subtype assigned by the GUSTO Classifier.

Participants in the standard care arm (and local investigators) will remain blinded to gene expression subtype category.

Outcomes will be collected at cystectomy and at 3, 6, and 12 months post-cystectomy.

6.1. Trial Overview

GUSTO will recruit over two phases: registration and randomisation.

Within the registration phase, patients will be identified for the study if they are considered to have confirmed MIBC (following TURBT) or clinically probable MIBC based on cystoscopic assessment (prior to TURBT).

Those patients with confirmed MIBC following TURBT will be registered into the study and their diagnostic tumour samples will be sent for gene expression microarray analysis and tumour subtype characterisation using the GUSTO Classifier to determine gene expression subtypes: basal and neuronal, luminal infiltrated and luminal / luminal papillary. Registration into the trial does not commit the patient to the trial treatment interventions, only to tumour subtype characterisation, eligibility assessment and data collection.

Sites will be informed whether the patient has been allocated a tumour gene expression subtype and can be assessed for eligibility for randomisation. Sites will not be informed of the tumour subtype prior to randomisation. Patients who are eligible for and who provide consent for the main trial participation will be randomised into the study.

For full details on the registration phase – GO TO SECTION 9.4

For full details on the randomisation phase – GO TO SECTION 9.10

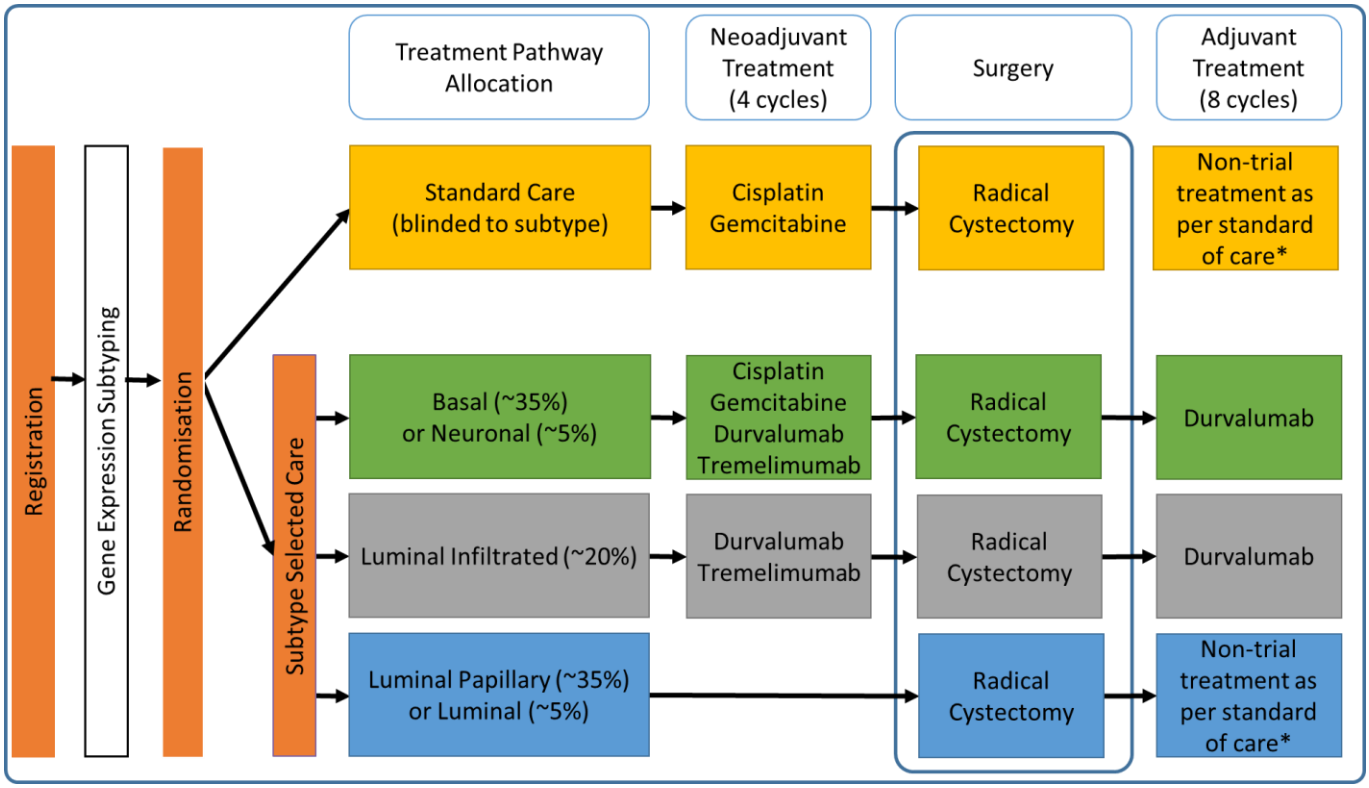
Patients randomised to the **Standard Care Arm** will receive 4 cycles of cisplatin and gemcitabine neoadjuvant chemotherapy, prior to radical cystectomy. Adjuvant treatment will be permitted if considered appropriate by the treating investigator. This treatment would not be considered trial treatment and is determined as per local standard of care. Patients randomised to this arm (and the treating investigators) will remain blinded to the tumour gene expression subtype during study participation.

Patients randomised to the **Gene Expression Subtype-Guided Arm** will have treatment guided by their gene expression subtype determined using the GUSTO classifier. Treatment will consist of one of three treatment options and will be allocated according to subtype with expected proportions as follows (45):

a) **Basal** (~35%) and n**euronal** (~5%) MIBC patients will receive neoadjuvant systemic combination immunotherapy (durvalumab and tremelimumab) and NAC (cisplatin and gemcitabine) prior to radical cystectomy followed by adjuvant durvalumab.
b) **Luminal infiltrated** (~20%) MIBC patients will receive neoadjuvant immunotherapy (durvalumab and tremelimumab) prior to radical cystectomy followed by adjuvant durvalumab.
c) Luminal / luminal papillary (~40%) MIBC patients will proceed direct to radical cystectomy without neoadjuvant systemic therapy. Adjuvant treatment will be permitted if considered appropriate by the treating investigator. This treatment would not be considered trial treatment and is determined as per local standard of care.

All patients who receive neoadjuvant systemic therapy, whether this is with chemotherapy, immunotherapy, or both, will each receive four 21-day cycles of treatment. The dosing schedule is described in Section 11.3 and is consistent with that shown to be tolerable in previous studies, with modest alterations to comply with a 12-week systemic treatment duration in all patients (23, 37).

Figure 2: Treatment pathway allocations within the GUSTO trial.



*Patients in the standard care arm and gene expression subtype-guided arm within the luminal papillary / luminal gene expression subtype may receive adjuvant treatment if considered appropriate by the treating investigator. This treatment would not be considered trial treatment and is determined by the treating investigator.

7. Aims and Objectives

7.1. Aims

The overarching aim of the GUSTO trial is to obtain information to understand whether this approach could improve outcomes, and therefore to guide the design of a subsequent phase III trial with respect to:

- the feasibility of gene expression subtyping within routine NHS care;
- the distribution of gene expression subtypes within UK NHS populations;
- the heterogeneity of the intermediate endpoint (pCR as the primary efficacy endpoint in GUSTO) with respect to subtype following standard of care treatment (NAC);
- to assess the activity of gene expression subtype guided neoadjuvant treatments for MIBC.

By providing data on disease-free survival and other time-to-event secondary endpoints, and incorporating adjuvant immunotherapy, the study will also accrue data that will inform a subsequent phase III study to incorporate the use of adjuvant treatment for those with residual disease.

7.2. Objectives

GUSTO has been designed with three interim stages. Stage 1 will assess the feasibility of recruitment and the hypothesis that it is feasible to embed gene expression subtype stratification into the clinical pathway for patients with MIBC. Stage 2 will confirm the feasibility of recruitment and assess the sample size assumptions. Stage 3 will assess whether there is evidence of a treatment benefit i.e. improved pCR rate post-cystectomy for patients in the gene expression subtype-guided arm. The trial will also determine whether the combination of neoadjuvant chemotherapy and immunotherapy, or the omission of neoadjuvant chemotherapy, affects the operative outcomes from radical cystectomy (RC).

Each stage of the trial has a separate set of objectives:

Stage 1

- To assess the feasibility of gene expression subtype stratification by assessing the:
  - recruitment of patients;
  - time to return of gene expression subtype allocation;
  - success rate of gene expression subtype allocation.

Stage 2

- To confirm the feasibility of recruitment of patients
- To assess the assumptions concerning the proportion of each gene expression subtype.
- To assess the proportion of patients attaining pCR in the MIBC standard care arm by gene expression subtype.
- To confirm / carry out a re-estimation of the sample size for stage 3.

Stage 3: Primary objectives

- To assess the pCR rate in each gene expression subtype within the gene expression subtype-guided arm post-cystectomy. This will be used to inform whether the interventions should be taken forward into a confirmatory phase III trial.

Stage 3: Secondary objectives

- To assess the activity of gene expression subtype-guided neoadjuvant treatments for MIBC in comparison to standard care on:
  - Disease-free survival (DFS) at 12 months post-RC randomisation and at the end of trial
  - Overall survival (OS) at 12 months post-RC and at the end of trial
  - Metastasis-free survival at 12 months post-RC and at the end of trial
  - Event-free survival at 12 months post-RC and at the end of the trial
- Treatment compliance
- Histological outcomes: TNM stages in the TURBT and RC specimens
- Patient-reported Quality of life (as measured by EQ-5D-3L and EORTC QLQ-C30) assessed at RC, 6 months and 12 months post-RC.
- To evaluate patient acceptability to registration and randomisation.
- To evaluate toxicity and tolerability of trial drug treatment.
- To evaluate the outcomes from recovery after radical cystectomy in the gene expression subtype-guided arms as determined by:

  - Time to RC
  - Safety of RC - blood loss, length of stay, readmission, complications and adverse events - Clavien-Dindo grade of complications
  - Pathological completeness:
    - Nodes - number in total and number that have cancer
    - Margins - positive (involved) or negative (clear)
    - Location of positive margin - Urethra. Ureteric. Circumferential. Soft tissue
- To evaluate the outcome (OS, DFS, metastasis-free survival, event-free survival) at 12 months post-RC for all consenting patients registered into the study.
- To assess the pCR stage by gene expression subtype in the gene expression subtype arms of the trial.
- To re-evaluate the stage 1 and 2 feasibility objectives

Gene expression secondary objectives

- To evaluate the RNA quality and mass/yield from the laboratory protocols
- To evaluate the gene expression subtyping success rate, re-test rate and repeat assay success rate
- To investigate the time from patient consent to TURBT sample dispatch
- To evaluation the time to RNA extraction, processing & gene expression subtyping

Stage 3: Exploratory objectives

- To assess the PD-L1 status of tumour and infiltrating immune cells in pre-existing diagnostic samples in all patients
- To compare concordance of predicted subtypes for each sample across classifiers
- To assess the prediction of various MIBC classifiers with respect to pathologic complete response and 12 month survival

Translational Research

- Blood and tissue samples will be collected for future research.

7.3. Multi-stage design outcomes, timings and progression criteria

7.3.1. Stage 1

7.3.1.1. Recruitment Feasibility

To assess feasibility of recruitment, recruitment from all sites within the first 6 months of the trial opening will be summarised. Progression criteria using ‘Red/Amber/Green’ targets have been set, where reaching the ‘Green’ target should allow recruitment of 320 participants (the target randomised sample size) into the trial within 3 years; reaching the ‘Amber’ target should allow the target sample size to be reached within approximately 3 years, with potential modifications to trial procedures; and reaching the ‘Red’ target indicates the trial may not be able to recruit the target sample size in an acceptable timeframe.

- The ‘Green’ target is the randomisation of ≥30 patients with ≥5 sites open
- The ‘Amber’ target is the randomisation of 10 to 29 patients with 3 or 4 sites open. If the ‘Amber’ target is reached, potential modifications will be considered in discussion

with the DMEC, TSC and the funder, including opening additional UK sites, further trial publicity or potential adjustment to the eligibility criteria.
- If ≤10 patients are randomised and <3 sites are open, this criterion will be graded as 'Red' and discussions will be had with the DMEC, TSC and the funder and the trial may be stopped.

Analysis (see Section 17.3.2) will be undertaken once the trial has been open for 6 months and will include all patients randomised into the study from all sites.

7.3.1.2. Gene Expression Subtype Feasibility

To confirm feasibility of the gene expression subtype process within routine NHS care, the time to return of gene expression subtyping allocation and the success rate of gene expression subtyping will be assessed.

- The 'Green' target set for return of gene expression subtyping results is ≤14 days of sample receipt at central laboratory to receipt of gene expression subtype result at CTRU. If this target is not met this criterion will be graded as 'Red' and discussions will be had with the DMEC, TSC and the funder and the trial may not progress to Stage 2.

- The 'Green' target set for the success rate of gene expression subtyping is 90% of patients to be assigned a gene expression subtype. If this target is not met this criterion will be graded as 'Red' and discussions will be had with the DMEC, TSC and the funder and the trial may not progress to Stage 2.

Analysis (see Section 17.3.1.2) will be undertaken once the trial has been open for 6 months and will include all patients registered into the study from all sites.

7.3.2. Stage 2

7.3.2.1. Stage 2 Recruitment Feasibility

To confirm feasibility of recruitment, recruitment from all sites within 24 months of the trial opening will be assessed. Progression criteria using 'Red/Amber/Green' targets have been set, where reaching the 'Green' target should allow the targeted sample to be reached within 3 years; reaching the 'Amber' target should allow recruitment of 320 participants (the target randomised sample size) into the trial to be reached within approximately 3 years, with potential modifications to trial procedures; and reaching the 'Red' target indicates the trial may not be able to recruit the target sample in an acceptable timeframe.

- The 'Green' target is the randomisation of ≥176 patients with 20 sites open
- The 'Amber' target is the randomisation of 144 to 176 patients with 16 to 19 sites open. If the 'Amber' target is reached, potential modifications will be considered in discussion with the DMEC, TSC and the funder, including opening additional UK sites, further trial publicity or potential adjustment to the eligibility criteria.
- If ≤144 patients are randomised and <16 sites open, this criterion will be graded as 'Red' and discussions will be had with the DMEC, TSC and the funder and the trial may be stopped.

Analysis (see section 17.3.1.1) will be undertaken once the trial has been open for 24 months and will include all patients randomised into the study from all sites.

7.3.2.2. Stage 2 Assessment of Sample Size Assumptions

Stage 2 will also assess the assumptions concerning the proportion of each gene expression subtype and the proportion of patients achieving pathologically proved complete response (pCR) in the standard care arm.

This analysis (see Section 17.3.2) is expected to be undertaken at the same time as the stage 2 feasibility assessment (i.e. once the trial has been open for 24 months). However, if this is not possible it may be appropriate for the sample size re-estimation to be carried out later. It is planned that this analysis will include at least 100 patients (all patients registered and randomised into the study at that point). This analysis will facilitate sample size re-estimation (and may change the trial design) if the proportion of patients with a particular subtype is different to design assumptions or if the pCR rate in the standard care arm is greater than the assumed gene expression subtype pCR rate.

If analysis confirms the validity of the sample size assumptions (Section 16.1), the sample size will not be re-estimated and the trial will proceed to stage 3. If the rate of a gene expression subtype (across all randomised patients) is above the defined 95% confidence intervals, investigations into that gene expression subtype may be dropped. If the assumed pCR rate of a particular gene expression subtype in the standard care arm is below the lower limit of the 95% confidence interval then investigations into that gene expression subtype may be dropped.

If an increase in sample size is indicated, or any of the study questions are deemed unanswerable, the study will continue with potential modifications considered in discussion with the DMEC, TSC and the funder.

7.3.3. Stage 3

Stage 3 will run to trial completion and will assess the pCR rate in each gene expression subtype in both arms to determine if a confirmatory phase III trial should proceed. An interim assessment will take place once all randomised participants have completed RC. Final analysis (see Section 17.3.3) will take place once all patients have been followed up for 12 months post-RC. Both analyses will include all patients randomised into the study from all stages. Stage 3 will also re-assess the feasibility endpoints assessed in stages 1 and 2.

8. Trial Sites and Investigators

8.1. Trial sites

Trial sites will be required to have obtained local management approvals and undertake a site initiation meeting with the CTRU prior to the start of recruitment into the trial.

Each trial site must be able to comply with the following, as applicable to the trial activities taking place at the site:

- Collection, preparation and shipment of biological samples, trial treatments, imaging, clinical care, follow-up schedules and all requirements of the trial protocol.
- Requirements of the UK Policy Framework for Health and Social Care and amendments.
- Data collection requirements, including adherence to eCRF submission timelines as per the eCRF completion guidance.

- Collection and shipment of biological samples for gene expression subtype characterisation as per Section 9.8.
- Monitoring requirements as outlined in Section 18.

Interested sites will be required to complete a trial-specific feasibility questionnaire to confirm that they have adequate resources and experience to conduct the study.

8.1.1. PIC sites

Participant Identification Centres (PICs) will be included where required by the site. Patients may present to District General Hospitals for TURBT surgery and may be identified and referred to the trial site for approach regarding study participation. Patients may be provided with patient information documents by the PIC site and approached for consent by the GUSTO trial site staff. Formal eligibility assessment and recruitment will be undertaken by the GUSTO trial site.

8.2. Principal Investigators and Co-Investigators

Sites must have an appropriate Principal Investigator (PI) authorised by the site and ethics committee to lead and coordinate the work of the trial on behalf of the site.

As well as the local PI, it is also recommended that each site identifies a designated urology lead, oncology lead and histopathology lead.

Lead Histopathologist at each centre

Each centre should designate one person as lead histopathologist. This person will be required to ensure that histological confirmation of TURBT samples is carried out in good time and notify the relevant nurse contact so that the patient can be registered into the study without delay. The histopathology lead will provide tissue and pathological reports for collection in the study.

Other investigators at site wishing to participate in the trial must be trained and approved by the PI. Investigators involved in the treatment and care of patients must be medical doctors and have experience of treating bladder cancer.

8.3. Training Requirements for Staff

All site staff must be appropriately qualified by education, training and experience to perform the trial related duties allocated to them, which must be recorded on the site authorised personnel log.

CVs for all staff must be kept up-to-date, signed and dated and copies (or statement of their location) held in the Investigator Site File (ISF) held at site. An up-to-date, signed copy of the CV for the PI must be forwarded to the CTRU prior to site activation.

GCP training is required for staff responsible for trial activities as appropriate. The frequency of repeat training may be dictated by the requirements of their employing institution, or 2 yearly where the institution has no policy, and more frequently when there have been updates to the legal or regulatory requirements for the conduct of clinical trials. Evidence of current GCP training for the PI must be forwarded to the CTRU prior to site activation.

8.4. Site Initiation

Before a site is activated, the CTRU trial team will arrange a site initiation with the site which, as a minimum the PI, pharmacy lead, histopathology lead and research nurse must participate. The site will be trained in the day-to-day management of the trial. Site initiation will be performed by tele/video conference or via the provision of pre-recorded training material which staff will be required to watch and log on the trial authorised personnel log. Any queries or questions arising from the training material can be addressed by email (GUSTO@leeds.ac.uk) or a call can be arranged between the site and CTRU.

Sites must inform the CTRU of any additional sites involved in the patient pathway. Recruiting sites which will be referring patients to a different site, for all or some of the trial activities, will not be activated until the relevant site involved is ready to be activated.

8.5. Essential Documentation

Once the CTRU trial team has received all the required essential documentation, the site has received their investigator and pharmacy site files and the site has been initiated, a site activation email will be issued to the PI and other research staff by CTRU. Sites must not approach any potential patients until they have received an activation email from CTRU.

9. Recruitment process

The process for providing information to patients about this study has a staged approach to reduce the possibility of information overload and allows plenty of time for consideration of participation. The process is summarised in Figure 3.

1. **Registration**: the patient is provided with PIS-1 and the Participant Supplementary Document. PIS-1 contains information about the nature of the research being considered and the need for analysis of the tumour tissue, with consent for release of the TURBT sample for analysis and gene expression subtyping. The Participant Supplementary Document contains general information regarding the trial and the data protection information. The Patient Summary may also be provided but this document is optional. These documents will usually be given at the consultation with the urologist when discussing the requirement for TURBT to establish MIBC. Alternatively, if the patient is approached following confirmation of MIBC, they may be given at the consultation with the oncologist. Once written consent (paper or electronic) for registration, and eligibility has been confirmed (confirmed MIBC with an intention to treat by neoadjuvant chemotherapy and radical cystectomy with curative intent as determined by the local site urology, oncology and pathology teams), the patient is registered into the study and a unique registration number is allocated to the patient.

2. **Randomisation**: once the gene expression subtype has been determined, the patient is provided with PIS-2 and the Participant Supplementary Document. PIS-2 contains information about standard information on randomised controlled trials, general issues regarding unwanted side-effects and toxicity from treatment and further details of the potential advantages and disadvantages of the arms between which the patient will be randomised. PIS-2 can be provided when considered appropriate for the individual patient, e.g. with PIS-1, but must be provided in a timely fashion prior to final consent and randomisation using the gene expression subtype results. The Participant Supplementary Document is also provided alongside PIS-2. The Patient Summary may also be provided but this document is optional.

9.1. Patient Screening

All trial trial sites will be required to complete regular Screening Logs of all patients screened. Anonymised data will be recorded and will include whether or not the patient was eligible for participation. For patients who were not eligible or who do not go on to be registered, the reason for ineligibility / non-registration will be recorded. However, the right of the patient to refuse consent without giving reasons will be respected.

The research team at trial research sites will be required to complete screening logs for:

- all patients presenting to the urology team with suspected bladder cancer and requiring a TURBT;
- all patients not screened prior to TURBT and subsequently found to have MIBC and considered for radical treatment.

Documented reasons for ineligibility or declining participation will be closely monitored by the CTRU as part of a regular review of recruitment progress. Screening forms should be returned to the CTRU on a monthly basis. Anonymised information will be collected including:

- age
- sex
- ethnicity
- whether the patient is registered to the trial

Screened patients who are not registered either because they are ineligible or because they decline participation will also have the following information recorded:

- the reason not eligible for study participation, OR
- the reason eligible but declined

9.2. Informed consent

The Principal Investigator (PI) retains overall responsibility for the informed consent of participants at their site. The PI must ensure that any person delegated responsibility to participate in the informed consent process is duly authorised on the GUSTO authorised personnel log, trained and competent to participate according to the ethically approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki 1996.

The right of the patient to refuse consent without giving reasons will be respected. Further, participants will be told that they remain free to withdraw from the trial at any time without giving reasons and without prejudicing any further treatment. The participant must also be provided with a contact point where they may obtain further information about the trial. 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 and according to any timelines requested by the CTRU.

Informed consent must be obtained prior to the participant undergoing procedures specifically for the purposes of the trial which are out-with standard routine care at the trial site. Patients may be invited to give consent to registration and TURBT sample processing prior to confirmation of MIBC: those patients who are found not to have MIBC will not be registered

into the study and will have no further involvement in the study. If consent is given using paper forms, the signed consent form will be marked to make it clear that they were not registered into the study and will be stored with their medical notes.

Site staff are responsible for ensuring that an appropriate member of staff has made dated entries in the patient's medical notes relating to the informed consent process (i.e. information given, consent signed, re-consent etc.).

Remote documented consent is an option within the GUSTO study and informed consent can be taken using the following:

- Face-to-face consent during a clinic visit
- Remote consent on paper consent form posted to the participant
- Remote eConsent on an electronic database (REDCap)

Verbal consent to the remote consent process will be obtained at the start of the telephone call. Participants will be provided with information on the consent process to help inform their decision on the method of consent. A record of the consent process detailing the date of screening call, consent and all those present (including mode of informed consent process) will be kept in the participant's medical notes. The mode of information consent used will depend on patient preference, and their accessibility to a device to support the eConsent process.

Information provided to participants will also include the potential to collect longer term routine data, should further funding become available.

Patients approached regarding registration will be provided with Participant Information Sheet-1 (PIS-1) and the Participant Supplementary Document. The Patient Summary may also be provided but this document is optional. Patients will have as long as they feel necessary to consider trial entry and will be given an opportunity to discuss the trial with their family, friends, and other healthcare professionals before they are asked whether they would be willing to take part in the study.

Patients will have the option to provide written informed consent for registration without delay (i.e. on the same day) as registration will not impact upon treatment choices (consent for registration is for gene expression subtyping and data collection only). Consent for registration may be taken by an appropriately qualified member of the trial team (including nurse and other healthcare professionals) who has received GCP training and is authorised by the PI on the trial delegation log to take this consent.

Participants approached regarding randomisation will be provided with Participant Information Sheet-2 (PIS-2 and the Participant Supplementary Document. The Patient Summary may also be provided but this document is optional. Following information provision, patients will have as long as they need to consider participation, normally a minimum of 24 hours, and will be given the opportunity to discuss the trial with their family and healthcare professionals before they are asked whether they would be willing to take part in the trial. Consent for randomisation may be taken by an appropriately qualified medically qualified healthcare professional who has received GCP training and is authorised by the PI on the trial delegation log to take this consent.

Where the patient is able to provide informed consent but is unable to sign or otherwise mark the consent form, provision for completion of the consent form by a witness will be made. This

should be a carer, friend/family member, or a local member of the clinical team who is independent of the research team. In this instance, consent must be obtained remotely or in-person.

Face to face consent (during a clinic visit)

Participants will provide informed consent on a paper consent form. The original signed copy will be retained at site in the Investigator Site File and a copy will be provided to the participant at the end of their visit. A further copy will be sent to CTRU and a final copy will be filed in the medical records (as per local practice).

Remote consent (on paper form posted to participant)

Participants will be posted the patient information sheets / consent materials in advance of the remote visit (telephone or video call). At the start of the call, the researcher will verify the participants' identity by asking the potential participant to confirm their initials and date of birth. The researcher will use this to verify identity by matching details in the hospital records.

The researcher will discuss the trial and what it involves, and will discuss each point on the consent form with the participant. The participant will be asked to complete all questions and add signature to the form. The consent form will be signed by the participants at home and posted back to site for the investigator to sign (using the date that consent was given). Following the consent call, the researcher must complete a GUSTO Remote Consent Proforma, to indicate the date and time that consent was received and that the researcher was happy that the patient was able and informed to consent. The original signed copy of the consent form and the GUSTO Remote Consent Proforma will be retained at site and a copy of the completed consent form will be provided to the participant. Copies of the consent form and the GUSTO Remote Consent Proforma will be sent to CTRU and final copies will be filed in the hospital notes (as per local practice).

Remote E-consent (using an electronic database (REDcap))

Participants who plan to consent using e-consent will provide verbal consent for their name, initials, date of birth, NHS/CHI number and email address to be disclosed to CTRU through the REDCap system (the e-consent system in this trial). If the participant does not consent to this disclosure they will be unable to use e-consent and alternatives will be discussed with them (i.e. written consent). The participant will be emailed the Participant Information Sheets prior to the remote visit (telephone or virtual call). If the participant prefers, they can request a posted copy of their information sheet.

At the start of the consent call, the researcher will verify the participants' identity by asking the potential participant to confirm their initials and date of birth. Identity is confirmed by matching details provided by participant with hospital records. The researcher will discuss the trial and what it involves, and will discuss each point on the consent form with the participant. The participant will be asked to complete all questions and add signature to the form, and submit the form. The researcher will open the form and complete the sign-off. Following the consent call, the researcher must complete a GUSTO Remote Consent Proforma, to indicate the date and time that consent was received and that the researcher was happy that the patient was able and informed to consent.

A PDF of the completed consent form will be available for the participant, and the site researcher will save copies of the consent form produced by REDcap and the GUSTO Remote Consent Proforma in the Investigator Site File; further copies will be filed in the hospital notes (as per local practice). A copy of the GUSTO Remote Consent Proforma will be sent to CTRU.

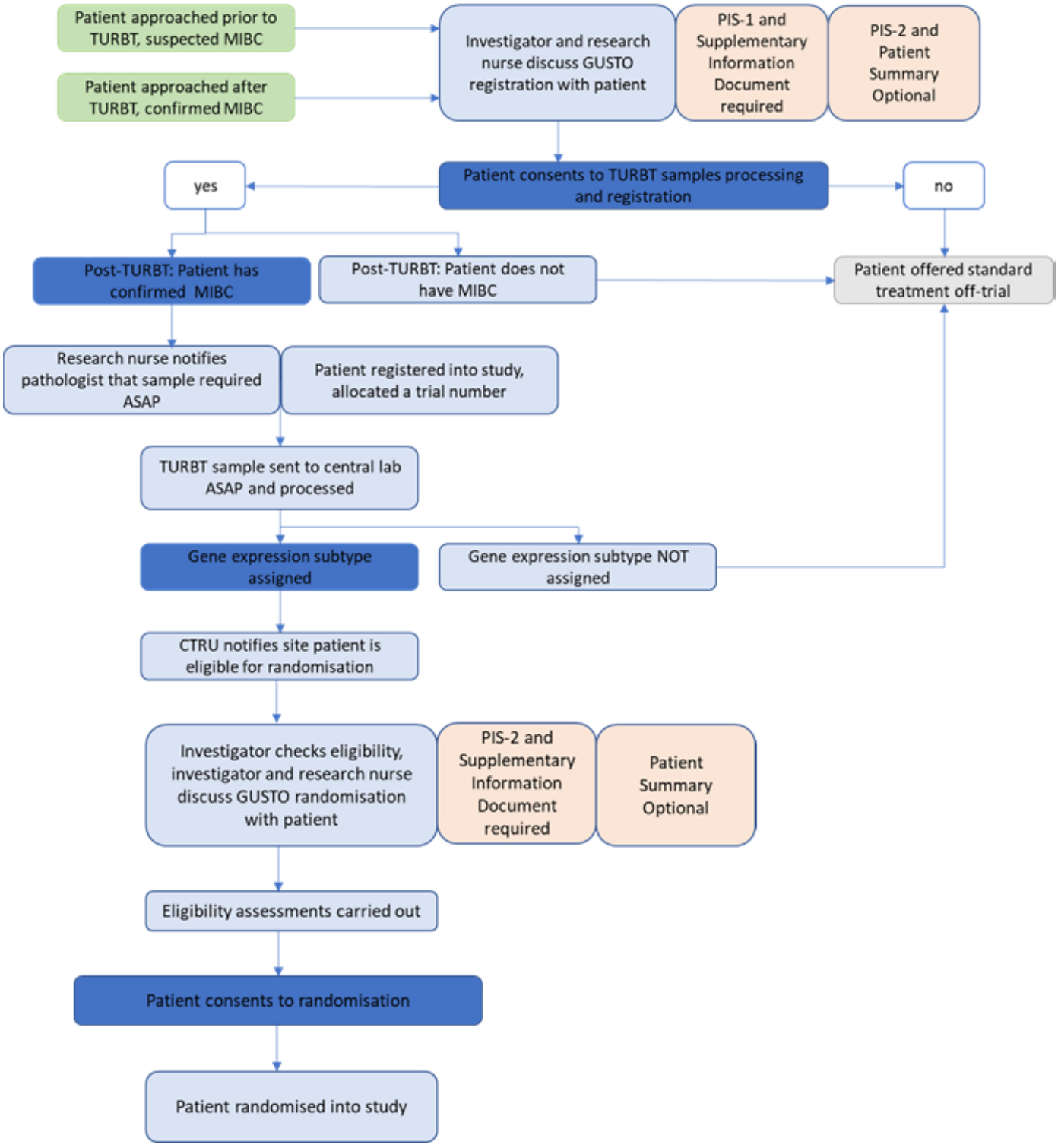
Where it is not possible for the participant to complete all questions and sign the form during the call, they will be asked to do this as soon as possible after the call. The site researcher will follow-up with the participant in the event that the form is not completed.

9.2.1. Loss of capacity following informed consent

Where valid informed consent is obtained from the participant and the participant subsequently becomes unable to provide ongoing informed consent by virtue of physical or mental incapacity, the consent previously given when capable remains legally valid.

Participants who lose capacity after informed consent has been obtained will continue with protocol treatment and assessments in consultation with the PI and participant's carer / family with the participant's best interests foremost in the decision-making process. Ongoing collection of safety and follow-up data will continue via the clinical care team for inclusion in the trial analysis in order to preserve the integrity of the trial's intention to treat analysis and fulfil regulatory requirements specifically for pharmacovigilance purposes.

Figure 3: Patient flow and procedures



9.3. Patient Identification and Approach

Participant recruitment will vary by site, dependent upon local arrangements and patient pathways. These will be established during site set-up, and strategies will be put in place to maximise identification and recruitment of potential participants. Participant identification is proposed as described in Figure 3.

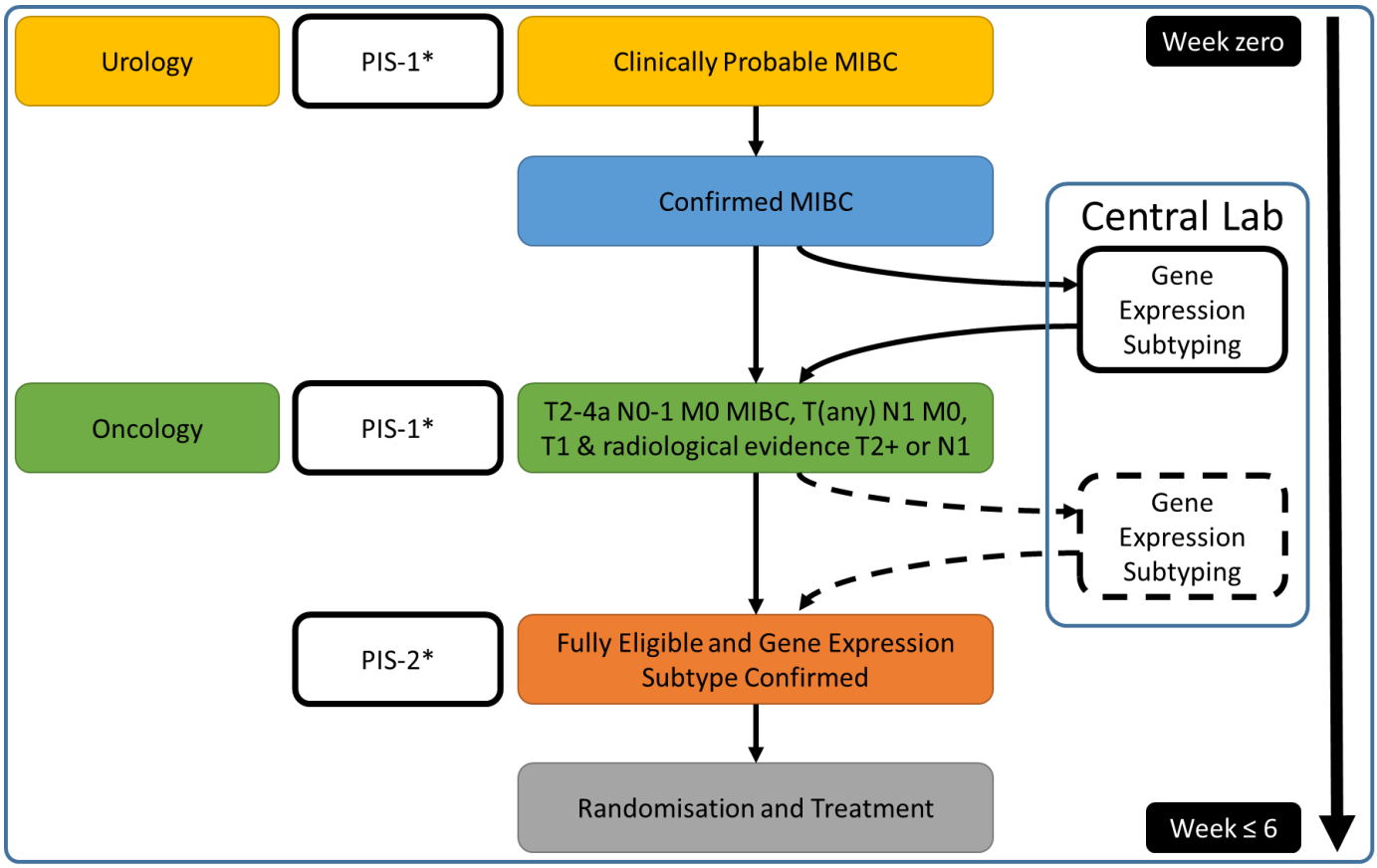
9.4. REGISTRATION

9.5. Initial approach and eligibility assessment for registration

Patients may be approached regarding the study at two different points in the clinical care pathway (see Figure 3):

- **Prior to formal histological MIBC confirmation:** This is the preferred approach to trial entry: where a urologist has an expectation of aggressive bladder cancer (MIBC probable), patients may be approached to provide consent for registration ahead of their transurethral resection of a bladder tumour (TURBT) before confirmation of MIBC is known. This allows a sample to be provided for gene expression subtyping once MIBC is confirmed (MIBC with an intention to treat by neoadjuvant chemotherapy and radical cystectomy with curative intent as determined by the local site urology, oncology and pathology teams) and the patient has been registered into the study. Such patients will be provided with PIS-1 (consent for registration) and Participant Supplementary Document and consent may be given immediately. Remote consent may be given to reduce delays in gene expression subtyping. Patients may also be provided with PIS-2 (consent for randomisation) and the Patient Summary and patients will have the opportunity to consider the information and provide consent for randomisation into the main study at their subsequent visit with an oncologist. Patients should be registered into the study as soon as possible following confirmation of MIBC by the pathologist.
- **Following MIBC confirmation**: where MIBC is already confirmed, patients may be approached at their oncology visit and provided with PIS-1 (consent for registration) and Participant Supplementary Document and consent may be given immediately. It may be appropriate to approach patients by telephone or video call if they are not expected in the clinic within an appropriate timeframe. Remote consent may be given to reduce delays in gene expression subtyping and start of treatment. Patients may also be provided with PIS-2 (consent for randomisation) and the Patient Summary and patients will have the opportunity to consider the information and provide consent for randomisation into the main study at the next clinic visit.

Figure 4 GUSTO trial consent and screening processes.



* Participant Supplementary Document also required. PIS-2 optional at the point that PIS-1 is provided. Patient Summary optional at each stage.

9.6. Eligibility for Registration

Eligibility waivers to the inclusion and exclusion criteria are not permitted. Queries in relation to the eligibility criteria must be addressed prior to registration. Patients are eligible for the trial if all the inclusion criteria are met and none of the exclusion criteria applies.

9.6.1. Inclusion criteria for registration

1. Age ≥ 18
2. Eastern Co-operative Oncology Group (ECOG) performance status 0 or 1
3. Currently considered for neoadjuvant chemotherapy and radical cystectomy with curative intent and suitable for all protocol defined treatment (chemotherapy and immunotherapy as defined for all treatment groups in this protocol)
4. Confirmation of MIBC (full report not required):
   - high grade pure or mixed urothelial (transitional) cell carcinoma which is at least T1 on histology AND radiological evidence of T2+ N1 cancer

   OR

   - high grade pure or mixed urothelial (transitional) cell carcinoma which is at least T2 on histology
5. Written informed consent for registration (PIS-1 and Participant Supplementary Document)

9.7. Registration Process

Informed written consent / eConsent for registration must be obtained prior to registration (see Section 9.2), subject to the patient meeting the eligibility criteria for registration (Section 9.6). Consenting patients should be registered into the study as soon as possible following histological confirmation of MIBC.

Registration will be performed centrally using the CTRU automated 24-hour online system. To register using the web, a staff email address, a site code and Personal Identification Number (PIN) will be required. Authorisation codes and PINs will be provided by the CTRU once all the necessary documentation has been received at CTRU and the site has been fully approved. Please note codes and PINs should be kept confidential.

Participants may only be registered into the trial by an authorised member of staff at the trial research site, as detailed on the Authorised Personnel Log. The following information will be required:

- Site code
- Confirmation of written informed consent/eConsent for registration
- Confirmation of eligibility for registration
- Participant details, including initials, sex, date of birth and NHS or CHI number, email address (for provision of electronic quality of life questionnaires)

Register the participant using
Web registration
https://lictr.leeds.ac.uk/webrand/

Once registration is complete, the system will allocate the participant a unique 5 digit trial number. This number together with the centre number will form the participant ID number. Confirmation of participant registration will be emailed to the research site.

The trial number will be used for the purpose of participant identification, tissue and blood sample identification and data collection during the study.

An email confirmation of registration and the allocated trial number will be sent to the site staff who performed the registration, the lead histopathologist and Principal Investigator by the CTRU. These notifications are generated and sent automatically from the CTRU and should be reviewed by the recipients and filed in the GUSTO Investigator Site File.

After registration, the site staff will:

- Add the unique participant trial number to the registration consent form
- Make sufficient copies of the signed registration consent form
- Give the patient a copy of their signed Consent Form, PIS-1 and Participant Supplementary Document. Also provide a copy of PIS-2.

- File the original (wet ink) consent form (if paper consent) in the ISF and file a copy in the patient's medical notes.
- Return a copy of the completed registration consent form and Remote Consent Proforma (if relevant) to CTRU (by secure file transfer – contact CTRU Data Manager for details), in line with the terms of the ethically approved consent form
- Record the patient details on the GUSTO Patient Log.
- Ensure that the local lead histopathologist is informed that the TURBT sample should be dispatched to the central laboratory as soon as possible.

Practical notes for sample collection:

- Once MIBC has been confirmed, the TURBT sample will be dispatched immediately to Sheffield as soon as possible following registration to avoid delays in obtaining gene expression subtype results. The GUSTO Laboratory Manual provides full details on this process.
- Tumour blocks must be labelled with the trial number generated at registration, the local pathology laboratory accession number and the patient's name and sent to the central laboratory in Sheffield. The trial number will be a unique identifier and the primary way in which the patient will be identified and should be used in all correspondence throughout the registration and subsequent trial periods. The full patient name is only collected as part of consent and sample tracking as part of NHS standard processes (from site to central laboratory).
- No treatment or trial allocation will be performed at this point.

Practical notes for randomisation

- Investigators should be cognisant of radiology requirements for randomisation – patients should have had a CT scan a maximum of 12 weeks prior to randomisation.

9.7.1. Registered Patients Not Proceeding to Randomisation

Registered patients who are not randomised either because they are found to be ineligible or because they decline further participation will have the following information recorded if they consent to this optional data collection and consent to this data collection is not withdrawn.

All information collected after screening will be collected remotely (without the need for patients who do not proceed beyond registration to be contacted or reviewed in person) by the site research team:

- TURBT sample histopathology details
- Gene expression subtyping result obtained (yes/no)
- Gene expression subtype if available
- The reason they did not proceed to randomisation
- Date of cancer relapse or last review
- Date of death or last review
- Cystectomy date, if done
- pCR status post-cystectomy

- Neoadjuvant treatment (yes/no, details)
- Adjuvant treatment (yes/no, details)

9.8. Tissue Block Collection and Handling

Sites must provide formalin-fixed paraffin-embedded (FFPE) tissue block(s) from TURBT for all patients registered into GUSTO. This is required for gene expression subtype profiling prior to randomisation into the study. It is imperative that tumour samples are sent to the designated central laboratory in Sheffield as soon as possible following histological confirmation of MIBC to avoid delays in obtaining the gene expression subtype and consequently treatment allocation.

It is suggested that, for patients consented prior to TURBT, the local lead histopathologist sends confirmation to the research nurse as soon as possible following examination of the tumour sample, it is not necessary to wait for the pathology report to be finalised. Patients who have consented to registration must be registered into the study as soon as possible once MIBC has been confirmed as a trial number will be allocated at registration and is required to identify the tumour sample.

Samples being transferred from site to the central laboratory must be labelled with the patient's GUSTO trial number, the local pathology laboratory accession number and the patient's full name. Ensure that the samples are clearly and indelibly labelled. Please refer to the Laboratory Manual for details on sample packaging and dispatch.

An anonymised copy of the pathology report (with trial number included) must be provided to CTRU but is not required on sample submission.

Tissue blocks will be sent by First Class post to the GUSTO study team at Sheffield Teaching Hospitals NHS Foundation Trust. Full details are included in the GUSTO Laboratory Manual. Upon receipt, the laboratory will confirm receipt of samples with site and CTRU.

Sections and tissue microarray sampling will be taken from the FFPE blocks and stored for use in the study and for future research (see Section 12.24). FFPE blocks will be returned to sites. Any sampling for GUSTO will be done under the supervision of the trial's central pathologist who will ensure that there is sufficient material for diagnostic use remaining.

9.9. Gene Expression Subtype Characterisation by GUSTO Classifier

The gene expression microarray data for each patient will be uploaded to the Decipher Bladder platform (identified by GUSTO trial number, patient initials and central pathology laboratory accession number only).

9.9.1.1. Gene Expression Subtype Results

The gene expression subtype results will be provided by Decipher to the central laboratory and to CTRU, Leeds.

The CTRU will confirm by email to the PI, lead histopathologist and research nurse contact at site whether the patient has been assigned a gene expression subtype but the subtype will not be disclosed.

If the patient is considered to meet the inclusion criteria specified in Section 9.11, the research team at site will arrange for the patient to be approached regarding randomisation and will ensure that all required eligibility assessments required for the study (such as ECG and pregnancy testing if relevant) are carried out.

9.9.1.2. Blinding in standard care arm

Patients randomised to the standard care arm (and the treating investigators) will remain blinded to the gene expression subtype during study participation. This is made clear in the Patient Information Documents. The gene expression subtype may be disclosed on patient request following completion of study participation.

9.10. RANDOMISATION

9.11. Eligibility for Randomisation

Eligibility waivers to the inclusion and exclusion criteria are not permitted. Queries in relation to the eligibility criteria must be addressed prior to randomisation. Patients are eligible for the trial if all the inclusion criteria are met and none of the exclusion criteria applies.

9.11.1. Inclusion criteria for randomisation

1. Diagnosed with MIBC staged as either T2-4a N0 M0, T(any) N1 M0 or T1 on histology with radiological evidence of T2+ or N1
2. Planned for neoadjuvant chemotherapy and radical cystectomy with curative intent and suitable for all protocol defined treatment (chemotherapy and immunotherapy as defined for all treatment groups in this protocol)
3. Confirmation of a pure or mixed urothelial (transitional cell) carcinoma (UC) tumour histology based on local institutional pathology reporting
4. ECOG performance status 0 or 1
5. Estimated creatinine clearance rate (using the Cockcroft-Gault formula) of >40 ml/min according to local institutional standard methods for estimation: patients with creatinine clearance ≥ 60 ml/min are eligible for full dose cisplatin; patients with impaired creatinine clearance (40-60 ml/min) are eligible only if split dose cisplatin 35 mg/m^2 is given on days 1 and 8 of neoadjuvant treatment.
6. Adequate haematological parameters
    a. Haemoglobin ≥ 90 g/L
    b. Neutrophil count ≥ 1.5 x10^9 /L

   c. Platelets ≥ 100 $x10^9$ /L

7. Adequate biochemical parameters
   a. Bilirubin ≤ 1.5 x ULN unless due to Gilbert's syndrome
   b. ALT and/or AST ≤ 1.5 x ULN (both ALT **and** AST are recommended)
8. Body weight >30 kg
9. Life expectancy of at least 12 weeks
10. For women of childbearing potential, negative blood serum pregnancy test and adequate contraceptive precautions (Section 9.12)
11. For men of reproductive potential, effective contraception if the risk of conception exists (Section 9.12)
12. Written informed consent for randomisation (PIS-2 and Participant Supplementary Document)
13. Patients must be able and willing to comply with the terms of the protocol for the duration of the study including treatment, trial visits and assessments

9.11.2. Exclusion criteria for randomisation

1. A bladder tumour where a gene expression subtype classification cannot be made
2. A delay in TURBT sample processing such that it is >4 weeks from receipt of TURBT sample (from initial or repeat surgery if relevant) at central laboratory to receipt of gene expression subtype result at site
3. Known or suspected allergy or hypersensitivity reaction to any of the components of study treatment or their excipients for any of the treatment groups in this protocol
4. Active infection likely to impact safety of treatment delivery for any of the treatment groups in this protocol or radical cystectomy. This includes known active tuberculosis, hepatitis B (known positive HBsAg result), hepatitis C, or human immunodeficiency virus (positive HIV 1/2 antibodies). Patients with a past or resolved HBV infection (defined as the presence of hepatitis B core antibody [anti-HBc] and absence of HBsAg) are eligible. Patients positive for hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction is negative for HCV ribonucleic acid (RNA)
5. Active documented autoimmune or inflammatory disorders, including but not limited to, inflammatory bowel disease (e.g., colitis or Crohn's disease), systemic lupus erythematosus, sarcoidosis, Wegener syndrome (granulomatosis with polyangiitis), Graves' disease, rheumatoid arthritis and uveitis. The following are exceptions to this criterion: vitiligo, alopecia, hypothyroidism that is stable on hormone replacement, any chronic skin condition that does not require systemic therapy and patients with coeliac disease controlled by diet alone.
6. Major surgical procedure (as defined by the treating clinician) within 28 days prior to randomisation

7. Coronary artery bypass graft, angioplasty, vascular stent, myocardial infarction, unstable angina pectoris or congestive cardiac failure (New York Heart Association > grade 2) within the last 6 months
8. Mean QT interval corrected for heart rate ≥470 ms calculated from 3 ECGs (within 15 minutes at 5 minutes apart)
9. Uncontrolled concurrent illness, including but not limited to, ongoing or active infection, symptomatic congestive heart failure, uncontrolled hypertension, unstable angina pectoris, cardiac arrhythmia, interstitial lung disease, serious chronic gastrointestinal conditions associated with diarrhoea, or psychiatric illness/social situations that would limit compliance with study requirement, substantially increase risk of incurring AEs or compromise the ability of the patient to give written informed consent
10. Any unresolved toxicity NCI CTCAE Grade ≥2 from previous anticancer therapy with the exception of alopecia, vitiligo, and the laboratory values defined in the inclusion criteria
11. Current or prior use of immunosuppressive medications within 14 days prior to randomisation including, but not limited to, systemic corticosteroids at doses exceeding 10 mg/day of prednisolone or equivalent, methotrexate, azathioprine, and tumour necrosis factor-α blockers. Permitted exceptions include: use prior to imaging procedures in patients with contrast allergies, use of inhaled, topical, and intranasal corticosteroids
12. Radiotherapy treatment to more than 30% of the bone marrow or with a wide field of radiation within 4 weeks of the first dose of study drug
13. A current separate other malignancy. Current non-melanoma skin cancer, cervical carcinoma in situ or incidental localised prostate cancer is permissible. Other prior malignancy is acceptable if treatment within the GUSTO trial would be given with curative intent in the view of the principal investigator and the local multi-disciplinary team
14. Any concurrent chemotherapy, biologic, or hormonal therapy for cancer treatment. Concurrent use of hormonal therapy for non-cancer-related conditions (e.g., hormone replacement therapy) is acceptable
15. Women who are breastfeeding
16. History of allogenic organ transplantation
17. History of leptomeningeal carcinomatosis
18. History of primary immunodeficiency
19. Receipt of live attenuated vaccine within 30 days prior to randomisation.
20. Prior randomisation or treatment in a previous durvalumab clinical study regardless of treatment arm assignment.
21. Patients who have received prior anti–PD-1 (including durvalumab), anti PD-L1 or anti CTLA-4 (including tremelimumab):

a. Must not have experienced a toxicity that led to permanent discontinuation of prior immunotherapy.

b. All AEs while receiving prior immunotherapy must have completely resolved or resolved to baseline prior to screening for this study.

c. Must not have experienced a ≥Grade 3 immune related AE or an immune related neurologic or ocular AE of any grade while receiving prior immunotherapy. NOTE: Patients with endocrine AE of ≤Grade 2 are eligible if they are stably maintained on appropriate replacement therapy and are asymptomatic.

d. Must not have required the use of additional immunosuppression other than corticosteroids for the management of an AE, not have experienced recurrence of an AE if re-challenged, and not currently require maintenance doses of > 10 mg prednisone or equivalent per day.

22. Any contraindication to treatment with cisplatin, gemcitabine or durvalumab as described within the respective local SmPCs.

23. Participation in another clinical study with an investigational product during GUSTO trial participation and follow-up.

9.12. Birth Control and Pregnancy Testing

Women of childbearing potential (see Appendix 3 for definition) should be advised to use a highly effective method of contraception while receiving neoadjuvant treatment. All women will receive a hysterectomy as part of radical cystectomy surgery therefore contraception measures during adjuvant treatment are not required.

Pregnancy testing must be undertaken **prior to randomisation** and as per local practice for patients receiving chemotherapy. Pregnancy testing must include a highly sensitive serum pregnancy test prior to randomisation and urine or serum pregnancy testing must be used prior to day 1 of each treatment cycle during trial treatment. A final pregnancy test (serum or urine) is required for women of child-bearing potential. This is required prior to radical cystectomy or, if it occurs sooner, 90 days after the last dose of durvalumab, cisplatin or gemcitabine or 180 days after the last dose of tremelimumab, whichever occurs latest or prior to radical cystectomy if cystectomy occurs before this point is reached.

Male patients who are sexually active with a woman of childbearing potential should be advised to use a highly effective method of contraception while receiving treatment and until 180 days after the last dose of tremelimumab or 90 days after the last trial treatment dose (durvalumab, cisplatin or gemcitabine) (if seminal vesicles were not removed during radical cystectomy).

Investigators should advise male patients to seek advice on conservation of sperm prior to treatment, due to potential anti-fertility effect of chemotherapy which could be irreversible.

9.13. Prior and Concurrent Participant in Other Clinical Trials

Participation in other interventional clinical trials is not permitted. However, participation in non-therapeutic registry studies or questionnaire-based studies is permitted. Questions about

potential clinical trials can be addressed to the Chief Investigators via CTRU prior to randomisation.

9.14. Randomisation Process

Informed written consent for randomisation must be obtained prior to randomisation.

Randomisation will be performed centrally using the CTRU web-based system and will be carried out by CTRU with information provided by site and the central laboratory.

An authorised member of staff at the trial research site (as detailed on the Authorised Personnel Log) will enter the Randomisation Form eCRF and inform CTRU that the information is available for randomisation.

The following information will be required at randomisation:

- Site code (assigned by CTRU) of the research site
- Participant details, including initials and date of birth (which must match details provided at registration)
- Confirmation of eligibility for randomisation
- Confirmation of date of written informed consent for randomisation
- Confirmation of completion of baseline quality of life questionnaires
- Disease stage
- Performance status
- Sex

To randomise the patient, CTRU staff will access the web-based system, enter the patient identifiers and the system will refer to the information entered by site staff on to the Randomisation Form and the gene expression subtype result on the central lab database.

9.14.1. Treatment allocation

Patients will be randomised on a 1:1 basis to receive either standard neoadjuvant chemotherapy (cisplatin and gemcitabine) prior to radical cystectomy or to have radical cystectomy with neoadjuvant and adjuvant chemotherapy and/or immunotherapy treatment determined based on the gene expression subtype assigned by the GUSTO Classifier.

A computer-generated minimisation programme that incorporates a random element will be used to ensure treatment groups are well-balanced for the following participant characteristics, details of which will be required for randomisation:

- Disease stage
    - T2 N0
    - T3-T4 N0
    - T(any), N1
- Performance Status
    - 0
    - 1
- Sex

  - Male
  - Female
- Gene expression subtype (CTRU will hold this information, sites will be notified whether a gene expression subtype has been assigned)
  - Basal
  - Neuronal
  - Luminal Infiltrated
  - Luminal Papillary
  - Luminal

Once randomisation is complete, the system will allocate the treatment allocation for that participant. The treatment allocation will be either:

Standard care: NAC (cisplatin and gemcitabine) prior to radical cystectomy

OR

- Gene expression subtype guided treatment:
  - Basal and neuronal subtypes: to receive combination NAC (cisplatin and gemcitabine) combined with neoadjuvant immunotherapy (durvalumab and tremelimumab) prior to radical cystectomy and followed by adjuvant durvalumab. Adjuvant treatment is permitted at the investigator's discretion but this is not trial treatment.
  - Luminal infiltrated subtype: to receive neoadjuvant immunotherapy (durvalumab and tremelimumab) prior to radical cystectomy and followed by adjuvant durvalumab.
  - Luminal/luminal papillary subtype: to proceed direct to radical cystectomy without neoadjuvant systemic therapy. Adjuvant treatment is permitted at the investigator's discretion but this is not trial treatment.

An e-mail confirmation of the participant's randomisation into the trial will be sent to the Principal Investigator, nominated research team members and pharmacist by the CTRU. These notifications are generated and sent automatically from the CTRU and once reviewed by the recipients, filed in the GUSTO Investigator Site File. The participant's details should be added to the Participant Log.

Upon randomisation, participants will be given a trial-specific participant card, which will have the trial title, participant trial number, contact details of the Principal Investigator and out of hours contact details in cases of emergency.

10. Trial Medicinal Product Management

Please refer to the GUSTO Pharmacy Manual for full details of the trial IMP management requirements.

Within the trial the following are classed as IMPs:

- Cisplatin: Generic product is acceptable for use, off-the-shelf supply
- Gemcitabine: Generic product is acceptable for use, off-the-shelf supply
- Durvalumab: Manufactured by AstraZeneca, trial supply
- Tremelimumab: Manufactured by AstraZeneca, trial supply

10.1. Cisplatin and gemcitabine composition

Cisplatin and gemcitabine are typically available as concentrate solutions for infusion.

10.1.1. Supply, storage and handling of cisplatin and gemcitabine

Cisplatin and gemcitabine are commercially available and should be sourced locally as per standard practice at the investigator site. As individual sites may use different brands or manufacturers for these drugs, each site is responsible for placing the most recent Summary of Product Characteristics (SmPC) for the brand being used at site in the local pharmacy folder or a file note that makes reference to the electronic source. Trial-specific labels will not be used for cisplatin or gemcitabine.

Descriptive information for cisplatin and gemcitabine can be found in the package inserts. Storage and handling of cisplatin and gemcitabine should be as described in the SmPC for the relevant brand used. Study treatment with cisplatin and gemcitabine should be administered according to the institutional standards at each site. Temperature monitoring and handling of temperature deviations will be as per local standard practice.

There will be no re-imbursement to sites for cisplatin and gemcitabine used during trial treatment.

Accountability of cisplatin and gemcitabine is as per local standard practice.

10.2. Durvalumab and tremelimumab composition

Durvalumab will be supplied as a 500mg vial (50 mg/mL) concentrate for solution for infusion. Please refer to the durvalumab Summary of Product Characteristics (SmPC) for full composition information.

Tremelimumab will be supplied as a 25 mg vial (20 mg/mL) concentrate for solution for infusion. Please refer to the tremelimumab Investigator Brochure (IB) for full composition information.

10.2.1. Supply and distribution of durvalumab and tremelimumab

Durvalumab and tremelimumab will be provided to sites free of charge for use in this clinical trial. Both IMPs will be supplied by AstraZeneca, labelled for trial use, stored and distributed to sites by Royal Free London NHS Foundation Trust.

Durvalumab and tremelimumab will be labelled with a trial-specific label in accordance with Directive 2001/20/EC and the Medicines for Human Use (Clinical Trials) Regulations 2004 (as amended).

Drug orders will be managed by CTRU and full details provided in the GUSTO Pharmacy Manual.

10.2.2. Storage

Once received at site, all trial IMP stock must be documented as received in accordance with the GUSTO Pharmacy Manual provided within the GUSTO Pharmacy Site File.

Durvalumab and tremelimumab vials must be stored at 2°C to 8°C and must not be frozen. Vials must be kept in their original packaging until time of preparation to prevent prolonged light exposure.

Doses of durvalumab and tremelimumab for administration must be prepared by the pharmacy staff members delegated by the Investigator (or an appropriate designee trained in study drug preparation), using aseptic technique and following local regulations and site requirements.

Stability requirements (included total time from durvalumab and tremelimumab preparation to administration) are provided in the GUSTO Pharmacy Manual and must be adhered to.

Temperature monitoring records must be kept. Stock affected by temperature deviations must be quarantined and reported to CTRU.

The trial supply of durvalumab and tremelimumab must not be used for any purpose other than that outlined in this protocol and should be stored in a secure ring-fenced location within the site pharmacy. Receipt of durvalumab and tremelimumab at trial centres and trial stock dispensed to participants must be recorded on the GUSTO Accountability and Dispensing Logs. Accountability logs will be provided by CTRU. The accountability logs must be retained in the relevant section of the Pharmacy Site File (PSF) (or a statement of its location). Local logs may be used if approved by CTRU. These completed logs will be returned to CTRU upon request, to facilitate central IMP reconciliation.

Unused durvalumab and tremelimumab should only be destroyed following authorisation from CTRU. Copies of accountability logs should be sent to CTRU with the request to destroy. Destruction should take place according to standard operating practice and local regulatory and environmental requirements. A record of any such destruction must be filed in the Pharmacy Site File and a copy sent to the CTRU.

10.2.3. Preparation and administration

Doses of durvalumab and tremelimumab must be prepared using aseptic technique. Full details regarding preparation are given in the GUSTO Pharmacy Manual which must be adhered to at all times. Further details are provided in the durvalumab SmPC and tremelimumab IB respectively.

Durvalumab and tremelimumab infusions are to be administered through an IV administration set with a 0.2 or 0.22 µm filter; acceptable configurations include an IV set containing an in-line filter or the attachment of a separate filter to the distal end of the IV tubing.

The durvalumab and tremelimumab infusion time is 1 hour ± 10 minutes; however, if there are interruptions, the total allowed time must not exceed 8 hours with the infusion bag kept at room temperature, otherwise a new dose must be prepared from new vials.

The administration of other drugs through the same infusion line at the same time is not permitted.

The IV line should be flushed with a volume equal to the IV line volume, according to local practices, to ensure the full dose is administered. Infusion time does not include the final flush time.

For patients having both immunotherapy and chemotherapy administrations, these may be administered over 2 days where this is required for logistical reasons due to the duration of administration. Immunotherapy is to be administered on day 1 and chemotherapy to be administered on day 2 of the cycle.

10.2.4. Prescribing

Trial sites are responsible for creating trial-specific prescriptions with the following information and must be signed by an authorised member of staff:

- Trial Name: GUSTO
- STH20710/IRAS 1005487
- Principal Investigator: site to add name
- 'Clinical Trial'
- Participant Trial Identifier (ID)

11. Treatment and Intervention Details

11.1. Gene Expression Subtyping Procedure

The trial will utilise the GUSTO Classifier for subtyping MIBC. This is defined as:

- a laboratory assay to determine RNA levels in the tumour tissue
- the Decipher platform and TCGA2 classification system which determine the expression of specific genes and assigns a relative weight to each gene in a patient's tumour to a given gene expression subtype

The laboratory assay is based on the Affymetrix Microarray (54). This is a high-density oligonucleotide microarray which is used to carry out a transcriptomic analysis of bladder tumour tissue. The assay will be carried out centrally by the GUSTO Laboratory team at the Sheffield Teaching Hospitals NHS Foundation Trust and the Genomics Core Facility of the Sheffield Institute for Translation Neuroscience (SITraN) in collaboration with Decipher (Veracyte).

Formalin-fixed paraffin-embedded tissue sample from TURBT will be transferred to the central Lab where standard 4 micron sections will be cut and stained with haematoxylin and eosin. The trial pathologist will mark areas of viable tumour and these will be macrodissected from

adjacent tissue sections. The resulting material will be de-paraffinised, rehydrated and RNA extracted. RNA will be labelled and hybridised on a GeneChip1 HuEx array (Affymeytrix). Microarrays will be scanned and gene expression subtyped using the Decipher bladder platform.

The results will be used to classify patients into study eligible categories: basal, neuronal, luminal infiltrated, luminal, luminal papillary and unclassifiable (ineligible). Unclassifiable cases will have a second attempt at subtyping starting from fresh sections from the FFPE block subject to timeline considerations (within 28 days from receipt of the first TURBT block). In cases where a repeat TURBT surgery is performed (according to local standard practice) and the tissue block from the repeat surgery is required for subtyping, the 28 day timeline will commence from receipt of the appropriate TURBT block at the central lab. At all times in the trial FFPE blocks will only be sampled if there is judged to be sufficient material remaining.

The results will be provided to the CTRU to be utilised in eligibility assessment. CTRU will confirm to the registering centre whether the patient is eligible or ineligible for randomisation based on whether the patient has MIBC with an eligible subtype.

11.1.1. Blinding to gene expression subtype result in the standard care arm

All patients registered into the study will have their TURBT sample analysed to determine a gene expression subtype, where possible. For patients in the standard care arm, the patient, clinician and trial staff at the trial sites will remain blinded to the results of this classification. Gene expression subtype will be available following end of trial.

11.1.2. Time to gene expression subtype result

It is anticipated that the time from TURBT sample receipt at the central laboratory to receipt of the gene expression subtype result by the central laboratory will be less than 2 weeks.

In the event that the time to receipt of the gene expression subtype result is greater than 4 weeks, the CTRU will advise the trial site that the patient will be ineligible for randomisation and that they should proceed to standard care treatment.

11.2. Surgery

11.2.1. Radical Cystectomy

Radical cystectomy should be performed at each cancer centre according to local practice and by surgeons and clinical teams specialising in this operation and service. According to national and international guidelines, surgery should include removal of adjacent organs and regional lymph nodes (61, 62). Exceptions to this surgical plan are to be recorded on the surgical treatment CRF.

Surgery should be performed as soon as possible after completion of, and recovery from, neoadjuvant therapy and is recommended within 14 to 56 days (but no more than 12 weeks) after the last dose of neoadjuvant chemotherapy/immunotherapy. Surgery for patients in the luminal papillary arm should be within 6 weeks of randomisation.

Procedures can be performed by either an open, laparoscopic or robotic route as per usual practice within that unit. Surgeons should avoid undertaking surgery whilst on their learning curve for a new modality.

Radical cystectomy should include removal of adjacent organs. In males, this includes the prostate and seminal vesicles. In women, this should include a section of adjacent anterior vaginal wall, the uterus, cervix and fallopian tubes and, if no bladder reconstruction is planned, the urethra. Oophorectomy is optional, as per local practice and individualised for each patient. *Exceptions to this surgical plan are acceptable with prior approval from CTRU.*

11.2.2. Pelvic Lymphadenectomy

Pelvic lymphadenectomy should be performed in all cases. The template for lymphadenectomy should include, at least, the regional lymph nodes up to the level of the ureteric crossing of the common iliac vessels. This includes the obturator fossa, the external iliac and internal iliac nodes. A more extended lymphadenectomy is acceptable. Excised lymphatic tissue should be submitted for local histological analysis as per local standard practice.

Urinary reconstruction: Reconstruction through all routes is acceptable. It is anticipated this will mostly include ileal conduit and orthotopic neobladder.

Perioperative care: Should be carried out as per ERAS protocols (63) and standard practice.

Postoperative care: Post-operative care is to be carried out as per standard practice.

11.3. IMP treatment

The IMP treatment in the study includes the following by arm and gene expression subtype:

Treatment arm and gene expression subtype	**Neoadjuvant systemic treatment (IMPs)**	**Adjuvant systemic treatment (IMPs)***
Standard care arm	Cisplatin Gemcitabine	Adjuvant treatment if considered appropriate by treating clinician (not trial treatment)
Subtype-guided: Basal Neuronal	Cisplatin Gemcitabine Durvalumab Tremelimumab	Durvalumab
Subtype-guided: Luminal Infiltrated	Durvalumab Tremelimumab	Durvalumab

Subtype-guided: Luminal Luminal Papillary	None	Adjuvant treatment if considered appropriate by treating clinician (not trial treatment)

* Adjuvant therapy is recommended to begin as soon as the patient recovers from, and within 28 to 120 days after, radical cystectomy.

Dosing will apply as follows (neoadjuvant cycles are 21 days in duration, adjuvant cycles are 28 days):

Standard care arm

- Neoadjuvant
  - Cisplatin: 70 mg/m^2 on day 1, every 21 days, for 4 cycles [or split dose 35 mg/m^2 on day 1 and day 8 for patients with impaired creatinine clearance (40-60 ml/min)]
  - Gemcitabine: 1000 mg/m^2 on days 1 and 8, every 21 days, for 4 cycles
- Adjuvant treatment is permitted if considered appropriate by the treating clinician (not trial treatment).

Basal and Neuronal subtypes

- Neoadjuvant
  - Cisplatin: 70 mg/m^2 on day 1, every 21 days, for 4 cycles
  - Gemcitabine: 1000 mg/m^2 on days 1 and 8, every 21 days, for 4 cycles
  - Durvalumab: 1500 mg on day 1, every 21 days, for 4 cycles
  - Tremelimumab: 75 mg on day 1, of cycles 1 and 3 only
- Adjuvant
  - Durvalumab: 1500 mg on day 1, every 28 days, for 8 cycles

Luminal Infiltrated subtype

- Neoadjuvant
  - Durvalumab: 1500 mg on day 1, every 21 days, for 4 cycles
  - Tremelimumab: 75 mg on day 1, of cycles 1 and 3 only
- Adjuvant
  - Durvalumab: 1500 mg on day 1, every 28 days, for 8 cycles

Luminal and Luminal Papillary subtypes

- Adjuvant treatment is permitted if considered appropriate by treating clinician (not trial treatment)

Surface Area Calculation: Body Surface Area (BSA) should be used to calculate cisplatin and gemcitabine doses. Local institutional policy for which BSA formula to use is acceptable (and will be reviewed during set up procedures for each site). Actual body weight should be used to calculate BSA. Weight should be monitored with each cycle of treatment and if there is a change of greater than 10% from baseline then BSA should be recalculated and doses adjusted accordingly.

Dose banding: Dose banding for cisplatin and gemcitabine to +/- 6% of the calculated dose per surface area, and consistent with the Chemotherapy Dose Standardisation Initiative developed by NHS England's Medicine Optimisation and Chemotherapy CRGs, is permitted where it is local practice to do so.
(https://www.england.nhs.uk/commissioning/spec-services/npc-crg/group-b/b02/dose-banded-chemotherapy-standardised-product-specifications/)

11.3.1. Immunotherapy administration

For patients allocated to receive neoadjuvant immunotherapy consisting of durvalumab in combination with tremelimumab: tremelimumab should be administered first and the durvalumab infusion should start approximately 1 hour (maximum 2 hours) after the end of the tremelimumab infusion. Standard infusion time for each is 1 hour, however if there are interruptions during infusion, the total allowed time must not exceed 8 hours at room temperature per infusion. If there are no clinically significant concerns after the first cycle, then, at the discretion of the treating clinician, all other cycles of durvalumab can be given immediately after the tremelimumab infusion has finished.

For patients allocated to receive both immunotherapy and chemotherapy (for basal and neuronal subtype treatment selection), it is recommended that a 60 minute observation period occurs after tremelimumab and durvalumab is administered and prior to chemotherapy, at least for Cycle 1. If no issues are observed following tremelimumab and durvalumab administration during the first cycle, subsequent reduction of the observation period may occur at the Investigator's discretion.

Patients must be monitored during and after infusion with durvalumab and tremelimumab. In the event of a ≤Grade 2 infusion-related reaction, the infusion rate of study drug may be decreased by 50% or interrupted until resolution of the event and re-initiated at 50% of the initial rate until completion of the infusion. For patients with a ≤Grade 2 infusion related reaction, subsequent infusions may be administered at 50% of the initial rate. Acetaminophen and/or an antihistamine (e.g., diphenhydramine) or equivalent medications per institutional standard may be administered at the discretion of the investigator. If the infusion related reaction is ≥Grade 3 or higher in severity, study drug will be discontinued. Standard infusion time is one hour, however if there are interruptions, the total allowed time must not exceed 8 hours at room temperature (otherwise requires new infusion preparation).

For management of patients who experience an infusion reaction, please refer to the toxicity and management guidelines in Appendix 4.

11.3.2. Concomitant medications

11.3.2.1. Supportive care

Local supportive care protocols including, but not limited to, anti-emetics, fluid hydration protocols, use of prophylactic granulocyte colony stimulating factor (G-CSF) and prophylactic medications for infusion reactions should be followed. Such policies will be reviewed by the TMG during site set up.

The administration of inactivated viruses, such as those in the influenza vaccine are permitted during trial treatment.

11.3.2.2. Excluded concomitant medications

- Any concurrent chemotherapy, radiotherapy, immunotherapy, or biologic or hormonal therapy for cancer treatment other than those under investigation in this study are not permitted during trial treatment. Concurrent use of hormonal therapy for non-cancer-related conditions (e.g., hormone replacement therapy) is acceptable.
- Immunosuppressive medications including, but not limited to, systemic corticosteroids at doses exceeding 10 mg/day of prednisone or equivalent, methotrexate, azathioprine, and tumor necrosis factor-α blockers. Permitted exceptions include:
    - use prior to imaging procedures in patients with contrast allergies
    - use of inhaled, topical, and intranasal corticosteroids
    - use of immunosuppressive medications for the management of adverse reactions to trial treatment
    - short-term premedication for patients receiving cisplatin/gemcitabine/durvalumab/tremelimumab combination treatment where the local standard practice regimen requires the use of steroids for documented hypersensitivity reactions and has been reviewed by the GUSTO TMG during site set-up
    - a temporary period of steroids is permitted if clinically indicated and considered to be essential for the management of non-immunotherapy related events experienced by the patient (e.g., chronic obstructive pulmonary disease, radiation, nausea, etc.).
- Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) (should not be concomitantly, should be used with caution in the 90 days post last dose of immunotherapy). Increased incidences of pneumonitis (with third generation EGFR TKIs) and increased incidence of transaminase increases (with first generation EGFR TKIs) has been reported when durvalumab has been given concomitantly.
- Live attenuated vaccinations (should not be given until 30 days after last dose of immunotherapy)
- Herbal and natural remedies which may have immune-modulating effects.

Patients receiving trial immunotherapy treatment should not donate blood during trial treatment or for at least 90 days after receipt of the final dose of durvalumab or tremelimumab.

11.3.3. Treatment Toxicities

11.3.3.1. Immunotherapy toxicity

Monoclonal antibodies directed against immune checkpoint proteins, such as PD-L1 as well as those directed against PD-1 or CTLA-4, aim to boost endogenous immune responses directed against tumour cells. By stimulating the immune system, however, there is the potential for adverse effects on normal tissues.

Most adverse drug reactions seen with the immune checkpoint inhibitor class of agents are thought to be due to the effects of inflammatory cells on specific tissues. These risks are generally events with a potential inflammatory or immune mediated mechanism and which may require more frequent monitoring and/or unique interventions such as

immunosuppressants and/or endocrine therapy. These immune mediated effects, can occur in nearly any organ system, and are most commonly seen as gastrointestinal AEs such as colitis and diarrhoea, pneumonitis/interstitial lung disease (ILD), hepatic AEs such as hepatitis and liver enzyme elevations, skin events such as rash and dermatitis and endocrinopathies including hypo- and hyper-thyroidism.

11.3.3.2. Allergic reactions to immunotherapy

As with any antibody, allergic reactions to durvalumab and tremelimumab dose administration are possible. Appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available, and study personnel must be trained to recognize and treat anaphylaxis. The study site must have immediate access to emergency resuscitation teams and equipment in addition to the ability to admit patients to an intensive care unit if necessary.

11.3.3.3. Infusion-related reactions to immunotherapy

In the event of a ≤Grade 2 infusion-related reaction, the infusion rate of durvalumab or tremelimumab may be decreased by 50% or interrupted until resolution of the event and re-initiated at 50% of the initial rate until completion of the infusion.

For patients with a ≤Grade 2 infusion related reaction, subsequent infusions may be administered at 50% of the initial rate. Acetaminophen and/or an antihistamine (e.g., diphenhydramine) or equivalent medications per local standard practice may be administered at the discretion of the treating clinician.

If the infusion related reaction is ≥Grade 3 or higher in severity, durvalumab and/or tremelimumab must be discontinued. Standard infusion time is one hour, however if there are interruptions, the total allowed time must not exceed 8 hours at room temperature (otherwise requires new infusion preparation). For management of patients who experience an infusion reaction, please refer to the toxicity and management guidelines in Appendix 4.

11.3.3.4. Durvalumab toxicity

Expected adverse reactions to durvalumab include, but are not limited to, diarrhoea/colitis, pneumonitis/interstitial lung disease (ILD), endocrinopathies (ie, events of hypophysitis/hypopituitarism, adrenal insufficiency, hyper- and hypothyroidism, type I diabetes mellitus (which may present with diabetic ketoacidosis), and diabetes insipidus), hepatitis/increases in transaminases, nephritis/increases in creatinine, rash/dermatitis (including pemphigoid, myocarditis, myositis/polymyositis, immune thrombocytopenia, infusion-related reactions, hypersensitivity reactions, pancreatitis, encephalitis serious infections, subcutaneous injection site reaction, and other rare or less frequent inflammatory events including neuromuscular toxicities (eg, Guillain-Barré syndrome, myasthenia gravis).

In monotherapy clinical studies, AEs at an incidence of ≥ 20% include events such as fatigue and decreased appetite. Approximately 10% of participants discontinued the drug due to an AE.

For information on all identified and potential risks with durvalumab please always refer to the current version of the durvalumab SmPC.

11.3.3.5. Tremelimumab toxicity

Expected adverse reactions to tremelimumab monotherapy include, but are not limited to, gastrointestinal effects (colitis, diarrhoea, enterocolitis, intestinal perforation and large intestinal perforation); endocrine disorders (hypo- and hyperthyroidism, hypophysitis and adrenal insufficiency); clinical manifestations of pancreatitis (elevations in lipase and amylase); skin effects (rash and pruritus); hepatic events (including autoimmune and immune-mediated hepatitis and liver enzyme elevations); pneumonitis and ILD; neurotoxicity (including encephalitis, encephalopathy, peripheral motor and sensory neuropathies and Guillain-Barré syndrome); thrombocytopenia, anaemia and neutropenia; infusion related reactions and hypersensitivity/anaphylactic reactions; renal events (including tubulointerstitial nephritis/autoimmune nephritis and acute kidney injury, autoimmune arthritis, Sjogren's syndrome, giant cell temporal arteritis and ulcerative colitis; hyperglycaemia and diabetes mellitus.

In monotherapy clinical studies, AEs reported at an incidence of ≥ 20% include events such as diarrhoea, nausea, fatigue, pruritus, decreased appetite, rash, vomiting and dyspnoea. Approximately 16% of participants experienced an AE that resulted in permanent discontinuation of tremelimumab, and approximately 45% of participants experienced an SAE.

For information on all identified and potential risks with tremelimumab please always refer to the current version of the tremelimumab IB.

11.3.3.6. Durvalumab and tremelimumab combination toxicity

The types of risks with the combination of durvalumab and tremelimumab (based on an equivalent durvalumab dose of 20 mg/kg and a tremelimumab dose of 1 mg/kg) are similar to those for durvalumab monotherapy with additional risks of amylase increased, lipase increased, intestinal perforation and large intestinal perforation, pulmonary embolism which are unique risks for the durvalumab and tremelimumab combination.

For information on all identified and potential risks with the durvalumab and tremelimumab combination please always refer to the current version of the durvalumab IB.

In durvalumab and tremelimumab combination studies at the dose of durvalumab 20 mg/kg and tremelimumab 1 mg/kg, AEs reported at an incidence of ≥ 20% included events such as fatigue, diarrhoea, nausea, decreased appetite, pruritus, dyspnoea, constipation and anaemia. Please see the current version of the durvalumab IB for a detailed summary of combination therapy data, including AEs, SAEs, and CTCAE Grade 3 to 5 events reported across the durvalumab program, including durvalumab in combination with tremelimumab.

Approximately 15% of participants experienced an AE that resulted in permanent discontinuation of study intervention, and approximately 16% of participants experienced an SAE that was considered to be related to durvalumab and tremelimumab by the study investigator.

11.3.4. Dose Modifications

Participants must be monitored for toxicity and toxicities graded according to NCI CTCAE, version 5.0 (see Appendix 1).

Every effort should be made to ensure patients complete four cycles of neoadjuvant therapy (with the exception of subtype-guided Luminal and Luminal Papillary patients), across all treatment arms in the study, if conditions allow.

Toxicities should be treated with maximum supportive care according to local practice and the suggested immunotherapy toxicity management guidelines detailed in Appendix 4.

Patients should be thoroughly evaluated to exclude alternative etiology (e.g., disease progression, concomitant medications, infections).

Changes to planned chemotherapy and immunotherapy administration should be fully documented in the patient's notes with clear reasoning for the approach taken.

If neoadjuvant chemotherapy or immunotherapy are being given in isolation, and are discontinued, then patients should proceed to cystectomy as soon as clinically appropriate.

If neoadjuvant chemotherapy or immunotherapy are being co-administered, and either, but not both, require either omission or discontinuation, then patients may continue to complete neoadjuvant treatment cycles (of the other modality) providing the rules regarding treatment delay are respected.

Where dose delays are required for reasons not relating to toxicity (i.e. logistical arrangements) these should be as minimal as possible and not more than 21 days.

11.3.4.1. Dose modifications for cisplatin and gemcitabine

Dose modifications are permitted for cisplatin and gemcitabine, regardless of treatment arm.

Dose reductions, omissions, delays or discontinuations should be made according to local institutional practice. In general, if treatment will not be administered, it is recommended to delay Day 1 treatment (cisplatin + gemcitabine, +/- immunotherapy) but to omit Day 8 (gemcitabine) treatment.

In the event that creatinine clearance drops below 60 mL/min (but ≥ 40 mL/min), the cisplatin dose must be fractionated into split dose 35 mg/m^2 administrations on days 1 and 8 of each cycle for management of renal toxicity.

11.3.4.2. Dose delays during neoadjuvant chemotherapy

If Day 1 of a neoadjuvant chemotherapy treatment cycle is delayed longer than 21 days, regardless of cause, then it is recommended to consider proceeding direct to cystectomy. MDT discussion and documentation of this decision is recommended.

If neoadjuvant chemotherapy treatment is delayed for longer than 42 days, treatment should be discontinued.

11.3.5. Dose modifications for durvalumab and tremelimumab

Dose reductions are <u>not permitted</u> for durvalumab and tremelimumab (apart from weight-based dosing described below). If immunotherapy cannot be administered due to toxicity, it

must be omitted or discontinued (or delayed only where it is co-administered with delayed chemotherapy).

Toxicities should be treated with maximum supportive care according to local practice and the toxicity management guidelines detailed in Appendix 4. The toxicity management guidelines in Appendix 4 detail the management of immune-mediated reactions, infusion-related reactions, and non-immune-mediated reactions for durvalumab and tremelimumab. Patients should be thoroughly evaluated and appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other aetiologic causes of the immune-mediated adverse event. Serologic, immunologic, and histologic (biopsy) data, as appropriate, should be used to support an immune-mediated adverse event diagnosis. In the absence of a clear alternative etiology, events should be considered potentially immune related.

Weight-based dosing should be utilized for patients whose body weight falls to <30 kg.

Durvalumab: If a patient's weight falls to ≤30 kg weight-based dosing at 20 mg/kg should be prepared using an IV bag selected such that the final concentration is within 1 to 15 mg/mL.

Tremelimumab: If a patient's weight falls to ≤30 kg weight-based dosing at 1 mg/kg should be prepared using an IV bag selected such that the final concentration is within 0.1 to 10 mg/mL

Once the patient's weight improves to above 30 kg (>30 kg), the patient should start resume receiving the full fixed dose of durvalumab and tremelimumab (as relevant).

In the absence of a clear alternative aetiology, all events should be considered potentially immune related. Toxicity management for immunotherapy is at the discretion of the treating clinician and decisions should be clearly documented. Suggested guidance is detailed in Appendix 4.

11.3.5.1. Dose omissions during neoadjuvant immunotherapy

If Day 1 of a neoadjuvant immunotherapy treatment cycle is omitted such that treatment does not occur for longer than 21 days (so once two consecutive doses have been omitted), regardless of cause, then it is recommended to consider proceeding direct to cystectomy. MDT discussion and documentation of this decision is recommended. Periods without scheduled treatment of longer than 42 days (once three doses have been omitted) will result in neoadjuvant treatment being discontinued.

On cycles where both durvalumab and tremelimumab are scheduled (neoadjuvant cycles 1 and 3) any omissions will apply to both drugs.

Where immunotherapy doses are omitted, it is advised to resume dosing on Day 1 of the subsequent scheduled cycle where this is possible. The duration of the neoadjuvant portion of the trial, and time to cystectomy, should therefore be preserved.

If immunotherapy is omitted then administration should be coordinated to resume with the next Day 1 of a chemotherapy cycle where they are being co-administered.

Patients who discontinue neoadjuvant chemotherapy, or immunotherapy, or both, may still receive adjuvant durvalumab if they had been allocated to receive it and if this remains clinically appropriate according to the toxicity management guidelines detailed in Appendix 4.

11.3.5.2. Dose omissions during adjuvant treatment

If planned doses are not given during adjuvant treatment, then immunotherapy doses that would have been scheduled to have been administered during the delay will be omitted such that adjuvant treatment is still completed over 32 weeks (eight cycles of four weeks). If three consecutive doses are omitted due to treatment related toxicity then the patient should permanently discontinue immunotherapy.

11.4. Discontinuation of trial treatment

In line with usual clinical care, cessation or alteration of regimens at any time will be at the discretion of attending clinicians or the participants themselves.

An individual patient must discontinue further trial treatment (durvalumab, tremelimumab, gemcitabine, or cisplatin) if any of the following occur:

- Adverse events, or emergent co-morbidity, that, in the opinion of the Investigator, contraindicates further dosing
- Pregnancy or intent to become pregnant
- Non-compliance with the study protocol that, in the opinion of the Investigator, warrants withdrawal from treatment
- Initiation of alternative anti-cancer therapy including another investigational agent other than treatment described in this protocol
- Clinical progression and treating clinician's determination that the patient is no longer benefiting from treatment
- Disease progression that precludes the patient from cystectomy or during adjuvant treatment
- Radical cystectomy is not performed for any reason

All randomised participants withdrawn from trial treatment or prescribed alternative treatment will still attend for follow-up assessments unless unwilling to do so and CRFs will continue to be completed. The exception to this is patients who do not receive radical cystectomy (see Section 12.13).

Follow-up data will continue to be collected, including data collection through linkage to NHS data systems, unless the patient explicitly states they do not wish to contribute further data to the study.

The PI, or delegate, should make every effort to ensure that the specific wishes of any participant who wishes to withdraw consent for further involvement in the trial are defined and documented using the Withdrawal eCRF in order that the correct processes are followed by the CTRU and site following the withdrawal of consent.

It should be made clear to any participant specifically withdrawing consent for further data collection in a CTIMP that data pertaining to safety will continue to be collected for regulatory reporting purposes and will be included in any safety analysis. In addition, it is suggested that

the participant is made aware of the fact that if any significant new information becomes available in regard to the treatment they have received in the trial it may be necessary to contact them in the future. It is not possible to withdraw data which has already been collected.

The Treatment Discontinuation eCRF should be completed **within 24 hours** of the research team becoming aware of a patient discontinuing trial treatment.

Patients will be considered lost to follow-up only if no contact has been established by the time the study is completed, such that there is insufficient information to determine the patient's status at that time.

12. Assessments, Samples and Data Collection

Investigations in this trial will use the results of local assessments. Data will be collected by electronic data capture (EDC) using the MACRO database which will be managed by the CTRU at the University of Leeds. Missing and discrepant data will be flagged and additional data validations raised as appropriate by the CTRU data management team.

Data must only be entered by trained personnel, authorised to do so by the Principal Investigator, as recorded on the trial-specific Authorised Personnel Log. Login details will be provided for these personnel only and should not be shared with others.

Some paper records such as copies of pathology reports and baseline CT/MRI scan reports will be requested by CTRU during the course of the trial. It is the responsibility of staff at trial sites to obliterate all personal identifiable data prior to sending to CTRU. Reports should only include trial number, initials and date of birth to identify the participant. The exception to this is the participant consent form, where the participant name and signature must not be obliterated. If signed consent forms are posted to CTRU, they must be sent in a separate envelope and not accompanied by any CRFs or other documents containing clinical data.

All Serious Adverse Events (SAEs), Suspected Unexpected Serious Adverse Reactions (SUSARs) and Unexpected Serious Complications (USCs) must be reported within 24 hours of becoming aware of the event.

Participant-completed Quality of Life questionnaires will be completed on using REDCap as default with paper CRFs available if required. Baseline questionnaires may be completed using paper CRFs.

Trial sites will be expected to maintain a file of essential trial documentation (Investigator Site File, ISF), which will be provided by the CTRU. The eCRFs and participant-completed Quality of Life questionnaires will contain the participant's unique trial number, date of birth, and initials.

12.1. Schedule of Events

12.1.1. Schedule of events for STANDARD CARE ARM patients (neoadjuvant chemotherapy only)

Trial Phase/Intervention		Pre-registration[1]	Pre-randomisation	NAC	NAC	NAC	NAC	NAC	NAC	90 days post end of treat-ment[2]	Surgery	FU	FU	FU
Timepoint				C1D1	C1D8	C2D1; C3D1	C2D8; C3D8	C4D1	C4D8		RC	3 month F/U	6 month F/U	12 Month F/U
Clinical Procedures/ Assessments	Consent	X PIS-1[3]	X PIS-2[3]											
	Surgery		X[4]								X[4]			
	Eligibility criteria review	X	X											
	ECOG performance status		X	X		X		X				X	X	X
	Medical history		X											
	Physical examination		X	X		X		X				X	X	X
	Weight and vital signs[5]		X			X		X						
	Height		X											
	ECG		X[6]	X[6]	X[6]	X[6]		X[6]		X[6]				
	CT or MRI Imaging		X[7]							X[8]			X[9]	X[9]
	Chemotherapy			X	X	X	X	X	X					
	AE assessment				X	X	X	X	X	X				
	Clavien-Dindo assessment										X	X		
	Concomitant medication		X	X		X		X						
	Translational blood sample			X[10]							X[10]	X		X
	Tissue sample collection	X[11]									X			
	Non-trial treatment details											X	X	X
Central laboratory assessment	Gene expression subtype allocation		X											
Local laboratory assessments	Serum biochemistry[12] & FBC		X	X[13]	X	X	X	X	X	X				
	Pregnancy test[14]		X	X		X		X		X				
	PD-L1 test											X[15]	X[15]	X[15]
Data collection	QoL[16]		X										X	X
	CRF completion		X	X		X		X		X	X	X	X	X

[1] Pre-screening may occur at any time following an initial cystoscopy at which the patient is deemed likely to have organ confined muscle invasive bladder cancer by the responsible urologist

[2] This may be completed in clinic or over the telephone 90 days (+/-7 days) following permanent end of trial treatment.
[3] and Participant Supplementary Document
[4] The pathological staging from the TURBT and RC sample should be recorded and provided as an anonymised copy of the pathology report
[5] Vital signs including blood pressure, pulse, temperature and respiration rate
[6] During screening, ECG should be undertaken within 28 days prior to randomisation. Any clinically significant abnormalities detected require triplicate ECG results. Subsequent ECG monitoring during the treatment period should be conducted if it is clinically indicated..
[7] CT scan or MRI (according to local practice) of chest, abdomen and pelvis as per local site standard practice but no longer than 12 weeks prior to randomisation
[8] Cross sectional imaging after completion of neoadjuvant treatment and prior to RC should be done as per standard practice (not required within 28 days of end of treatment)
[9] CT scan or MRI (according to local practice) of chest, abdomen and pelvis at 6 and 12 months post-RC which can occur up to 3 weeks prior to the trial visit
[10] blood samples to be taken prior to starting NAC and post-NAC but prior to RC
[11] TURBT sample collection for gene expression subtype allocation (dispatched ASAP)
[12] Serum biochemistry including renal (including Na+, K+, urea, creatinine), liver (including ALT and/or AST (both are recommended), ALP and bilirubin), bone (including serum albumin and calcium) profiles, amylase and/or lipase
[13] Not required if screening assessments were performed within 7 days prior to day 1
[14] Highly sensitive serum pregnancy test required pre-randomisation, serum or urine pregnancy test required on day 1 of each neoadjuvant treatment cycle and at 90 days post end of trial treatment or prior to radical cystectomy, whichever comes first
[15] Local PD-L1 results to be collected if available
[16] EQ-5D-3L and QLQ C30

12.1.2. Schedule of events for BASAL and NEURONAL subtype patients in the gene expression subtype arm (chemotherapy + immunotherapy)

Trial Phase/Intervention		Pre-registration[1]	Pre-randomisation	NAC; IO	NAC	NAC; IO	NAC	NAC; IO	NAC	90 days post end of treatment[3]	Surgery	IO	FU	IO	90 days post end of treatment[3]	FU	FU
Timepoint				C1D1[2]	C1D8	C2D1[2]; C3D1[2]	C2D8; C3D8	C4D1[2]	C4D8		RC	C5D1	3 month F/U	C6D1 to C8D1		6 month F/U	12 month F/U
Clinical Procedures/ Assessments	Consent	X PIS-1[4]	X PIS-2[4]														
	Surgery		X[5]								X[5]						
	Eligibility criteria review	X	X														
	ECOG performance status		X	X		X		X				X	X	X		X	X
	Medical history		X														
	Physical examination		X	X		X		X				X	X	X		X	X
	Weight and vital signs[6]		X			X		X				X		X			
	Height		X														
	ECG		X[7]	X[7]		X[7]		X[7]				X[7]		X[7]	X[7]		
	CT or MRI Imaging		X[8]							X[9]						X[10]	X[10]
	Chemotherapy			X	X	X	X	X	X								
	Immunotherapy			X[11]		X[11]		X[11]				X[12]		X[12]			
	AE assessment				X	X	X	X	X	X				X	X		
	Clavien-Dindo assessment										X		X				
	Concomitant medication		X	X		X		X				X		X			
	Translational blood sample			X[13]							X[13]		X				X
	Tissue sample collection	X[14]									X						
Central laboratory assessment	Gene expression subtype allocation		X														
Local laboratory assessments	Serum biochemistry[15] & FBC		X	X[16]	X	X	X	X	X	X		X		X	X		
	Cortisol and thyroid profile			X		X		X				X		X			
	Pregnancy test[17]		X	X		X		X		X							
	PD-L1 test												X[18]			X[18]	X[18]
Data collection	QoL[19]		X													X	X
	CRF completion		X	X		X		X		X	X	X	X	X	X	X	X

[1] Pre-screening may occur at any time following an initial cystoscopy at which the patient is deemed likely to have organ confined muscle invasive bladder cancer by the responsible urologist
[2] Treatment administration may be split over 2 days to accommodate logistical arrangements if consistent with local standard practice
[3] This may be completed in clinic or over the telephone 90 days (+/-7 days) following permanent end of trial treatment.
[4] and Participant Supplementary Document
[5] The pathological staging from the TURBT and RC sample should be recorded and provided as an anonymised copy of the pathology report
[6] Vital signs including blood pressure, pulse, temperature and respiration rate
[7] During screening, ECG should be undertaken within 28 days prior to randomisation. Any clinically significant abnormalities detected require triplicate ECG results. Subsequent ECG monitoring during the treatment period should be conducted if it is clinically indicated..
[8] CT scan or MRI (according to local practice) of chest, abdomen and pelvis as per local site standard practice but no longer than 12 weeks prior to randomisation
[9] Cross sectional imaging after completion of neoadjuvant treatment and prior to RC should be done as per standard practice (not required within 28 days of end of treatment)
[10] CT scan or MRI (according to local practice) of chest, abdomen and pelvis at 6 and 12 months post-RC which can occur up to 3 weeks prior to the trial visit
[11] Durvalumab given every 21 days, tremelimumab given at C1D1 and C3D1
[12] Durvalumab given every 28 days
[13] blood samples to be taken prior to starting NAC and post-NAC but prior to RC
[14] TURBT sample collection for gene expression subtype allocation (dispatched ASAP)
[15] Serum biochemistry including renal (including Na+, K+, urea, creatinine), liver (including ALT and/or AST (both are recommended), ALP and bilirubin), bone (including serum albumin and calcium) profiles, amylase and/or lipase
[16] Not required if screening assessments were performed within 7 days prior to day 1
[17] Highly sensitive serum pregnancy test required pre-randomisation, serum or urine pregnancy test required on day 1 of each neoadjuvant treatment cycle and prior to radical cystectomy or, if it occurs sooner, at 180 days after the last dose of tremelimumab or 90 days post end of trial treatment (durvalumab, cisplatin and gemcitabine), whichever occurs latest or prior to radical cystectomy if cystectomy occurs before this point is reached
[18] Local PD-L1 results to be collected if available
[19] EQ-5D-3L and QLQ C30

12.1.3. Schedule of events for LUMINAL INFILTRATED subtype patients in the gene expression subtype arm (immunotherapy only)

Trial Phase/Intervention		Pre-registration[1]	Pre-randomisation	IO	IO	IO	90 days post end of treat-ment[2]	Surgery	IO	FU	IO	90 days post end of treat-ment[2]	FU	FU
Timepoint				C1D1	C2D1; C3D1	C4D1		RC	C5D1	3 month F/U	C6D1 to C8D1		6 month F/U	12 Month F/U
Clinical Procedures/ Assessments	Consent	X PIS-1[3]	X PIS-2[3]											
	Surgery		X[4]					X[4]						
	Eligibility criteria review	X	X											
	ECOG performance status		X	X	X	X			X	X	X		X	X
	Medical history		X											
	Physical examination		X	X	X	X			X	X	X		X	X
	Weight and vital signs[5]		X		X	X			X		X			
	Height		X											
	ECG		X[6]	X[6]	X[6]	X[6]			X[6]		X[6]	X[6]		
	CT or MRI Imaging		X[7]				X[8]						X[9]	X[9]
	Immunotherapy			X[10]	X[10]	X[10]			X[11]		X[11]			
	AE assessment				X	X	X				X	X		
	Clavien-Dindo assessment							X		X				
	Concomitant medication		X	X	X	X			X		X			
	Translational blood sample			X[12]				X[12]		X				X
	Tissue sample collection	X[13]						X						
Central laboratory assessment	Gene expression subtype allocation		X											
Local laboratory assessments	Serum biochemistry[14] & FBC		X	X[15]	X	X	X		X		X	X		
	Cortisol and thyroid profile			X	X	X			X		X			
	Pregnancy test[16]		X	X	X	X	X							
	PD-L1 test									X[17]			X[17]	X[17]
Data collection	QoL[18]		X										X	X
	CRF completion		X	X	X	X	X	X	X	X	X	X	X	X

[1] Pre-screening may occur at any time following an initial cystoscopy at which the patient is deemed likely to have organ confined muscle invasive bladder cancer by the responsible urologist
[2] This may be completed in clinic or over the telephone 90 days (+/-7 days) following permanent end of trial treatment.
[3] and Participant Supplementary Document
[4] The pathological staging from the TURBT and RC sample should be recorded and provided as an anonymised copy of the pathology report
[5] Vital signs including blood pressure, pulse, temperature and respiration rate
[6] During screening, ECG should be undertaken within 28 days prior to randomisation. Any clinically significant abnormalities detected require triplicate ECG results. Subsequent ECG monitoring during the treatment period should be conducted if it is clinically indicated..
[7] CT scan or MRI (according to local practice) of chest, abdomen and pelvis as per local site standard practice but no longer than 12 weeks prior to randomisation
[8] Cross sectional imaging after completion of neoadjuvant treatment and prior to RC should be done as per standard practice (not required within 28 days of end of treatment)
[9] CT scan or MRI (according to local practice) of chest, abdomen and pelvis at 6 and 12 months post-RC which can occur up to 3 weeks prior to the trial visit
[10] Durvalumab given every 21 days, tremelimumab given at C1D1 and C3D1
[11] Durvalumab given every 28 days
[12] blood samples to be taken prior to starting NAC and post-NAC but prior to RC
[13] TURBT sample collection for gene expression subtype allocation (dispatched ASAP)
[14] Serum biochemistry including renal (including Na+, K+, urea, creatinine), liver (including ALT and/or AST (both are recommended), ALP and bilirubin), bone (including serum albumin and calcium) profiles, amylase and/or lipase
[15] Not required if screening assessments were performed within 7 days prior to day 1
[16] Highly sensitive serum pregnancy test required pre-randomisation, serum or urine pregnancy test required on day 1 of each neoadjuvant treatment cycle and prior to radical cystectomy or, if it occurs sooner, at 180 days after the last dose of tremelimumab or 90 days post end of trial treatment (durvalumab, cisplatin and gemcitabine), whichever occurs latest or prior to radical cystectomy if cystectomy occurs before this point is reached
[17] Local PD-L1 results to be collected if available
[18] EQ-5D-3L and QLQ C30

12.1.4. Schedule of events for LUMINAL PAPILLARY or LUMINAL subtype patients in the gene expression subtype arm (direct to surgery, optional adjuvant treatment not part of trial treatment)

Trial Phase/Intervention		Pre-registration[1]	Pre-randomisation	Surgery	FU	FU	FU
Timepoint				RC	3 month F/U	6 month F/U	12 Month F/U
Clinical Procedures/ Assessments	Consent	X PIS-1[2]	X PIS-2[2]				
	Surgery		X[3]	X[2]			
	Eligibility criteria review	X	X				
	ECOG performance status		X		X	X	X
	Medical history		X				
	Physical examination		X		X	X	X
	Weight and vital signs[4]		X				
	Height		X				
	ECG		X[5]				
	CT or MRI Imaging		X[6]			X[7]	X[7]
	AE assessment						
	Clavien-Dindo assessment			X	X		
	Concomitant medication		X				
	Translational blood sample			X[8]	X		X
	Tissue sample collection	X[9]		X			
	Non-trial treatment details				X	X	X
Central laboratory	Gene expression subtype allocation		X				
Local laboratory assessments	Serum biochemistry[10] & FBC		X				
	Pregnancy test[11]		X				
	PD-L1 test				X[12]	X[12]	X[12]
Data collection	QoL[13]		X			X	X
	CRF completion		X	X	X	X	X

[1] Pre-screening may occur at any time following an initial cystoscopy at which the patient is deemed likely to have organ confined muscle invasive bladder cancer by the responsible urologist
[2] and Participant Supplementary Document
[3] The pathological staging from the TURBT and RC sample should be recorded and provided as an anonymised copy of the pathology report
[4] Vital signs including blood pressure, pulse, temperature and respiration rate
[5]Within 28 days prior to randomisation. Any clinically significant abnormalities detected require triplicate ECG results. ECG monitoring must be conducted during the treatment period if clinically indicated.
[6]CT scan or MRI (according to local practice) of chest, abdomen and pelvis as per local site standard practice but no longer than 12 weeks prior to randomisation
[7] CT scan or MRI (according to local practice) of chest, abdomen and pelvis at 6 and 12 months post-RC which can occur up to 3 weeks prior to the trial visit
[8] blood sample to be taken prior to RC
[9] TURBT sample collection for gene expression subtype allocation (dispatched ASAP)
[10] Serum biochemistry including renal (including Na+, K+, urea, creatinine), liver (including ALT and/or AST (both are recommended), ALP and bilirubin), bone (including serum albumin and calcium) profiles, amylase and/or lipase
[11] Highly sensitive serum pregnancy test required pre-randomisation
[12] Local PD-L1 results to be collected if available
[13] EQ-5D-3L and QLQ C30

12.2. Pre-registration eligibility assessments

The following investigations must be performed to establish eligibility for registration:

- Confirmation of MIBC with pure or mixed urothelial (transitional) cell carcinoma. This includes:
  - High grade pure or mixed urothelial (transitional) cell carcinoma which is at least T1 on histology AND radiological evidence of T2+ or N1
  - High grade pure or mixed urothelial (transitional) cell carcinoma which is at least T2 on histology

12.3. Pre-randomisation eligibility and baseline assessments

The following investigations and assessments must be carried out **prior to randomisation. Written informed consent must be obtained before any assessments that are not part of standard care**.

- Gene expression subtype allocation using TURBT sample: once MIBC has been confirmed by the local pathologist, the pathology sample must be sent to the central laboratory in Sheffield ASAP.
- CT or MRI scan (as per local site standard practice but no longer than 12 weeks prior to randomisation). An anonymised copy of the report will be collected post-randomisation.
- ECG (within 28 days prior to randomisation). Any clinically significant abnormalities detected require triplicate ECG results. ECG monitoring must be conducted during the treatment period if clinically indicated.

The following assessments must be carried out within 7 days prior to randomisation:

- FBC, serum biochemistry (including Na+, K+, urea, creatinine), liver (including ALT and/or AST (both are recommended) and bilirubin), bone (including serum albumin and calcium) profiles, amylase and/or lipase
- Negative pregnancy test (if the participant is a woman of childbearing potential). This must be a highly sensitive serum pregnancy test.
- Physical examination
- Weight and vital signs (including blood pressure, pulse, temperature and respiration rate)
- Height
- ECOG performance status
- Medical history (including details of concomitant disease and medication)
- Completion of the baseline QoL questionnaires by the participant (to be completed after consent for randomisation but before randomisation)

The following should be collected:

- Translational blood samples prior to neoadjuvant treatment start for patients in standard care arm and patients with basal, neuronal and luminal infiltrated subtypes in gene expression subtype-guided arms (refer to GUSTO Laboratory Manual for full details).
- An anonymised copy of the pathology report from the TURBT will be collected.

12.4. Neoadjuvant treatment: pre-treatment assessments

Neoadjuvant treatment should start as soon as possible after randomisation (within 21 days of randomisation) for standard care arm and for patients in gene expression subtype-guided arm and assigned basal, luminal infiltrated and neuronal subtypes.

The following assessments must be carried out **within 7 days** prior to cycle 1 day 1 and **within 3 days** prior to day 1 of subsequent cycles of neoadjuvant treatment:

- FBC, serum biochemistry (including Na+, K+, urea, creatinine), liver (including ALT and/or AST (both are recommended) and bilirubin), bone (including serum albumin and calcium) profiles, amylase and/or lipase on day 1 and day 8 of each cycle
- Negative pregnancy test prior to day 1 of each treatment cycle (if the participant is a woman of childbearing potential). This may be a serum or urine pregnancy test.
- Cortisol and thyroid profile (day 1 of each cycle of durvalumab and/or tremelimumab treatment only)
- Physical examination (targeted examinations from cycle 2 onwards are acceptable)
- Weight and vital signs (including blood pressure, pulse, temperature and respiration rate)
- Concomitant medication (day 1 of each cycle only)
- ECOG performance status
- Toxicity assessment: collection of any adverse events that have occurred since the previous treatment cycle (cycle 2 of neoadjuvant treatment onwards)
- ECG monitoring must be conducted during the treatment period if clinically indicated.

The following assessments must be carried out **within 3 days** prior to day 8 of chemotherapy treatment:

- FBC, serum biochemistry (including Na+, K+, urea, creatinine), liver (including ALT and/or AST (both are recommended) and bilirubin), bone (including serum albumin and calcium) profiles, amylase and/or lipase on day 8 of each cycle
- Toxicity assessment: collection of any adverse events that have occurred since the previous treatment (cycle 1 day 8 of neoadjuvant treatment onwards)
- Patients receiving durvalumab and tremelimumab should be contacted at day 8 for all neoadjuvant treatment cycles to ensure early identification and management of toxicities. This contact can be by telephone call if the patient is not attending clinic.
- ECG monitoring must be conducted during the treatment period if clinically indicated.

Investigations / assessments carried out at pre-randomisation do not need to be repeated if done within 7 days prior to the start of trial treatment.

12.5. Neoadjuvant immunotherapy treatment assessments

Patients receiving their **first immunotherapy infusion** should be closely monitored. Blood pressure and pulse should be measured before, during, and after each infusion at the following times (based on a 60-minute infusion):

- Prior to the infusion starting (measured once from approximately 30 minutes before up to 0 minutes [i.e., the beginning of the infusion])
- Approximately 30 minutes during the infusion (halfway through infusion)
- At the end of the infusion (approximately 60 minutes ±5 minutes)

If the infusion takes longer than 60 minutes, then blood pressure and pulse measurements should follow the principles as described above or be taken more frequently if clinically indicated.

A 1-hour observation period is recommended after the first infusion of durvalumab.

For **subsequent infusions**, patients should continue to be carefully monitored.

- Blood pressure, pulse and other vital signs should be measured prior to the start of the infusion.

- Blood pressure and other vital signs should be measured during and post infusion as per local standard practice and as clinically indicated.

12.6. Post-neoadjuvant treatment assessments

The following assessment **must** be carried out at 90 days (+/- 7 days) post last dose of neoadjuvant treatment for all patients who received immunotherapy:

- FBC, serum biochemistry (including Na+, K+, urea, creatinine), liver (including ALT and/or AST (both are recommended) and bilirubin), bone (including serum albumin and calcium) profiles, amylase and/or lipase

The following assessments should be carried out as relevant:

- Pregnancy testing:
    - A final pregnancy test (serum or urine) is required for women of child-bearing potential. This is required prior to radical cystectomy or, if it occurs sooner, 90 days after the last dose of durvalumab, cisplatin or gemcitabine or 180 days after the last dose of tremelimumab, whichever occurs latest or prior to radical cystectomy if cystectomy occurs before this point is reached.
- AE assessment:
    - For patients in the standard care/basal/neuronal/luminal infiltrated arm who received neoadjuvant trial chemotherapy and/or immunotherapy: an AE assessment is required at 90 days (+/- 7 days) post last dose of neoadjuvant treatment. This assessment may be completed in clinic or over the telephone.

12.7. Pre-, intra- and post-RC assessments

Radical cystectomy should take place within 2 to 8 weeks (14 to 56 days) and no more than 12 weeks (84 days) post last dose of neoadjuvant treatment or within 6 weeks (42 days) of randomisation for patients in the gene expression-guided arm and assigned a luminal/luminal papillary subtype.

The following assessments should be carried out prior to RC :

- Cross sectional imaging (CT or MRI as per local standard practice) after completion of neoadjuvant treatment and prior to RC should be done as per standard practice.
- Translational blood samples (for consented patients) to be taken as close as possible prior to RC (after completion of NAC if relevant) (refer to GUSTO Laboratory Manual for full details).

The following assessments should be carried out as part of intra-operative data collection (within 6 weeks of RC):

- Assessment of intra-operative complications from medical notes or as assessed at the time of operation
- Assessment of patient status at discharge
- Assessment of post-operative complications at discharge
- Post-operative details including return to theatre, post-operative intensive care unit (ITU) / high dependency unit (HDU) admission or radiological intervention
- The pathological staging from the RC sample should be recorded and an anonymised copy of the pathology report provided

The following assessments should be carried out post-RC (at 3 months post RC):

- Assessment of post-operative complications since discharge

- Post-operative details including return to theatre, post-operative intensive care unit (ITU) / high dependency unit (HDU) admission or radiological intervention
- Assessment of post-operative complications
- Readmission within 90 days of RC
- Pathological disease stage (N and M)
- Translational tissue collection (RC sample) for patients who have consented to future research

12.8. Adjuvant treatment: pre-treatment assessments

Adjuvant treatment should start no earlier than 4 weeks (28 days) and no more than 17 weeks (120 days) post-RC for patients in the basal, luminal infiltrated and neuronal subtypes.

The following assessments must be carried out **within 7 days** prior to cycle 5 day 1 and **within 3 days** prior to day 1 of subsequent cycles:

- FBC, serum biochemistry (including Na+, K+, urea, creatinine), liver (including ALT and/or AST (both are recommended), ALP and bilirubin), bone (including serum albumin and calcium) profiles, amylase and/or lipase
- Cortisol and thyroid profile
- Physical examination (targeted examinations from cycle 6 onwards are acceptable)
- Weight and vital signs (including blood pressure, pulse, temperature and respiration rate)
- Concomitant medication
- ECOG performance status
- Toxicity assessment: collection of any adverse events that have occurred since the start of the previous treatment cycle (cycle 6 onwards)
- ECG monitoring must be conducted during the treatment period if clinically indicated.

12.9. Post-adjuvant treatment assessments

The following assessment **must** be carried out at 90 days (+/- 7 days) post last dose of adjuvant treatment for all patients who received immunotherapy:

- FBC, serum biochemistry (including Na+, K+, urea, creatinine), liver (including ALT and/or AST (both are recommended) and bilirubin), bone (including serum albumin and calcium) profiles, amylase and/or lipase

The following assessments should be carried out as relevant:

- Pregnancy testing:
    - For women of child-bearing potential in the basal/neuronal/luminal infiltrated arm who received adjuvant durvalumab and did not receive a radical cystectomy including hysterectomy: a final pregnancy test (serum or urine) is required 90 days after the last dose of durvalumab.
- AE assessment:
    - For patients in the basal/neuronal/luminal infiltrated arm who received adjuvant trial immunotherapy: an AE assessment is required at 90 days (+/- 7 days) day 1 of last adjuvant treatment cycle. This assessment may be completed in clinic or over the telephone.

12.10. Post-RC follow-up assessments

Follow-up will take place at 3, 6 and 12 months post-RC (+/- 3 weeks).

The following assessments will be undertaken at each follow-up visit:

- Physical examination
- ECOG performance status
- Progression status or date of last review
- Date of death or last review
- Post-operative surgical complication collection (at 3 months post-RC only)
- CT or MRI scan at 6 and 12 month follow-up visits
- Translational blood samples (for consented patients) to be taken at 3 and 12 month follow-up visits (refer to GUSTO Laboratory Manual for full details).
- Treatment details for participants in the standard care arm and luminal/luminal papillary arm patients receiving non-trial treatment
- Local PD-L1 assessment results for all patients (if available)
- TMN staging from RC pathology report (at 3 months post-RC only)

Any patients still alive at their 12 month post-RC visit will be followed up at the time point coinciding the timepoint when all participants randomised have reached is 12 months post-RC.

12.11. Duration of follow-up

All evaluable trial participants will be followed up until all patients have reached 12 months post-RC. For patients who are more than 12 months post-RC, this data will be collected by the trial site from medical records relating to standard clinic visits.

Follow-up data collection will be from standard clinic visits and will include:

- Progression status or date of last review
- Date of death or date of last review
- Anti-cancer treatment administered after end of trial treatment

The further collection of outcome data beyond this defined follow up is planned subject to additional funding and ethical approval. This will be carried out by remote data capture such as NHS Digital.

12.12. Data collection for registered non-randomised patients

All evaluable consenting trial participants registered in the trial but not proceeding to randomisation will have the following data collected:

- Progression status or date of last review
- Date of death or last review
- RC date if done
- pCR status post-RC
- Neoadjuvant chemotherapy treatment (yes/no and details of treatment received)
- Adjuvant treatment (yes/no and details of treatment received)

This data will be collected at the time point coinciding with the timepoint when all participants randomised have reached is 12 months post-RC. This data collection will be from data recorded in the patients' notes during standard clinic visits.

The further collection of outcome data beyond this defined follow up is planned subject to additional funding and ethical approval. This will be carried out by remote data capture such as NHS Digital.

12.13. Data collection for participants who do not receive RC

Participants in the gene expression subtype-guided arm assigned to receive adjuvant immunotherapy who do not receive RC must not receive adjuvant durvalumab treatment.

Participants in any treatment arm who do not receive RC will not be required to attend trial follow-up visits.

These participants will be followed up until the end of the trial and data collection will be from the participant notes from standard of care visits. This data will be collected at the time point coinciding with the timepoint when all participants randomised have reached is 12 months post-RC. The following data will be collected:

- Progression status or date of last review
- Date of death or last review
- Treatment (yes/no and details of treatment received)

12.14. Disease progression

Any death and cause of death will be reported on a death specific CRF. Any instances of locoregional disease or metastatic cancer will be recorded on the follow-up CRFs at 3, 6 and 12 months post-RC and at the timepoint when all participants randomised have reached is 12 months post-RC (if applicable).

Participants who have progressed will be followed up at the end of the trial and the following data will be collected:

- Date of death or last review
- Progression status or date of last review
- Radiological staging

12.15. CT/MRI imaging

CT/MRI scans are to be carried as per local standard practice at the following timepoints:

- no longer than 12 weeks prior to randomisation
- after completion of neoadjuvant treatment and prior to RC
- at 6 months post-cystectomy
- at 12 months post-cystectomy

An anonymised copy of the CT/MRI scan report for the scan carried out prior to randomisation will be collected by CTRU for central monitoring.

Scans at these timepoints are considered to be part of standard practice. RECIST reporting is not required.

Patients will have additional CT/MRI scans beyond 12 months post-cystectomy as part of standard practice. These scans are not considered to be part of the study and this timepoint will not be reached for all patients on study. Data on standard practice scans outside of the scans listed above may be collected for some patients if these occur within the trial follow-up period.

12.16. Assessment of efficacy – central review of pathology reports

All patients that undergo randomisation will be followed up to assess pCR post-RC. pCR is assessed locally and the pathology report confirming this response is collected for central verification of this result.

Pathology reports must be anonymised and identified with the patient's trial number, date of birth and initials. Pathology reports should be transferred to CTRU with 6 weeks of RC.

12.17. Quality of life

For all participants who are randomised into the study, health related quality of life will be assessed at randomisation and at 6 and 12 months post-RC using the EQ-5D-3L and EORTC QLQ-C30 questionnaires.

12.18. Adverse Events and Serious Adverse Events

Adverse events relating to IMP will be collected at treatment visits and at 90 days post last dose of IMP and will graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Specific expected post-operative complications will be collected at 3 months post-surgery and will be graded using the Clavien-Dindo classification (64).

Details of intra-operative complications will be collected within 6 weeks post-surgery and will be graded using the ClassIntra classification (65).

Serious adverse events will be collected at all treatment and follow-up visits within the reporting period (90 days post last dose of IMP and 30 days post-RC).

12.19. Pregnancies

All protocol treatment must be stopped immediately if a pregnancy in a female participant occurs or is suspected.

All pregnancies and suspected pregnancies of a participant, or of a male participant's partner, must be reported from the date of randomisation to:

- for female participants: the date of radical cystectomy including hysterectomy
- for partners of male participants:
  - 90 days following last dose of trial chemotherapy and durvalumab monotherapy (such as adjuvant immunotherapy treatment).
  - 180 days following last dose of trial durvalumab and tremelimumab combination treatment (neoadjuvant immunotherapy treatment).

Pregnancies should be reported to the CTRU using the 'Notification of Pregnancy' eCRF within 24 hours of the trial site becoming aware of the pregnancy or suspected pregnancy.

The CTRU will report pregnancies in participants or the partners of participants who received trial immunotherapy treatment to AstraZeneca along with any follow-up information. All information passed on to AstraZeneca will be link anonymised.

All pregnancies will be followed-up until the outcome is known.

Pregnancy itself is not regarded as an SAE unless there is a suspicion that the trial IMP may have interfered with the effectiveness of a contraceptive medication. The outcome of a pregnancy my meet the definition of an SAE, see Section 13.1 for the definition of an SAE.

12.20. Deaths

All deaths occurring from registration until the end of follow-up (the time point coinciding with the final participant's 12-month post-RC visit) will be collected. Deaths must be recorded on the Notification of Death Form eCRF and submitted to the CTRU within 5 days of the site research team becoming aware of the death.

Where additional information is provided to CTRU, such as a postmortem report, this will be passed on to AstraZeneca and will be link anonymised.

12.20.1. Deaths requiring expedited reporting

At the end of the trial, sites will be contacted to provide data on any subsequent deaths and survival data. This will be captured via a Notification of Death eCRF.

In addition to completing a Notification of Death eCRF, suspected treatment-related deaths and deaths with an unknown cause must be notified to the CTRU via the Serious Adverse Event (SAE) eCRF (in accordance with Section 13) within 24 hours of the site becoming aware.

12.21. End of trial

The end of trial is defined as the date of the collection of the last participant's last data item.

12.22. Trial data and documentation held at sites

Trial sites will be expected to maintain a file of essential trial documentation (Investigator Site File), which will be provided by the CTRU.

Each research site is responsible for maintaining source data for their trial patients in their medical notes, and for entering data on electronic CRFs (eCRFs) in a trial-specific database.

All entries on the eCRF, including corrections, must be made by an authorised member of trial staff. Research site staff will also provide trial patients with copies of the relevant quality-of-life questionnaires for completion at each required time-point.

Research sites will submit eCRFs, within two weeks of the data being collected/the assessment taking place. A number of CRFs require expedited reporting to the CTRU within 24 hours:

- SAE Report
- SUSAR Report
- USC Report
- Withdrawal Notification
- Death Notification (for suspected treatment-related deaths and deaths with an unknown cause). Other deaths to be reported within 5 days of the site becoming aware.
- Pregnancy Notification
- Treatment Discontinuation
- Protocol Deviation Notification

Site staff are responsible for ensuring the eCRFs completed do not contain any other personal identifiable data.

It is the responsibility of the site to ensure that copies of pathology reports are anonymised prior to sending to CTRU: all personal identifiable data should be obliterated and trial number plus date of birth and initials should be used to identify the participant (as well as any other required identifiers, e.g. pathology laboratory accession number).

Following receipt of the completed eCRFs, the CTRU will contact sites on a regular basis to resolve any missing or discrepant data.

12.23. Protocol deviations

The CTRU undertake to adopt all reasonable measures to record data in accordance with the protocol. Under practical working conditions, however, some minor variations may occur due to circumstances beyond the control of the CTRU. All such deviations will be documented on the study records, together with the reason for their occurrence. Where appropriate, deviations will be detailed in the published report.

12.23.1. Overdose

Any overdose of a study patient with durvalumab or tremelimumab, with or without associated AEs/SAEs, is required to be reported as a Protocol Violation within 24 hours of becoming aware. If the overdose results in an AE, the AE must also be recorded as an AE. Overdose does not automatically make an AE serious, but if the consequences of the overdose are serious, for example death or hospitalization, the event is serious and must be recorded and reported as an SAE. There is currently no specific treatment in the event of an overdose of durvalumab or tremelimumab.

12.24. Translational Research

12.24.1. Tissue samples

FFPE blocks from TURBT surgery will be collected from all patients registered into GUSTO. These samples will be processed for trial purposes (gene expression subtype characterisation and potentially for PD-L1 immunohistochemistry).

Sections from FFPE blocks from TURBT will be held at the central laboratory for PD-L1 immunohistochemistry for trial purposes. The batched processing of these samples will not be time sensitive as they are not required to inform treatment and therefore the results will not be made available to patients.

The study is translationally rich and it is intended that tissue samples from TURBT, RC and any biopsy of metastatic deposits from registered and randomised patients will be collected for future research into bladder cancer, subject to patient consent. Consent for future research is optional and refusal will not preclude trial entry.

Subject to further funding being obtained, FFPE blocks from RC surgery and any biopsy of metastatic deposits will be collected for future research from consenting patients (registered and randomised patients). This tissue collection is not time sensitive and will proceed on request from CTRU (it will not be collected as standard following RC, sites will be advised by CTRU when this tissue collection is in a position to proceed). Sections from FFPE block from TURBT will be collected for translational research purposes from consenting patients (registered and randomised patients).

FFPE blocks from TURBT and RC surgery will be sampled by section and tissue microarray sampling and these samples will be stored for the purpose of future research at The Sheffield Biorepository (HTA license #12182 for the storage of human tissue for the purposes of research) once the ethical approval for GUSTO or linked translational projects has expired. Any sampling for GUSTO will be done under the supervision of the trial pathologist who will ensure that there is sufficient material for diagnostic use remaining. The original blocks will be returned to the laboratory of origin. Note that tumour samples can also be temporarily returned to site at any time during the study upon request to the CTRU, e.g. for further clinical testing.

Subject to further funding being obtained, fresh tissue sampling from TURBT and cystectomies may also be done at selected sites (subject to agreement with the trial site). Fresh tissue will be snap frozen in liquid nitrogen or otherwise preserved and stored in The Sheffield Biorepository once the ethical approval for GUSTO or linked translational projects has expired.

Translational tissue samples will be processed for storage and stored at the central laboratory for translational analysis. Translational analysis of these samples will be subject to separate ethical and/or regulatory review and approval as required. Analysis is anticipated to include circulating tumour DNA and other promising biomarkers such as peripheral blood mononuclear cells (PBMCs). Results of this analysis will not be provided to local investigators and trial patients.

Full details of the microarray analysis, gene expression subtype characterisation and laboratory quality assurance are detailed in the Laboratory SOPs held by the central laboratory.

Biological samples collected from participants as part of this study will be transported, stored, access and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities shall at least meet the requirements as set out in the 2004 Human Tissue Act.

12.24.2. Blood samples

Translational blood samples will be collected prior to NAC, post-NAC (for consenting patients in standard care arm and patients with basal, neuronal and luminal infiltrated subtypes in gene expression subtype-guided arms) and prior to RC, at 3 months post-RC and at 12 months post-RC. These blood samples should be stored at room temperature and dispatched to the central laboratory at the University of Sheffield. The samples should be dispatched within 24 hours of collection. Please refer the GUSTO Laboratory Manual for full details.

Translational blood samples will be processed for storage and stored at the central laboratory for translational analysis. Translational analysis of these samples will be subject to separate ethical and/or regulatory review and approval as required. Analysis is anticipated to include circulating tumour DNA and other promising biomarkers such as peripheral blood mononuclear cells (PBMCs). Results of this analysis will not be provided to local investigators and trial patients.

It is the responsibility of the trial site to ensure that samples are appropriately labelled in accordance with the trial procedures to conform with the 2018 General Data Protection Regulation. Biological samples collected from participants as part of this study will be transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes, and such activities shall at least meet the requirements as set out in the 2004 Human Tissue Act.

12.25. Gene expression classifier comparison

Expression data (obtained by central laboratory using the microarray protocol) will be used as input to other published single sample MIBC sub-classifiers (TCGA, Consensus, etc.).

Decipher will also provide classification reports for other single sample MIBC sub-classifiers including their commercial classifier.

13. Pharmacovigilance

13.1. General definitions

The standard definitions in Table 1 are taken from the 'Detailed guidance on the collection, verification and presentation of adverse event / reaction reports arising from clinical trials on medicinal products for human use ("CT-3")'. A copy is provided in the Investigator Site File and may be obtained at:

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

Table 1: Definitions of adverse events/reactions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial subject which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with participation in the study, whether or not considered related to the study interventions. A laboratory finding that is not clinically significant is not reportable as an AE. A worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a pre-existing condition that is temporally associated with the study interventions is also an adverse event. AEs are collected for all participants that have been randomised into the study. Reporting of AEs will begin from randomisation. After initiation of study treatment, all AEs will continue to be reported until 90 days after last dose of trial IMP or until initiation of new systemic anti-cancer therapy, whichever occurs first (relevant for patients in all other arms). For luminal/luminal papillary patients AEs will continue to be reported until 30 days after the date of cystectomy or the date of planned cystectomy (if the patient does not undergo cystectomy).
Adverse Reaction (AR)	All untoward and unintended responses to an IMP related to trial treatment where a possible causal relationship is indicated. This definition also covers medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product. This definition implies a reasonable possibility of a causal relationship between the event and the IMP. This means there are facts (evidence) or arguments to suggest a causal relationship. ARs are collected for all participants that have received trial IMP. Reporting of ARs will begin from initiation of trial treatment. After initiation of study treatment, all ARs will continue to be reported until 90 days after last dose of trial IMP or until initiation of new systemic anti-cancer therapy, whichever occurs first.
Serious Adverse Event (SAE)	Any untoward medical occurrence or effect that: • Results in death,

	• Is life-threatening, • Requires hospitalisation or prolongation of existing hospitalisation (prolongation of in-patient hospitalisation post-RC due to Clavien-Dindo Grade 3b or higher complications), • Results in persistent or significant disability or incapacity, • Results in a congenital anomaly or birth defect, • Jeopardises the subject or may require an intervention to prevent one of the above characteristics/consequences – herein referred to as 'Other important medical events' These characteristics/consequences have to be considered at the time of the event. For example, regarding a life-threatening event, this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. Medical and scientific judgement must be exercised in deciding whether an event is "serious" in accordance with these criteria. After randomisation but prior to initiation of study treatment, only SAEs caused by a protocol-mandated intervention (e.g., invasive procedures such as biopsies, discontinuation of medications) should be reported. After initiation of study treatment, all SAEs will continue to be reported until 90 days after last dose of trial IMP or until initiation of new systemic anti-cancer therapy, whichever occurs first.
Serious Adverse Reaction (SAR)	Reference is made to the criterion of "Seriousness" above in relation to SAE and the definition of AR. SARs are collected for all participants that have received trial treatment. Reporting of SARs will begin from initiation of trial treatment. After initiation of study treatment, all SARs will continue to be reported until the end of trial.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	An adverse reaction, the nature or severity of which is not consistent with the applicable Reference Safety Information (Section 13.2). The term "severity" is used here to describe the intensity of a specific event. This has to be distinguished from the term "serious". Reports which add significant information on the specificity, increase of occurrence, or severity of a known, already documented serious adverse reaction constitute unexpected events. SUSARs are collected for all participants that have received trial treatment. Reporting of SUSARs will begin from initiation of trial treatment. After initiation of trial treatment, all SUSARs will continue to be reported until the end of trial.
Complications	A complication is defined as an untoward medical event in a participant, which has a causal relationship to surgical intervention as part of the study (see Section 11.2).

	A complication can therefore be any unfavourable and unintended sign, symptom or disease temporally associated with surgery, whether or not considered related to the study interventions. Complications are collected for all randomised participants that have radical cystectomy as part of the study. Reporting of complications will begin from RC and will continue until 3 months post-RC.
Serious Complications (SC)	Reference is made to the criterion of "Seriousness" as defined in relation to SAEs and implies a reasonable possibility of a causal relationship between the event and RC. SCs are collected for all randomised participants that have received RC. Reporting of SCs will begin from RC until 6 weeks after RC.
Unexpected Serious Complications (USC)	A serious complication of RC, the nature or severity of which is not consistent with the applicable Reference Safety Information (Section 13.2.2). The term "severity" is used here to describe the intensity of a specific event. This has to be distinguished from the term "serious". Reports which add significant information on the specificity, increase of occurrence, or severity of a known, already documented serious complication constitute unexpected events. USCs are collected for all randomised participants that undergo RC during the trial. A USC meets the definition of a SUSAR except that is not related to an IMP; essentially, USCs are SAEs that are unexpected and related to RC only. USCs are collected as per the timings of SUSARs. USCs are reported to REC only, not to MHRA. Reporting of USCs will begin from RC until 6 weeks after RC.

13.2. Reference Safety Information

Expectedness of a serious adverse reaction (where a possible causal relationship is indicated) will be routinely assessed by CTRU upon receipt (i.e. not by site). SAEs, SARs, SUSARs, SCs and USCs will be reviewed by the CI (or delegate) and expectedness will be checked at this review. If CTRU is not able to perform the expectedness review it will be performed by site.

The version of the SmPCs/IB to be used for the purposes of pharmacovigilance reporting will be supplied to sites by CTRU and filed in the Investigator Site File.

Please note that where the RSI does not explicitly state that expected SARs may be life-threatening / result in death, then such SARs which are life-threatening / result in death must be considered unexpected and reported as SUSARs.

13.2.1. Reference safety information for IMP

The Reference Safety Information (RSI) in this trial is defined as:

- Gemcitabine, cisplatin, durvalumab, tremelimumab: Section 4.8 of the current trial-approved version of the applicable SmPC, supplied for use within the trial; (note this may not necessarily be the latest version of the SmPC and may be for a different brand of IMP for cisplatin and gemcitabine).

13.2.2. Reference safety information for radical cystectomy

Radical cystectomy has a well-established safety profile is a surgery carried out as part of standard of care in this patient population. For the purpose of assessing USCs, the following are expected events related to RC (66):

1. Bleeding - Anaemia requiring transfusion
2. Bleeding - Post-operative bleeding other than GI
3. Bleeding - Wound haematoma
4. Cardiac - Angina
5. Cardiac - Arrhythmia
6. Cardiac - Congestive heart failure
7. Cardiac - Hypertension
8. Cardiac - Hypotension
9. Cardiac - Myocardial infarction
10. Gastrointestinal - Anastomic bowel leak
11. Gastrointestinal - Clostridium difficile colitis
12. Gastrointestinal - Constipation
13. Gastrointestinal - Diarrhoea
14. Gastrointestinal - Emesis
15. Gastrointestinal - Gastrointestinal bleeding
16. Gastrointestinal - leus
17. Gastrointestinal - Small bowel obstruction
18. Gastrointestinal - Urolevo ileal obstruction
19. Infectious - Abscess
20. Infectious - Fever of unknown origin
21. Infectious - Systemic sepsis
22. Infectious - Urosepsis
23. Infectious - Urinary tract infection
24. Genitourinary - Renal failure
25. Genitourinary - Haematuria
26. Genitourinary - Stomal ischaemia
27. Genitourinary - Urethral obstruction / reconstruction of the upper urinary tract (RUT)
28. Genitourinary - Urinary leak
29. Genitourinary - Urinary fistula
30. Genitourinary - Urinary retention
31. Neurological - CVA
32. Neurological - Delirium / agitation
33. Neurological - Loss of consciousness
34. Neurological - Peripheral neuropathy
35. Neurological - Seizure
36. Neurological – transient ischemic attack (TIA)
37. Neurological - Vertigo
38. Miscellaneous - Acidosis
39. Miscellaneous - Decubitis ulcer

40. Miscellaneous - Dehydration
41. Miscellaneous - Lymphocele
42. Miscellaneous - Peripheral arterial ischaemia
43. Miscellaneous - Psychological illness
44. Miscellaneous - Thrombocytopenia
45. Pulmonary - Atelectasis
46. Pulmonary - Pleural effusion
47. Pulmonary - Pneumonia
48. Pulmonary - Pneumothorax
49. Pulmonary - Respiratory distress
50. Surgical - Bowel injury
51. Surgical - Incisional hernia
52. Surgical - Port-site hernia
53. Surgical - Retained foreign body
54. Surgical - Vascular injury
55. Surgical - Visceral injury
56. Thromboembolic – deep vein thrombosis (DVT)
57. Thromboembolic - Pulmonary embolism
58. Thromboembolic - Superficial phlebitis
59. Wound - Hernia
60. Wound - Wound dehiscence deep (facial)
61. Wound - Wound dehiscence superficial
62. Wound - Wound infection
63. Wound - Wound sepsis
64. Wound - Wound seroma
65. Other (specify)

13.3. Operation definition and reporting of AEs/ARs

Information about AE/ARs whether volunteered by the participant, discovered by investigator questioning or detected through physical examination, laboratory test or other investigation will be collected and recorded on the relevant eCRF.

AEs, ARs, SARs and SUSARs will be collected as specified in Section 13.1. Toxicity relating to IMPs will be recorded using Common Toxicity Criteria for Adverse Events version 5.0 (CTCAE v5.0).

Surgical complications, SCs and USCs will be collected as specified in Section 13.1. Intraoperative complications will be graded according to the ClassIntra classification (see Appendix 5). The system is divided into 6 grades (0, I, II, III, IV and V), reflecting the varying severity of complications. Post-operative complications will be graded according to the Clavien-Dindo classification and classified according to type (see Section 13.2.2) (64, 67). This is an acceptable and validated method of documenting surgical complications. The system is divided into 7 grades (I, II, IIIa, IIIb, IVa, IVb and V), reflecting the varying severity of complications (see Appendix 2).

The Clavien-Dindo classification grades the severity of complications by assigning a grade to the most severe complication;

I. Any deviation from the normal course without the need for pharmacological treatment,
II. Requiring pharmacological treatment including blood transfusions and total parenteral nutrition,
III. Requiring surgical, endoscopic or radiological intervention, IIIa. Intervention not under general anesthesia,
IIIb. Intervention under general anesthesia,
IV. Life-threatening complication,
IVa. Single organ dysfunction,
IVb. Multi organ dysfunction, and
V. Death of a patient (67, 68)

The Comprehensive Complication Index is a continuous measure which calculates the severity and grade of all experienced post-operative complications (69).

13.4. Operational definition – Serious Adverse Events

13.4.1. Hy's law

Hepatic function abnormality that fulfils the biochemical criteria of a potential Hy's Law case (occurrences of AST or ALT ≥3x Upper Limit of Normal (ULN) together with total bilirubin ≥2xULN with or without associated clinical manifestations) in a patient who received trial immunotherapy is required to be reported as an SAE as an 'other significant medical event'. Investigators are responsible for checking laboratory results for Hy's Law and taking action as required.

13.4.2. New cancers

The development of a new cancer should be regarded as an SAE. New primary cancers are those that are not the primary reason for the administration of the trial treatment and have been identified after the patient's inclusion in this study.

13.4.3. Adverse events of special interest

An adverse event of special interest (AESI) is one of scientific and medical interest specific to understanding of the IMP and may require close monitoring. An AESI event may be serious or non-serious. Serious AESIs require expedited reporting to CTRU as an SAE (see Section 13.1).

AESIs associated with durvalumab and tremelimumab include:

- Diarrhoea / Colitis and intestinal perforation
- Pneumonitis / ILD
- hepatitis / transaminase increases
- Endocrinopathies (i.e. events of hypophysitis/hypopituitarism, thyroiditis, adrenal insufficiency, hyper- and hypothyroidism and type I diabetes mellitus)
- Rash / Dermatitis
- Nephritis / Blood creatinine increases
- Pancreatitis / serum lipase and amylase increases
- Myocarditis

- Myositis / Polymyositis
- Other inflammatory responses that are rare/less frequent with a potential immune-mediated aetiology include, but are not limited to:
- Pericarditis
- Neuromuscular toxicities (such as Guillain-Barre syndrome and myasthenia gravis)
- Sarcoidosis
- Uveitis
- Other events involving the eye and skin
- Haematological events
- Rheumatological events
- Vasculitis
- Non-infectious meningitis
- Non-infectious encephalitis.
- It is possible that events with an inflammatory or immune mediated mechanism could occur in nearly all organs.

In addition, infusion-related reactions and hypersensitivity/anaphylactic reactions with a different underlying pharmacological aetiology are also considered AESIs.

13.4.4. Events not classed as SAEs/SCs

The following events do **not** require reporting as an SAE/SAR/SC, and will therefore not be subject to expedited reporting;

- Hospitalisation for disease progression.
- Planned hospitalisation (e.g. for RC where the inpatient stay is not considered prolonged or where prolongation of in-patient hospitalisation post-RC is due to Clavien-Dindo Grade 3 or lower complications)
- Disease-related deaths.
- Routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
- Any admission to hospital or other institution for general care where there was no deterioration in condition.
- Hospital admissions for palliative care.
- Treatment on an emergency, outpatient basis for an event **not** fulfilling any of the definitions of serious, as provided above and not resulting in hospital admission.

13.4.5. Recording and reporting of SAEs/SARs/SUSARs/SCs/USCs

SAEs, SARs, SUSARs, SCs and USCs must be recorded on the appropriate eCRF within 24 hours of the research staff becoming aware of the event. Site must also notify CTRU by email or by telephone.

For each SAE / SAR / SUSAR / SC / USC, the following information will be collected:

- full details in medical terms with a diagnosis, if possible
- duration (start and end dates if applicable)
- action taken (including concomitant medications)
- outcome

- causality (i.e., relatedness to trial drug/RC) in the opinion of the investigator
- whether or not the event would be considered expected or unexpected (see Section 13.2).

Any changes to key information must be reported to the CTRU within 24 hours of becoming aware. Key information includes:

- Main diagnosis/symptom
- Seriousness criteria
- Outcome
- Causality and expectedness

All SAEs/SARs/SCs must be reported on the electronic database **within 24 hours** of becoming aware of the event by completing an SAE eCRF

On receipt of the SAE report form, the CTRU will send an acknowledgement of the SAE via email to the relevant members of the trial team at the trial site. This acknowledgement will include an SAE code which should be included on all future correspondence regarding the SAE.

Serious Adverse Events will be followed up until resolution or until the end of trial.

13.4.6. Responsibilities

13.4.6.1. Principal Investigator:

- Checking for AEs, ARs and SCs when participants attend for treatment / follow-up.
- Using medical judgement in assigning seriousness and causality.
- Ensuring that all SAEs, SARs, SUSARs, SCs and USCs are recorded and reported to the CTRU within 24 hours of becoming aware of the event and provide further follow-up information as soon as available.
- Ensuring that SAEs, SARs, SUSARs, SCs and USCs are chased with CTRU, if a record of receipt is not received within 2 working days of initial reporting.
- Ensuring that AEs and SCs are recorded and reported to the CTRU in line with the requirements of the protocol.

13.4.6.2. Chief Investigator (CI) / delegate:

- Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.
- Using medical judgement in assigning seriousness, causality and expectedness of SAEs, where it has not been possible to obtain local medical assessment.
- Immediate review of all SUSARs and USCs.
- Review of specific SAEs and SARs in accordance with the trial risk assessment, and protocol, as detailed in the Trial Monitoring Plan.
- Assigning Medical Dictionary for Regulatory Activities (MedDRA) or Body System coding to all SAEs, SARs and USCs.
- Preparing the clinical sections and final sign-off of the Development Safety Update Report (DSUR).

13.4.7. CTRU:

- Central data collection and verification of AEs, ARs, SAEs, SARs, SUSARs, SCs and USCs according to the trial protocol onto a MACRO database.
- Using the RSI to assign expectedness of all SARs, SUSARs, SCs and USCs.
- Reporting safety information to the CI, delegate or independent clinical reviewer for the ongoing assessment of the risk / benefit according to the Trial Monitoring Plan.
- Reporting safety information to the independent oversight committee identified for the trial (Data Monitoring & Ethics Committee (DMEC) and Trial Steering Committee (TSC)) according to the Trial Monitoring Plan.
- Expedited reporting of SUSARs to the MHRA, REC and Sponsor within required timelines.
- Expedited reporting of USCs to REC and Sponsor within required timelines.
- Notifying Investigators of SUSARs and USCs that occur within the trial.
- Checking for (annually) and notifying Principal Investigators of updates to the Reference Safety Information for the trial.
- Preparing standard tables and other relevant information for the DSUR in collaboration with the Chief Investigator and ensuring timely submission to the MHRA and REC.
- Notify AstraZeneca of any SARs and SUSARs occurring in trial participants receiving durvalumab and/or tremelimumab using the CTRU SAE/SUSAR eCRF. Initials and date of birth will be removed from the CRF before sending to AstraZeneca. Participants will be identified by trial number only.
- Providing AstraZeneca with a quarterly line listing of SAEs reported in trial participants receiving durvalumab and tremelimumab. Participants will be identified by trial number only.
- Providing AstraZeneca with an annual listing of adverse events of special interest occurring in trial participants receiving durvalumab and/or tremelimumab. Participants will be identified by trial number only.
- Notify AstraZeneca of incidences of durvalumab and/or tremelimumab overdosing. Participants will be identified by trial number only.
- Notify AstraZeneca of pregnancies in trial participants receiving durvalumab and tremelimumab and in partners of trial participants receiving durvalumab and tremelimumab. Participants will be identified by trial number only.

13.4.8. Trial Steering Committee (TSC):

In accordance with the Trial Terms of Reference for the TSC, periodically reviewing safety data and liaising with the DMEC regarding safety issues.

13.4.9. Data Monitoring & Ethics Committee (DMEC):

In accordance with the Trial Terms of Reference for the DMEC, periodically reviewing unblinded overall safety data to determine patterns and trends of events, or to identify safety issues, which would not be apparent on an individual basis.

13.4.10. Pharmaceutical company (AstraZeneca)

Immediately notifying CTRU of any new significant safety information relating to the study drug which may alter the benefit risk balance of durvalumab and/or tremelimumab.

13.4.11. Decipher (Veracyte)

Immediately notifying CTRU of any new significant safety information relating to the Decipher Bladder platform which may alter the benefit risk balance of the platform.

14. Participant Questionnaires

Quality of life will be assessed in all randomised participants using validated instruments completed at appropriate and specified time points throughout the trial. Participants who consent to randomisation will be asked to complete questionnaires at baseline, 6 months and 12 months post-RC. Details on scoring methods will be included in the Statistical Analysis Plan.

Questionnaires to be completed include:

- EQ-5D-3L

The non-disease-specific EQ-5D-3L instrument (70) will be used to evaluate health related quality of life for patients. It consists of two pages, the EQ-5D descriptive system, which assesses five dimensions (Mobility, self-care, usual activities, pain/discomfort and anxiety/depression), each of which has three levels (No problems, some problems and extreme problems) and outputs a value which describes a patient's health, and the EQ visual analogue scale (EQ-VAS), which records a patient's self-rated health on a vertical analogue scale between endpoints "Best imaginable health state" and "Worst imaginable health state".

- EORTC QLQ-C30

The cancer specific EORTC QLQ-30 instrument (71) will be used to evaluate cancer related quality of life. The European Organisation for Research and Treatment of Cancer (EORTC) QLQ-C30 is a well-validated questionnaire developed to assess the quality of life of cancer patients by scoring patients on scales that assess global health status, social, physical and emotional functioning and common symptoms.

15. Endpoints

15.1. Primary Endpoints

<u>Stage 1</u>

- Recruitment
- Time to return of gene expression subtype allocation
- Gene expression subtype allocation success rate

<u>Stage 2</u>

- Recruitment
- Gene expression subtype distribution
- pCR rate in the standard care arm by gene expression subtype
- Confirmation / re-estimation of sample size

<u>Stage 3</u>

- pCR rate by gene expression subtype in the gene expression subtype-guided arm post-RC

15.2. Secondary Endpoints

Gene expression subtype endpoints

- RNA quality and mass/yield
- Gene expression subtype allocation re-test rate and repeat assay success rate
- Time from patient consent to TURBT sample dispatch
- Time to RNA extraction, processing & gene expression subtype allocation

Clinical secondary endpoints

- Disease-free survival at 12-months post-RC and at the end of the trial
- Overall survival at 12-months post-RC and at the end of the trial
- Metastasis-free survival at 12-months post-RC and at the end of the trial
- Event-free survival at 12-months post-RC and at the end of the trial
- Histological outcomes
- Quality of life at baseline, 6-months and 12-months post-RC
- Patient acceptability to registration and randomisation
- pCR stage by gene expression subtype in the standard and gene expression subtype-guided arms post-RC
- Outcomes from recovery after radical cystectomy in this stratified treatment cohort as determined by:
    - Time to RC
    - Safety of RC:
        - blood loss
        - Intra-operative complications and adverse events categorised using the ClassIntra classification
        - length of stay
        - readmission within 90 days of RC
        - post-operative complications and adverse events categorised using the Clavien-Dindo classification
    - Pathological completeness:
        - Nodes - number in total and number that have cancer
        - Margins - positive (involved) or negative (clear)
        - Location of positive margin - Urethra. Ureteric. Circumferential. Soft tissue
- To re-evaluate the stage 1 and 2 feasibility endpoints
- Disease-free survival at 12-months post-RC for all consenting patients registered into the study
- Overall survival at 12-months post-RC for all consenting patients registered into the study
- Metastasis-free survival at 12-months post-RC for all consenting patients registered into the study
- Event-free survival at 12-months post-RC for all consenting patients registered into the study

Pharmacological secondary endpoints

- Toxicity and tolerability
- Treatment compliance as assessed by dose omissions, dose delays, dose reductions and delays to RC

15.3. Exploratory Endpoints

- PD-L1 status of tumour and infiltrating immune cells in pre-existing diagnostic samples in all patients
- Cohen's Kappa will be used to measure the agreement between classifications from different MIBC classifiers using the same RNA profile.
- Concordance index (C-index) will be used to measure the correlation between predicted risk and observed time of death for each classifier.

15.4. Study Endpoint Definitions

Recruitment in stage 1 is defined as the number of patients randomised within the first 6 months of the trial opening.

Recruitment in stage 2 is defined as the number of patients randomised within the first 24 months of the trial opening.

Time to return of gene expression subtype allocation is defined as the time from the date the sample is received by the Sheffield central laboratory to the date the gene expression subtype allocation is entered onto the lab-specific database.

Gene expression subtype allocation success rate is defined as the proportion of samples sent to the Sheffield central laboratory which are successfully allocated a gene expression subtype.

Gene expression subtype distribution is defined as the proportion of samples (of those successfully allocated a gene expression subtype) in each gene expression subtype.

pCR rate by gene expression subtype is defined as the proportion of patients within each gene expression subtype who are defined as having a pathological complete response according to local pathologist report (pT0 (72)) at RC.

RNA Quality and mass/yield is defined as the quality and yield of the samples tested at Sheffield central laboratory. RNA quality will be measured using two measures: the 260:230 ratio and the 260:280 ratio. RNA yield will be measured in nanograms per microlitre (ng/uL).

Gene expression subtype allocation re-test rate and repeat assay success rate is defined as the proportion of samples sent to the Sheffield central laboratory which require retesting, and the proportion of those re-tested samples which are successfully allocated a gene expression subtype after re-testing.

Time from patient consent to TURBT sample dispatch is defined as the time from a patient consenting to gene expression subtyping to the time their TURBT sample is sent for gene expression profiling.

Time to RNA extraction, processing & gene expression subtype allocation is defined as the time from the sample being received in Sheffield to RNA extraction/processing/gene expression subtype allocation, respectively.

Disease-free survival for randomised participants is defined as the time from the date of RC to the first recurrence of disease post-RC, or death due to any cause or last follow-up. DFS

will be assessed in patients who undergo RC and are disease free at adjuvant baseline visit. If a participant has not had disease recurrence or is still alive at the time of analysis or lost to follow-up before disease recurrence/death is documented, they will be censored at the last date known alive. Participants discontinuing protocol treatment or receiving non-protocol treatment will still be followed for disease-free survival unless they explicitly withdraw consent. For consenting registered participants disease-free survival is defined similarly but from the date of registration.

Overall survival is defined as the time from randomisation to death from any cause or last follow-up. If a participant is still alive at the time of analysis or lost to follow-up before death is documented, they will be censored at the last date known alive. Participants discontinuing protocol treatment or receiving non-protocol treatment will still be followed for overall survival unless they explicitly withdraw consent. For consenting registered participants overall survival is defined similarly but from the date of registration.

Metastasis-free survival is defined as the time from randomisation to the first indication of a metastasis. If a participant has not had a metastasis at the time of analysis, or either died or was lost to follow-up before a metastasis is documented, they will be censored at the last date known alive without having developed a metastasis. Participants discontinuing protocol treatment or receiving non-protocol treatment will still be followed for metastasis-free survival unless they explicitly withdraw consent. For consenting registered participants metastasis-free survival is defined similarly but from the date of registration.

Event-free survival is defined as the time from randomisation to any of the following events: failure to undergo RC (at the date the decision is made), recurrent cancer, metastasis, death from bladder cancer or commencement of further treatment for disease relapse. If a participant has not had any of these events at the time of analysis or either died from another cause or was lost to follow-up before an event was documented, they will be censored at the last date known alive without having had any of these events occur. Participants discontinuing protocol treatment or receiving non-protocol treatment will still be followed for event-free survival unless they explicitly withdraw consent. For consenting registered participants event-free survival is defined similarly but from the date of registration.

Histological outcomes are defined as the Tumour-Node-Metastasis (TNM) stages (73) in both the TURBT and RC specimens. Additional parameters include Margin rate (negative or positive), location of positive margins (urethra, ureteric, circumferential, soft tissue), lymph node count and number of nodes containing cancer.

Quality of life will be determined by a participant's response to the EQ-5D-3L and EORTC QLQ-C30 questionnaires (see Section 14).

Patient acceptability to registration and randomisation are defined as the proportion of eligible patients agreeing to registration. Acceptability to randomisation is defined as the proportion of eligible registered patients agreeing to randomisation and the proportion of randomised participants who receive their randomised treatment.

pCR stage by gene expression subtype is defined as the proportion of participants within each gene expression subtype with:

- pComplete Response (no tumour)

- pPartial Response (down staging – MIBC to NMIBC)
- pNo response (MIBC to MIBC)
- pProgression (Tany N0 to Tany N1-2 or M1-2)

Time to RC is defined as the time from randomisation to the time of RC.

Safety of RC will be defined by the following:

- Blood loss,
- Intra-operative complications and adverse events categorised using the ClassIntra classification
- Length of stay,
- Readmission (number of patients who are re-admitted to hospital) within 90 days of RC
- Post-operative complications and adverse events categorised using the Clavien-Dindo classification

Pathological completeness, will be assessed using the following:

- Nodes, defined as the number in total and number that have cancer
- Margins, defined as the proportion positive (involved) or negative (clear)
- Location of positive margin - Urethra. Ureteric. Circumferential. Soft tissue

Toxicity and tolerability will be reported based on safety events, as graded by CTCAE V5.0 and Clavien-Dindo grade of complications where appropriate.

Treatment compliance is defined as the number of dose omissions, dose delays, dose reductions and delays to RC.

PD-L1 status of tumour and infiltrating immune cells in pre-existing diagnostic samples in all patients is defined as the result of the Ventana/Roche SP263 PD-L1 immunohistochemistry assay (74), and will give a dichotomous result of either:

- PD-L1 high/positive
- PD-L1 low/negative

Gene expression subtype classifier comparison is defined as any comparisons made between the Decipher Bladder (Veracyte, USA) commercial gene expression subtyping test and any alternative classifier systems/tests.

16. Statistical Considerations

16.1. Sample size

16.1.1. Primary comparisons

The total sample size will be fixed by the re-estimation procedure at the end of stage 2 based on estimates of the gene expression subtype proportions and their respective pCR rates in the standard care arm. However, it will not be fewer than the total number obtained from the calculations detailed below.

The design within each gene expression subtype is a single-arm assessment within the gene expression subtype arm, where the randomisation to the standard care arm will enable the correct design parameters to be estimates and a secondary unpowered randomised comparison to be conducted (see the Statistical Analysis Section 17). Any changes to the sample size will be determined by the independent Data Monitoring and Ethics Committee by examining data from the standard care arm. The calculations below are based on the best assumptions currently available.

For each gene expression subtype the calculations are as described by A'Hern (75) where the one-sided significance level is 0.1 and the power is 0.8 for the test and the average pCR rate in the standard care arm is assumed to be 0.35 (across all gene expression subtypes following NAC and RC (20, 45, 72).

Table 2: Sample size assumption in gene expression subtypes

Gene expression subtype	Luminal/ Luminal Papillary	Luminal Infiltrated	Basal
Assumed gene expression subtype distribution[1] (*% of patients in population*)	40	20	35
pCR rate in the standard care arm	0.15 (45, 47, 54)	0.3 (21, 76)	0.6 (45, 47, 54, 72)
Assumed pCR rate in the gene expression subtype arm	0.25	0.5	0.75
Non-inferiority margin for later study	0.05	N/A	N/A
Number required to have a pCR post-RC (*for a 10% one-sided significance level and 80% power*)	9/37 [2]	13/30	33/47
Total sample size required *(Accounting for 5% drop-out and assumed proportion of patients with this gene expression subtype)*	195	320	286

As detailed above, 320 patients need to be randomised into the trial. Each of the sample size calculations account for 5% of patients not being assessable for the primary outcome of the trial: pCR rate post-RC.

[1] Note that 5% of patients are assumed to have the neuronal gene expression subtype but as this group is small and has no evidence to describe the assumption in the standard care or gene expression subtype group, this comparison is not powered and will be described and not be subject to statistical testing.

[2] A phase II design for non-inferiority of type 4 as proposed by S-H Jung (Design of Phase II Non-inferiority Trials. Contemp Clin Trials Commun. 2017;7:23-7). It is hypothesised that the removal of NAC will result in a small net improvement of 0.10 in pCR rate, due to the removal of the delay prior to cystectomy. However, in a confirmatory non-inferiority study it is hypothesised that the acceptable margin would be 0.05. This leads to a single arm phase II trial to assess evidence for the confirmatory study where the target difference is the sum of the potential efficacy increase and the anticipated non-inferiority margin .(0.10+0.05=0.15).

16.1.2. Secondary comparisons

Two secondary powered comparisons will be reported, if the trial completes enrollment as expected, passing each feasibility stage. If the trial does not complete as planned then these comparisons will be reported as exploratory.

For the neoadjuvant phase, pCR rates will be compared between the standard care arm and the gene expression subtype-guided arm. It is assumed that the pCR rate in the standard care arm will be 0.35 and the pCR rate in the gene expression subtype-guided arm will be the weighted average in Table 2 above: ((0.4*0.25)+(0.2*0.5)+(0.35*0.75))/0.95 = 0. 4868421. At the one-sided 10% significance level, there will be 80% power to conclude evidence of activity in the gene expression subtype-guided arm compared to the standard of care arm accounting for the 5% drop-out as above.

For the adjuvant treatment phase, disease-free survival rate is considered to be a key secondary endpoint to assess whether there is sufficient treatment activity to continue gene expression subtype-guided care. Considering a single-arm assessment within the gene expression subtype-guided arm as a whole (rather than considering each individual gene expression subtype as above). It is thought that a 10% increase in the disease-free survival rate from 54% in the standard of care arm (77) to 64% in the gene expression subtype-guided care arm at 12 months post-RC is an acceptable increase. In order to have an 80% power of demonstrating that the one-sided 90% confidence interval of the disease-free survival rate at 12 months post-RC excludes 54%, 114 evaluable patients are required, where gene expression subtype-guided care will be deemed a “success” if at least 69 out of 114 patients are disease-free at 12 months post-RC.

16.2. Planned recruitment rate

Given the estimated gene expression subtype proportions and the anticipated success rate of the gene expression subtype test, it is estimated that 458 patients will be required to be registered.

320 participants will be randomised over 36 months from 20 UK centres. Once all centres are open, the randomisation target is 13 patients per month.

16.3. Analysis timelines

Stage 1:

- Analysis of stage 1 recruitment feasibility will be conducted once the trial has been open to recruitment for 6 months and will include all patients randomised into the trial from all sites.
- Analysis of stage 1 gene expression subtype feasibility will be conducted once the trial has been open to recruitment for 6 months and will include all patients registered into the trial from all sites.

Stage 2:

- Analysis of stage 2 recruitment feasibility will be conducted once the trial has been open for 24 months of recruitment and will include all patients randomised into the trial from all sites.

- The assessment of the sample size assumptions is expected to take place at the same time as the analysis of the stage 2 recruitment feasibility however if this is not feasible it may be appropriate for this analysis to take place later.

Stage 3:

- An interim assessment of pCR rate in each gene expression subtype will be conducted once all randomised participants have completed RC.
- Final analysis of the pCR rate in each gene expression subtype will take place once all randomised participants have been followed up for 12 months post-RC (after 36 months of recruitment, 18 months after analysis of stage 2). These timelines are subject to change based on updated recruitment rates. Analysis will commence after data cleaning and will take between 3 and 6 months depending on the number of analyses being performed.

17. Statistical Analysis

17.1. Statistical considerations

Statistical analysis is the responsibility of the CTRU statisticians. A full statistical analysis plan will be written before any analyses are undertaken. The analysis plan will be written in accordance with the current CTRU standard operating procedures and will be finalised and agreed by the following people: the trial statistician and supervising statistician, the Chief Investigator, the CTRU Scientific and Project Delivery Leads and the Senior Trial Manager. Any changes to the finalised analysis plan, and the reasons for changes, will be documented.

Analyses using registered participants (stage 1 gene expression subtype feasibility) will include all patients registered into the trial from all sites.

Analyses using randomised participants will be conducted on the intention-to-treat (ITT) population, where participants will be included according to their randomisation allocation, regardless of whether they prematurely discontinued treatment or did not comply with the regimen.

If there are a considerable number of protocol violators, a per-protocol analysis, where participants will be included according to the treatment they received, will be considered for the primary endpoint.

The safety population will consist of all participants who received at least one dose of neoadjuvant treatment or underwent RC.

17.2. Formal interim analyses

Statistical summaries will be presented to the Data Monitoring and Ethics Committee (DMEC) in strict confidence at yearly intervals as a minimum, in addition to the planned formal interim analyses relating to assessment of progression criteria. This committee, in the light of the interim data, and of any advice or evidence they wish to request, will advise the Trial Steering Committee (TSC) if there is proof beyond reasonable doubt that one treatment is superior (either across the trial or by gene expression subtype), or the trial should be stopped/changed/continue in accordance with the planned assessment of progression criteria.

17.3. Primary endpoint analyses

17.3.1. Stage 1

17.3.1.1. Recruitment Feasibility

This endpoint will be assessed once the trial has been open to recruitment for 6 months and will include all patients randomised into the trial from all sites. The number of patients randomised will be presented overall, by month and as a rate per month.

17.3.1.2. Gene Expression Subtype Feasibility

These endpoints will be assessed once the trial has been open to recruitment for 6 months and will include all patients registered into the trial from all sites.

When assessing time to gene expression subtype allocation, an exact one-sided (lower) 90% confidence interval will be calculated for the time to gene expression subtype classification for all patients who consent to genotyping. If the limit of the confidence interval for this is greater than 2 weeks (14 days) then the trial may not proceed to stage 2, as it may be concluded that genotyping assessment cannot be feasibly integrated into the clinical care pathway.

When considering the rate of successful gene expression subtyping, an exact one-sided (upper) 90% confidence interval will be calculated. If the limit of this confidence interval is less than 90%, then the trial may not proceed to stage 2, as it may be deemed to be too irreproducible a method to be incorporated into standard practice.

Confidence intervals will be calculated using the Clopper-Pearson method (78).

17.3.2. Stage 2

The endpoints of stage 2 will be assessed once the trial has been open to recruitment for 24 months.

If the assumptions made within the sample size calculation are within the confidence intervals of the two assessments below, then the sample size will not be re-estimated and the trial will continue with the proposed single-arm assessments for treatment activity with the standard arm continuing as a calibration arm to ensure a more precise estimate for the phase III trial.

17.3.2.1. Gene Expression Subtype pCR assumption

When assessing the pCR rate in the standard care arm by gene expression subtype, exact two-sided 95% confidence intervals will be calculated for each gene expression subtype and will include all patients in the standard care arm who were successfully allocated a gene expression subtype. These will be calculated using the Clopper-Pearson method. If the assumed pCR rate of a gene expression subtype falls below the lower limit of the confidence interval, then investigations into that gene expression subtype may be dropped, as sample size re-estimation would result in a smaller difference being investigated, which may cause a larger, unfeasible, sample size to be required.

17.3.2.2. Gene Expression Subtype Distribution assumptions

When assessing gene expression subtype distribution, exact two-tailed 95% confidence intervals will be calculated for each gene expression subtype and will include all patients who were successfully allocated a gene expression subtype. These will be calculated using the Clopper-Pearson method. If the assumed rate of a gene expression subtype is above the

upper limit of the confidence interval, then investigations into that gene expression subtype may be dropped, as sample size re-estimation would result in a smaller difference being investigated, which may cause a larger, infeasible, sample size to be required.

17.3.3. Stage 3

An interim assessment of gene-expression subtype pCR rate will take place once all randomised participants have completed RC or are known not to be proceeding to RC. Final analysis will take place once all randomised participants have been followed up for 12 months post-RC. At this later point, the stage 1 and 2 endpoints will be re-evaluated.

17.3.4. Gene Expression Subtype pCR rate

Treatment activity will be assessed in stage 3 by calculating the proportion of patients within each gene expression subtype-guided arm who have a pCR following RC. Where the proportion passes the pre-specified threshold, indicated in Table 2, the proposed treatment combination for that gene-expression subtype will be considered for inclusion in a phase III trial. The 95% confidence interval for pCR rate within the gene expression subtype-guided arm for each gene expression sub type will also be presented.

17.4. Secondary endpoint analyses

A secondary analysis on the primary outcome will be implemented in which the proportion of patients, within each gene expression subtype, experiencing a pCR post-RC will be compared across the trial arms using logistic regression adjusting for the stratification factors of the trial excluding centre. Parameter estimates, odds ratios and corresponding 95% confidence intervals, degrees of freedom, test statistics and p-values will be presented for each variable in the model. This analysis is not formally powered and should be considered exploratory.

The secondary time to event outcomes, including the key secondary endpoint disease-free survival, will be analysed using Cox proportional hazard regression adjusting for the stratification factors of the trial, excluding centre. Parameter estimates, hazard ratios and corresponding 95% confidence intervals, degrees of freedom, test statistics and p-values will be presented for each variable in the model.

Other secondary binary or categorical outcomes, will be compared between trial arms using similar methods as that described for the secondary analysis of the primary outcome indicated above.

Safety events will be summarised descriptively using CTCAE categories for events relating to IMP. For events relating to RC, ClassIntra and Clavien-Dindo grade of complications will be used for intra-operative and post-operative events respectively.

Treatment compliance will be summarised both through proportion of patients who experienced a non-compliant event or not, and by detail of the non-compliant event e.g. number of omissions, number of delays, number of reductions, length of delays.

Quality of life will be summarised using mean scores adjusted for baseline and two-sided 95% CIs for each EORTC QLQ-C30 module symptom, role and functioning domain at each assessment time point. Similar summaries will be produced using the EQ-5D and VAS questionnaires.

Outcomes not covered by the above relating to histological outcomes, acceptability to registration and randomisation, RC (safety and pathological completeness) and gene expression (RNA quality, mass/yield, retest rate and repeat assay success rate) will be summarised descriptively.

Any reassessment of stage 1 and 2 endpoints will be conducted using the same methods as originally used in stages 1 and 2.

17.5. Exploratory endpoint analysis

The methods for analysis of the exploratory endpoints will be detailed within the Statistical Analysis Plan.

18. Trial Monitoring

A trial monitoring plan will be developed and agreed by the Trial Management Group (TMG) and Trial Steering Committee (TSC) based on the trial risk assessment; this may include on site monitoring. Trial sites and PIs must agree to allow trial-related on-site monitoring, Sponsor audits and regulatory inspections by providing direct access to source data/documents as required. Patients are informed of this in the patient information sheet and are asked to consent to their medical notes being reviewed by appropriate individuals on the consent form.

CTRU will determine the appropriate level and nature of monitoring required for the trial. Risk will be assessed on an ongoing basis and adjustments made accordingly.

18.1. Data monitoring and Ethics Committee (DMEC)

An independent DMEC will review the safety and ethics of the study. A data monitoring analysis plan will be prepared and agreed with the DMEC Committee. Detailed un-blinded reports will be prepared by the CTRU for the DMEC at a minimum of yearly intervals and the committee will be required to review any formal interim analysis reports relating to assessment of progression criteria as detailed in Section 7.3.

18.2. Data monitoring

Data will be monitored for quality and completeness by the CTRU. Missing data will be chased until it is received, confirmed as not available, or the trial is at analysis. However missing data items will not be chased from participants (although missing questionnaires sometimes are). The CTRU and Sponsor will reserve the right to intermittently conduct source data verification exercises on a sample of participants, which will be carried out by staff from the CTRU.

Source data verification will involve direct access to patient notes at the hospital sites and the ongoing central collection of copies of consent forms and other relevant investigation reports. Pathology reports for TURBT and RC surgeries and baseline CT/MRI scan reports will be centrally reviewed as part of central data monitoring.

18.3. Clinical governance issues

To ensure responsibility and accountability for the overall quality of care received by participants during the study period, clinical governance issues pertaining to all aspects of routine management will be brought to the attention of the Trial Steering Committee and, where applicable, to individual NHS Trusts.

19. Quality Assurance and Ethical Considerations

19.1. Quality Assurance

The trial will be conducted in accordance with the principles of Good Clinical Practice (GCP) in clinical trials, as applicable under UK regulations, the UK Policy Framework for Health and Social Care Research and Scottish Executive Health Department Research Governance Framework for Health and Social Care 2006, and through adherence to CTRU Standard Operating Procedures (SOPs).

19.2. Serious breaches

CTRU and Sponsor have systems in place to ensure that serious breaches of GCP or the trial protocol are picked up and reported. Investigators are required to promptly notify the CTRU of a serious breach (as defined in Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 and amendments) that they become aware of. A 'serious breach' is a breach which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial.

In the event of doubt or for further information, the Investigator should contact the Senior Trial Manager at the CTRU.

19.3. Ethical and regulatory considerations

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, amended at the 52nd World Medical Association General Assembly, Edinburgh, Scotland, 1996. Informed written consent/eConsent will be obtained from the patients prior to registration into the study. The right of a participant to refuse participation without giving reasons must be respected. The participant must remain free to withdraw at any time from the study without giving reasons and without prejudicing their further treatment. The study will be submitted to and approved by a REC prior to entering patients into the study. The CTRU will provide the REC with a copy of the final protocol, patient information sheets, consent forms and all other relevant study documentation.

20. Confidentiality

All information collected during the course of the trial will be kept strictly confidential. Information will be held securely on paper and electronically at the CTRU. The CTRU will comply with all aspects of the 2018 General Data Protection Regulation and operationally this will include:

- consent from participants to record personal details including name, date of birth, NHS/CHI number, email address, GP name and address;
- appropriate storage, restricted access and disposal arrangements for participant personal and clinical details.
- consent from participants for access to their medical records by responsible individuals from the research staff or from regulatory authorities, where it is relevant to trial participation.

- consent from participants for the data collected for the trial to be used to evaluate safety and develop new research;
- full participant name will be collected when a participant is registered and randomised into the study
- full participant name, trial number and local laboratory sample accession number will be collected on the TURBT tissue samples and the TURBT Block Receipt & Return form used when samples are transferred from sites to the central laboratory.
- participant initials, trial number and local laboratory sample accession number will be collected on the cystectomy and/or biopsy tissue samples and blood samples and the Translational Block Receipt & Return form used when the samples are transferred from sites to the central laboratory.
- participant year of birth, trial number and central laboratory number will be uploaded to the Decipher platform.
- data collection forms that are transferred to or from the CTRU will be coded with a trial number and will include two participant identifiers, usually the participant's initials and date of birth.
- Participants' email addresses will be collected if the participant agrees to complete the quality of life questionnaires or provide consent electronically.
- where central monitoring of source documents by CTRU (or copies of source documents) is required (such as scans or local blood results), the participant's name must be obliterated by site before sending;
- where anonymisation of documentation is required, sites are responsible for ensuring only the instructed identifiers are present before sending to CTRU as per instructions given by CTRU in accordance with the trial procedures to conform with the 2018 General Data Protection Regulation.

If a participant withdraws consent from further trial treatment or from further collection of data, their data and samples will remain on file and will be included in the final trial analysis.

Published results will not contain any personal data that could allow identification of individual patients.

20.1. Trial record retention and archiving

Essential documents will be maintained at the CTRU and at the Investigator Sites in a way that will facilitate the management of the trial, audit and inspection. Documents should be securely stored and access restricted to authorised personnel. At the end of the trial, data will be securely archived in line with the Sponsor's procedures for a minimum of 15 years. Data held by the CTRU will be archived in the Sponsor archive facility and site data and documents will be archived at the trial sites. Following authorisation from the Sponsor, arrangements for confidential destruction will then be made.

21. Sponsorship and indemnity

The Sheffield Teaching Hospitals NHS Foundation Trust is the Sponsor and can only provide indemnity for harm caused by the negligent actions of its employees, through its membership of the Clinical Negligence Scheme for Trusts (CNST). The Trust is not able to provide "no fault" indemnity for non-negligent harm.

22. Study Organisational Structure

22.1. Individuals and Individual Organisations

Chief Investigator (CI) – The Chief Investigator is involved in the design, conduct, co-ordination and management of the trial. The Chief Investigator will have overall responsibility for the design and set-up of the trial, the investigational drug supply and pharmacovigilance within the trial.

Trial Sponsor – The Sponsor is responsible for trial initiation management and financing of the trial as defined by Directive 2001/20/EC. These responsibilities are delegated to the CTRU as detailed in the trial contract.

Clinical Trials Research Unit – The CTRU will have responsibility for conduct of the trial as delegated by the Sponsor in accordance with relevant GCP standards and CTRU SOPs. The CTRU will provide set-up and monitoring of trial conduct to CTRU SOPs, and the GCP Conditions and Principles as detailed in the UK Medicines for Human Use (Clinical Trials) Regulations 2006 including, randomisation design and service, database development and provision, protocol development, CRF design, trial design, source data verification, monitoring schedule and statistical analysis for the trial. In addition the CTRU will support ethical approval submissions, any other site-specific approvals and clinical set-up, ongoing management including training, monitoring reports and promotion of the trial. The CTRU will be responsible for the day-to-day running of the trial including trial administration, database administrative functions, data management, safety reporting and all statistical analyses.

Central pharmacy – Sponsor will contract with a third party to carry out the labelling, packaging and distribution of trial IMP.

Central laboratory– Sheffield Teaching Hospitals NHS Foundation Trust is responsible for gene expression subtyping of FFPE tissue using the Decipher platform. Sheffield Teaching Hospitals NHS Foundation Trust is also responsible for PD-L1 analysis which may involve using a third party service provider as per standard practice.

Central laboratory for collection of samples for future research – The University of Sheffield is responsible for the handling of blood and tissue samples collected during the study for use in future research.

22.2. Oversight and Trial Monitoring Groups

Trial Management Group (TMG) – The TMG, comprising the CI, CTRU team, other key external members of staff involved in the trial will be assigned responsibility for the clinical set-up, on-going management, promotion of the trial, and for the interpretation and publishing of the results. Specifically the TMG will be responsible for (i) protocol completion, (ii) eCRF development, (iii) obtaining approval from the REC, (iv) submitting a , (v) completing cost estimates and project initiation, (vi) nominating members and facilitating the TSC and DMEC, (vii) reporting of serious adverse events, (viii) monitoring of screening, recruitment, treatment and follow-up procedures, (ix) auditing consent procedures, data collection, trial end-point validation and database development.

Trial Steering Committee (TSC) – The TSC will provide overall supervision of the trial, in particular trial progress, adherence to protocol, participant safety and consideration of new

information. It will include an Independent Chair, not less than two other independent members and a consumer representative. The Chief Investigator and other members of the TMG may attend the TSC meetings and present and report progress. The Committee will meet annually as a minimum.

Data Monitoring and Ethics Committee (DMEC) – The DMEC will include independent membership and will review the safety and ethics of the trial by reviewing interim data during recruitment and follow-up and will report to the TSC. The Committee will meet annually as a minimum.

22.3. Patient and Public Involvement

Patient and public involvement (PPI) representatives are involved in the study at all stages.

One PPI representative is appointed to the Trial Management Group. This group is responsible for the set up and ongoing management of the trial. The PPI representative has input into the patient facing documents and study design and will be involved in decisions on trial progress and plans for dissemination of the research.

Two PPI representatives are appointed to the Trial Steering Committee, which provides independent oversight on trial safety and conduct and makes high level decisions about the trial.

A group of five PPI representatives form a Patient Advisory Group for the study. This group meets to discuss issues arising in the study that affect patients and has input into the patient facing documents to ensure that they are informative and meet the information needs of the patients.

23. Publication Policy

The trial will be registered with an authorised registry, according to the International Committee of Medical Journal Editors (ICMJE) Guidelines, prior to the start of recruitment.

The success of the trial depends upon the collaboration of all participants. For this reason, credit for the main results will be given to all those who have collaborated in the trial, through authorship and contributorship. Uniform requirements for authorship for manuscripts submitted to medical journals will guide authorship decisions. These state that authorship credit should be based only on substantial contribution to:

- conception and design, or acquisition of data, or analysis and interpretation of data,
- drafting the article or revising it critically for important intellectual content,
- and final approval of the version to be published,
- and that all these conditions must be met (www.icmje.org).

In light of this, the Chief Investigator, and relevant senior CTRU staff will be named as authors in any publication. In addition, all collaborators will be listed as contributors for the main trial publication, giving details of roles in planning, conducting and reporting the trial. The manuscript will be prepared by a writing group, appointed from amongst the Trial Management Group, and high accruing clinicians. All trial centres and clinicians will be acknowledged in this publication together with staff from the CTRU.

To maintain the scientific integrity of the trial, data will not be released prior to the first publication of the analysis of the primary endpoint, either for trial publication or oral presentation purposes, without the permission of the Trial Steering Committee and Sponsor. In addition, individual collaborators must not publish data concerning their participants which is directly relevant to the questions posed in the trial until the first publication of the analysis of the primary endpoint. No investigator may present or attempt to publish data relating to this trial without prior permission from the TMG and sponsor.

Authorship of any secondary publications e.g. relating to the various biological studies will reflect the intellectual and time input into these studies, and will not be the same as on the primary publication.

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Appendix 1 National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE)

Toxicities will be assessed based on the latest National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE).

A copy of NCI-CTCAE is provided in the Investigator Site File and may be obtained at: **http://evs.nci.nih.gov/ftp1/CTCAE/About.html**

Appendix 2 Clavien-Dindo Classification

Table 3: Classification of Surgical Complications - The Clavien-Dindo Classification

Grade	Definition
I	Any deviation from the normal post-operative course without the need for pharmacological treatment or surgical, endoscopic, and radiological interventions. Allowed therapeutic regimens are: drugs as antiemetics, antipyretics, analgesics, diuretics, electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside.
II	Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included.
III	Requiring surgical, endoscopic or radiological intervention.
IIIa	Intervention not under general anaesthesia.
IIIb	Intervention under general anaesthesia.
IV	Life-threatening complication (including CNS complications)* requiring IC/ICU management.
IVa	Single organ dysfunction (including dialysis).
IVb	Multi-organ dysfunction.
V	Death of a patient.

*Brain haemorrhage, ischemic stroke, subarachnoidal bleeding but excluding transient ischemic attacks. CNS, central nervous system; IC, intermediate care; ICU, intensive care unit

Appendix 3 Contraception Guidance

Definition of women of childbearing potential:

A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

Acceptable methods of contraception for women of childbearing potential:

- combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[1]:
  - oral
  - intravaginal
  - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation[1]:
  - oral
  - injectable
  - implantable [2]
  - intrauterine device (IUD)[2]
- intrauterine hormone-releasing system (IUS)[2]
- intrauterine device (provided coils are copper-banded)[2]
- bilateral tubal occlusion[2]
- vasectomised partner [2,3]
- sexual abstinence[4]

[1] Hormonal contraception may be susceptible to interaction with the IMP, which may reduce the efficacy of the contraception method

[2] Contraception methods that in the context of this guidance are considered to have low user dependency.

[3] Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success (confirmation of azoospermia).

[4] In the context of this guidance sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

Abstinence is acceptable only as true abstinence: when this is in line with the preferred and usual lifestyle of the patient. [Periodic abstinence (e.g., calendar, ovulation, symptothermal, postovulation methods), declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception].

Recommended contraception for non-sterilized male participants:

- Male condom plus spermicide[5]

[5] Even if the female partner is pregnant, male participants should still use a condom plus spermicide, as indicated above, if there is a concern about damaging the developing foetus from drug in ejaculate.

A male participant is considered sterilized if he has undergone a vasectomy and received medical assessment of the surgical success (confirmation of azoospermia).

Female partners (of childbearing potential) of male participants must also use a highly effective method of contraception from randomisation of the male participant until 180 days after the last dose of trial treatment.

Appendix 4 Dose Modification Guidance for Durvalumab and Tremelimumab

Dosing Modification and Toxicity Management Guidelines (TMGs) for Durvalumab Monotherapy, Durvalumab in Combination with other Products, or Tremelimumab Monotherapy –21 September 2022

General Considerations Regarding Immune-Mediated Reactions

These guidelines are provided as a recommendation to support investigators in the management of potential immune-mediated adverse events (imAEs). Immune-mediated events can occur in nearly any organ or tissue, therefore, these guidelines may not include all the possible immune-mediated reactions. Investigators are advised to take into consideration the appropriate practice guidelines and other society guidelines (e.g., National Comprehensive Cancer Network (NCCN), European Society of Medical Oncology (ESMO)) in the management of these events. Refer to the section of the table titled “Other -Immune-Mediated Reactions” for general guidance on imAEs not noted in the “Specific Immune-Mediated Reactions” section.

Early identification and management of imAEs is essential to ensure safe use of the study drug. Monitor patients closely for symptoms and signs that may be clinical manifestations of underlying imAEs. Patients with suspected imAEs should be thoroughly evaluated to rule out any alternative etiologies (e.g., disease progression, concomitant medications, infections). In the absence of a clear alternative etiology, all such events should be managed as if they were immune-mediated. Institute medical management promptly, including specialty consultation as appropriate. In general, withhold study drug/study regimen for severe (Grade 3) imAEs. Permanently discontinue study drug/study regimen for life-threatening (Grade 4) imAEs, recurrent severe (Grade 3) imAEs that require systemic immunosuppressive treatment, or an inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks of initiating corticosteroids.

Based on the severity of the imAE, durvalumab and/or tremelimumab should be withheld and corticosteroids administered. Upon improvement to Grade ≤ 1, corticosteroid should be tapered over ≥ 28 days. More potent immunosuppressive agents should be considered for events not responding to systemic steroids. Alternative immunosuppressive agents not listed in this guideline may be considered at the discretion of the investigator based on clinical practice and relevant guidelines. With longterm steroid and other immunosuppressive use, consider the need for glucose monitoring.

Considerations for Prophylaxis for Long Term use of Steroids for Patients Receiving Immune Checkpoint Inhibitor Immunotherapy

- **Infection Prophylaxis: Pneumocystis jirovecii pneumonia (PJP), antifungal and Herpes Zoster reactivation**
- **Gastritis: Consider prophylaxis for patients at high risk of gastritis (e.g. NSAID use, anticoagulation) when the patient is taking steroid therapy**
- **Osteoporosis: Consider measures for prevention and mitigation of osteoporosis.**

Dose modifications of study drug/study regimen should be based on severity of treatment-emergent toxicities graded per NCI CTCAE version in Appendix 1.

Relevant Society Guidelines for Management of imAEs

These society guidelines are provided as references to serve in support of best clinical practice and the Toxicity Management Gudielines. Please note, these were the current versions of these guidelines at the time of updating Toxicity Management Guidelines.

1. Brahmer JR, et al. Society for Immunotherapy of Cancer (SITC) clinical practice guideline on immune checkpoint inhibitor-related adverse events. J Immunother Cancer 2021;9:e002435

2.Schneider BJ, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American Society of Clinical Oncology (ASCO) Guideline Update. J Clin Oncol 2022;39(36):4073-4126.

3. Haanen JBAG, et al. Management of toxicities for immunotherapy: European Society for Medical Oncology (ESMO) clinical practice guidelines for diagnosis, treatment, and follow-up. Annals Oncol 2022;33(12):1217-1238.

4. Sangro B, et al. Diagnosis and management of toxicities from immune checkpoint inhibitors in hepatocellular carcinoma. J Hepatol 2020;72(2):320-341.

5. Thompson JA, et al. National Comprehensive Cancer Network Guidelines: Management of immunotherapy-related toxicities version 2.2023. Published February 28, 2023.

Specific Immune-Mediated Reactions			
Adverse Events	**Severity Grade of the Event**	**Dose Modifications**	**Toxicity Management**
Pneumonitis/ **Interstitial** **Lung Disease (ILD)**	**Any Grade** (Refer to NCI CTCAE in Appendix 1 for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** − Patients should be thoroughly evaluated to rule out any alternative aetiology with similar clinical presentation (e.g. infection, progressive disease) − Monitor patients for signs (e.g. tachypnoea) and symptoms of pneumonitis or ILD (new onset or worsening shortness of breath or cough). Evaluate patients with imaging and pulmonary function tests, including other diagnostic procedures as described below. − Suspected pneumonitis should be confirmed with radiographic imaging and other infectious and disease-related aetiologies excluded, and managed as described below. − Initial work-up may include clinical evaluation, monitoring of oxygenation via pulse oximetry (resting and exertion), laboratory work-up (including clinically relevant culture specimens to rule out infection), and high- resolution computed tomography (CT) scan. − Consider Pulmonary and Infectious Diseases consults.

	Grade 1	No dose modifications required. However, consider holding study drug/study regimen dose as clinically appropriate and during diagnostic work-up for other aetiologies.	**For Grade 1** − Monitor and closely follow up in 2 to 4 days for clinical symptoms, pulse oximetry (resting and exertion), and laboratory workup, and then as clinically indicated.
	Grade 2	Hold study drug/study regimen dose until Grade 2 resolution to Grade ≤1. • If toxicity improves to Grade ≤1, then the decision to reinitiate study drug/study regimen will be based upon treating physician's clinical judgment and after completion of steroid taper (<10 mg prednisone or equivalent).	**For Grade 2** − Monitor symptoms daily and consider hospitalization as clinically indicated. − Consider Pulmonary and Infectious Diseases Consults. - Promptly start systemic steroids (e.g., prednisone 1 to 2 mg/kg/day PO or IV equivalent). − Consider HRCT or chest CT with contrast. Repeat imaging study as clinically indicated. − If no improvement within 2 to 3 days, additional workup should be considered and prompt treatment with IV methylprednisolone 2 to 4 mg/kg/day started. − If no improvement within 2 to 3 days despite IV methylprednisolone at 2 to 4 mg/kg/day, promptly start immunosuppressive therapy such as tumor necrosis factor (TNF) inhibitors (e.g., infliximab at 5 mg/kg IV once, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using

			infliximab. Consider discussing with GUSTO Chief Investigator or Trial Physicians.
	Grade 3 or 4	Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** − Hospitalize the patient. − Promptly initiate empiric IV methylprednisolone 1 to 4 mg/kg/day or equivalent. − Obtain Pulmonary and Infectious Diseases Consults; consider discussing with GUSTO Chief Investigator or Trial Physicians, as needed. − Consider starting anti-infective therapy if infection is still a consideration on the basis of other diagnostic testing despite negative culture results.. − Supportive care (e.g., oxygen). − If no improvement within 2 days, additional workup should be considered and prompt treatment with additional immunosuppressive therapy such as TNF inhibitors (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). Caution: rule out sepsis and refer to infliximab label for general guidance before using infliximab.
Diarrhoea/Colitis	**Any Grade** (Refer to NCI CTCAE in Appendix 1 for defining the	**General Guidance**	**For Any Grade** − Patients should be thoroughly evaluated to rule out any alternative aetiology (e.g., disease progression,

	CTCAE grade/severity)		other medications, or infections), including testing for *Clostridium difficile* toxin, etc. − Monitor for symptoms that may be related to diarrhoea/enterocolitis (abdominal pain, cramping, or changes in bowel habits such as increased frequency over baseline or blood in stool) or related to bowel perforation (such as sepsis, peritoneal signs, and ileus). - Consider further evaluation with imaging study with contrast. - Consult gastrointestinal (GI) specialist for consideration of further workup. − **WHEN SYMPTOMS OR EVALUATION INDICATE AN INTESTINAL PERFORATION IS SUSPECTED, CONSULT A SURGEON EXPERIENCED IN ABDOMINAL SURGERY IMMEDIATELY WITHOUT ANY DELAY.** − **PERMANENTLY DISCONTINUE STUDY DRUG FOR ANY GRADE OF INTESTINAL PERFORATION.** − Steroids should be considered in the absence of clear alternative aetiology, even for lowgrade events, in order to prevent potential progression to higher grade events, including intestinal perforation. − Use analgesics carefully; they can mask symptoms of perforation and peritonitis.
	Grade 1	No dose modifications.	**For Grade 1** − Monitor closely for worsening symptoms.

			− Consider symptomatic treatment, including hydration, electrolyte replacement, dietary changes (e.g., American Dietetic Association colitis diet), loperamide, and other supportive care measures. − If symptoms persist, consider checking lactoferrin and/or calprotectin; if positive, treat as Grade 2 below. If negative and no infection, continue Grade 1 management.
	Grade 2	Hold study drug/study regimen until resolution to Grade ≤1 • If toxicity improves to Grade ≤1, then study drug/study regimen can be resumed after completion of steroid taper (<10 mg prednisone, or equivalent).	**For Grade 2** − Consider symptomatic treatment, including hydration, electrolyte replacement, dietary changes (e.g., American Dietetic Association colitis diet), and loperamide and/or budesonide. - Consider further evaluation with imaging study with contrast. - Consider consult of a gastrointestinal (GI) specialist for consideration of further workup. − Promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent. If no improvement within 3 days despite therapy with 1 to 2 mg/kg IV prednisone equivalent, reconsult GI specialist and, if indicated, promptly start additional immunosuppressant agent such as infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines.

			Caution: it is important to rule out bowel perforation and refer to infliximab label for general guidance before using infliximab. − **If perforation is suspected, consult a surgeon experienced in abdominal surgery immediately without any delay.** − Consider, as necessary, discussing with GUSTO Chief Investigator or Trial Physicians if no resolution to Grade ≤1 in 3 to 4 days.
	Grade 3 or 4	**Grade 3** • For patients treated with durvalumab monotherapy, hold study drug/study regimen until resolution to Grade ≤1; study drug/study regimen can be resumed after completion of steroid taper (<10 mg prednisone per day, or equivalent). • For patients treated with durvalumab in combination with other products (not tremelimumab), decision to be made at the discretion of the study investigator, in discussion with AstraZeneca Clinical Study Lead. • **<u>For patients treated with durvalumab in combination with tremelimumab or tremelimumab monotherapy</u>**: **A. Permanently discontinue tremelimumab for Grade 3 diarrhea/colitis. HOLD durvalumab until resolution to Grade ≤1; durvalumab**	**For Grade 3 or 4** − Urgent GI consult and imaging and/or colonoscopy as appropriate. - Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent. − Monitor stool frequency and volume and maintain hydration. − If still no improvement within 2 days, continue steroids and promptly add further immunosuppressants. (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). **Caution**: Ensure GI consult to rule out bowel perforation and refer to infliximab label for general guidance before using infliximab.

		alone can be resumed after completion of steroid taper (<10 mg prednisone per day or equivalent) **B. Permanently discontinue both durvalumab and tremelimumab for 1) Grade 4 diarrhea/ colitis or 2) Any grade of intestinal perforation**. **Grade 4** Permanently discontinue study drug/study regimen.	**− If perforation is suspected, consult a surgeon experienced in abdominal surgery immediately without any delay.**
Hepatitis **Infliximab should not be used for management of immune-related hepatitis.**	**Any Grade** (Refer to NCI CTCAE in Appendix 1 for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** − Patients should be thoroughly evaluated to rule out any alternative aetiology (e.g., viral hepatitis, disease progression, concomitant medications). − Monitor and evaluate transaminases (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP]) and total bilirubin.
	ALT or AST ≤3 x ULN or total bilirubin ≤1.5 x ULN	• No dose modifications. • If it worsens, then consider holding therapy.	− Continue transaminase and total bilirubin monitoring per protocol.
	ALT or AST >3 ≤5 x ULN or total bilirubin >1.5<3 x ULN	• Hold study drug/study regimen dose until ALT or AST < 3 x ULN or total bilirubin <1.5 x ULN. Resume study drug/study regimen after completion of	− Regular and frequent checking of transaminases and total bilirubin (e.g., every 1 to 2 days) until transaminases and total bilirubin elevations improve or resolve.

		steroid taper (<10 mg prednisone or equivalent). • Permanently discontinue study drug/study regimen for any case meeting Hy's law laboratory criteria (AST or ALT >3 × ULN AND bilirubin >2 × ULN without initial findings of cholestasis (i.e., elevated ALP) and in the absence of any alternative cause.	- Consider checking creatinine phosphokinase (CPK) and aldolase (to rule out myositis) − If no resolution to ALT or AST ≤ 3 x ULN or total bilirubin ≤ 1.5 x ULN in 1 to 2 days, consider discussing with GUSTO Chief Investigator or Trial Physicians, as needed. − If event is persistent (>2 to 3 days) or worsens, promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent.
	ALT or AST > 5- ≤ 10 x ULN	• Hold study drug/study regimen. Resume study drug/study regimen if elevations downgrade to ALT or AST ≤ 3 x ULN or total bilirubin ≤ 1.5 x ULN after completion of steroid taper (<10 mg prednisone, or equivalent). • **If in combination with tremelimumab, do not restart tremelimumab.**	− Promptly initiate empiric IV methylprednisolone at 1 to 2 mg/kg/day or equivalent. - Check CPK and aldolase (to rule out myositis) − Perform Hepatology Consult, abdominal workup, and imaging as appropriate. - If still no improvement within 2 to 3 days despite 1 to 2 mg/kg/day methylprednisolone IV or equivalent, promptly start treatment with an additional immunosuppressant.(e.g., mycophenolate mofetil 0.5 – 1 g every 12 hours then taper in consultation with hepatology consult or relevant practice guidelines). Discuss with GUSTO Chief Investigator or Trial Physicians if mycophenolate is not available. **Infliximab should NOT be used.**
	Concurrent ALT or AST >	Permanently discontinue study drug/study regimen.	− Promptly initiate empiric IV methylprednisolone at 1 to 2 mg/kg/day or equivalent.

	3 x ULN and total bilirubin > 2 x ULN ALT or AST > 10 x ULN OR total bilirubin > 3 x ULN		− If still no improvement within 2 to 3 days despite 1 to 2 mg/kg/day methylprednisolone IV or equivalent, promptly start treatment with an additional immunosuppressant.(e.g., mycophenolate mofetil 0.5 – 1 g every 12 hours then taper in consultation with hepatology consult or relevant practice guidelines). Discuss with GUSTO Chief Investigator or Trial Physicians if mycophenolate is not available. **Infliximab should NOT be used**. − Perform Hepatology Consult, abdominal workup, and imaging as appropriate.
Nephritis and/or renal dysfunction	**Any Grade** (Refer to NCI CTCAE in Appendix 1 for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** − Patients should be thoroughly evaluated to rule out any alternative aetiology (e.g., disease progression, infections, recent IV contrast, medications, fluid status). − Consider consulting a nephrologist. - Consider imaging studies to rule out any alternative aetiology. - Monitor for signs and symptoms that may be related to changes in renal function (e.g., routine urinalysis, elevated serum BUN and creatinine, decreased creatinine clearance, electrolyte imbalance, decreased urine output, or proteinuria). Follow urine protein/creatinine ratio every 3-7 days.
	Grade 1	No dose modifications.	**For Grade 1** − Monitor serum creatinine weekly and any accompanying symptoms.

			• If creatinine returns to baseline, resume regular monitoring per Section 12. • If creatinine worsens, depending on the severity, treat as Grade 2, 3, or 4. − Consider hydration, electrolyte replacement, and diuretics as clinically indicated. − Consider nephrologist consult if not resolved within 14 days, or earlier as clinically indicated.
	Grade 2	Hold study drug/study regimen until resolution to Grade ≤1 or baseline. • If toxicity improves to Grade ≤1 or baseline, then resume study drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent).	**For Grade 2** − Consider including hydration, electrolyte replacement, and diuretics as clinically indicated. - Follow urine protein/creatinine ratio every 3-7 days − Carefully monitor serum creatinine as clinically warranted. − Consult nephrologist and consider renal biopsy if clinically indicated. - Start prednisone 0.5 – 1 mg/kg/day if other causes are ruled out. − If event is persistent beyond 5 days or worsens, increase prednisone up to 2 mg/kg/day PO or IV equivalent. − If event is not responsive within 5 days or worsens despite prednisone at 1 to 2 mg/kg/day PO or IV equivalent, consider additional workup. When event returns to baseline, resume study drug/study regimen

			and routine serum creatinine monitoring per study protocol.
	Grade 3 or 4	Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** Carefully monitor serum creatinine daily. - Follow urine protein/creatinine ratio every 3-7 days. − Consult nephrologist and consider renal biopsy if clinically indicated. − Promptly start prednisone 1 to 2 mg/kg/day PO or IV equivalent. − If event is not responsive within 3 to 5 days of steroids or worsens despite prednisone at 1 to 2 mg/kg/day PO or IV equivalent, consider additional workup and prompt treatment with an immunosuppressant.
Dermatologic Adverse Events **(Including Pemphigoid)**	**Any Grade** (Refer to NCI CTCAE in Appendix 1 for defining the CTCAE grade/severity depending on type of skin rash)	**General Guidance**	**For Any Grade** − Patients should be thoroughly evaluated to rule out any alternative aetiology. − Monitor for signs and symptoms of dermatitis (rash and pruritus). − **HOLD STUDY DRUG GRADE 3 PEMPHIGOID OR SEVERE CUTANEOUS ADVERSE REACTION (SCAR)[1] IS SUSPECTED.** − **PERMANENTLY DISCONTINUE STUDY DRUG IF SCAR OR GRADE 3 PEMPIGOID IS CONFIRMED.**

	Grade 1	No dose modifications.	**For Grade 1** − Consider symptomatic treatment, including oral antipruritics (e.g., diphenhydramine or hydroxyzine) and topical therapy (e.g., emolient, lotion, or institutional standard).
	Grade 2	For persistent (>1 week) Grade 2 events, hold scheduled study drug/study regimen until resolution to Grade ≤1 or baseline. • If toxicity improves to Grade ≤1 or baseline, then resume drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent).	**For Grade 2** − Consider dermatology consult and skin biopsy, as indicated. − Consider symptomatic treatment, including oral antipruritics (e.g., diphenhydramine or hydroxyzine) and topical therapy − Consider moderate-strength topical steroid. - If no improvement of rash/skin lesions occurs within 1 week or is worsening despite symptomatic treatment and/or use of moderate strength topical steroid, consider discussing with GUSTO Chief Investigator or Trial Physicians, as needed, and promptly start systemic steroids such as prednisone 1 to 2 mg/kg/day PO or IV equivalent.
	Grade 3	**For Grade 3** • Hold study drug/study regimen until resolution to Grade ≤1 or baseline. If toxicity improves to Grade ≤1 or baseline, then resume drug/study	**For Grade 3** − Reconsult a dermatologist. Consider skin biopsy (preferably more than 1) as clinically feasible.

		regimen after completion of steroid taper (<10 mg prednisone, or equivalent).	− Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent. − Consider hospitalization. − Monitor the extent of rash [Rule of Nines]. −Consider, as necessary, discussing with GUSTO Chief Investigator or Trial Physicians.
	Grade 4	**For Grade 4** Permanently discontinue study drug/study regimen.	**For Grade 4** Reconsult a dermatologist. Consider skin biopsy (preferably more than 1) as clinically feasible. − Promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent. − Consider hospitalization. − Monitor the extent of rash [Rule of Nines]. Consider, as necessary, discussing with GUSTO Chief Investigator or Trial Physicians.
Endocrinopathy (e.g., hyperthyroidism, thyroiditis, hypothyroidism, type 1 diabetes mellitus, hypophysitis, hypopituitarism, and adrenal insufficiency)	**Any Grade** (Refer to NCI CTCAE in Appendix 1 for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** − Patients should be thoroughly evaluated to rule out any alternative aetiology (e.g., disease progression including brain metastases, or infections). − Consider consulting an endocrinologist for endocrine events.

			− Consider discussing with GUSTO Chief Investigator or Trial Physicians, as needed. − Monitor patients for signs and symptoms of endocrinopathies. (Non-specific symptoms include headache, fatigue, behaviour changes, mental status changes, photophobia, visual field cuts, vertigo, abdominal pain, unusual bowel habits, polydipsia, polyuria, hypotension, and weakness.) − Depending on the suspected endocrinopathy, monitor and evaluate thyroid function tests: thyroid stimulating hormone (TSH), free T3 and free T4 and other relevant endocrine and related labs (e.g., blood glucose and ketone levels, haemoglobin A1c (HgA1c)). If a patient experiences an AE that is thought to be possibly of autoimmune nature (e.g., thyroiditis, pancreatitis, hypophysitis, or diabetes insipidus), the investigator should send a blood sample for appropriate autoimmune antibody testing. − Investigators should ask subjects with endocrinopathies who may require prolonged or continued hormonal replacement, to consult their primary care physicians or endocrinologists about further monitoring and treatment after completion of the study.
	Grade 1	No dose modifications.	**For Grade 1** − Monitor patient with appropriate endocrine function tests. − For suspected hypophysitis/hypopituitarism, consider consulting an endocrinologist to guide assessment of early-morning adrenocorticotropin

			hormone (ACTH), cortisol, TSH and free T4; also consider gonadotropins, sex hormones, and prolactin levels, as well as cosyntropin stimulation test (though it may not be useful in diagnosing early secondary adrenal insufficiency). − If TSH < 0.5 × LLN, or TSH >2 × ULN, or consistently out of range in 2 subsequent measurements, include free T4 at subsequent cycles as clinically indicated and consider consultation of an endocrinologist.
	Grade 2, 3, or 4	• For Grade 2-4 endocrinopathies <u>other than hypothyroidism and type 1 diabetes mellitus (T1DM)</u>, consider holding study drug/study regimen dose until acute symptoms resolve. • Study drug/study regimen can be resumed once patient stabilizes and after completion of steroid taper (<10 mg prednisone, or equivalent). • Patients with endocrinopathies who may require prolonged or continued steroid replacement (e.g., adrenal insufficiency) can be retreated with study drug/study regimen if the patient is clinically stable as per investigator or treating physician's clinical judgement.	**For Grade 2, 3, or 4** − Consult endocrinologist to guide evaluation of endocrine function and, as indicated by suspected endocrinopathy and as clinically indicated, consider pituitary scan. − For all patients with abnormal endocrine work up, except those with isolated hypothyroidism or T1DM, and as guided by an endocrinologist, consider short-term corticosteroids (e.g., 1 to 2 mg/kg/day methylprednisolone or IV equivalent) and prompt initiation of treatment with relevant hormone replacement. − **Isolated hypothyroidism may be treated with replacement therapy, without study drug/study regimen interruption, and without corticosteroids.** − **Isolated T1DM may be treated with appropriate diabetic therapy, and without corticosteroids. <u>Only hold study drug/study regimen in setting of</u>**

			hyperglycemia when diagnostic workup is positive for diabetic ketoacidosis. − For patients with normal endocrine workup (laboratory assessment or magnetic resonance imaging (MRI) scans), repeat laboratory assessments/MRI as clinically indicated.
Amylase/Lipase **increased**	**Any Grade** (Refer to NCI CTCAE in Appendix 1 for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** − Patients should be thoroughly evaluated to rule out any alternative aetiology (e.g. disease progression, viral infection, concomitant medications, substance abuse). − For modest asymptomatic elevations in serum amylase and lipase, corticosteroid treatment is not indicated as long as there are no other signs or symptoms of pancreatic inflammation. − Assess for signs/symptoms of pancreatitis − Consider appropriate diagnostic testing (e.g., abdominal CT with contrast, MRCP if clinical suspicion of pancreatitis and no radiologic evidence on CT) - If isolated elevation of enzymes without evidence of pancreatitis, continue immunotherapy. Consider other causes of elevated amylase/lipase − If evidence of pancreatitis, manage according to pancreatitis recommendations
	Grade 1	No dose modifications.	
	Grade 2, 3, or 4	**For Grade 2, 3, or 4** In consultation with relevant gastroenterology specialist consider continuing study drug/study regimen if no clinical/radiologic evidence of pancreatitis ± improvement in amylase/lipase.	
Acute Pancreatitis	**Any Grade**	**General Guidance**	**For Any Grade**

	(Refer to NCI CTCAE in Appendix 1 for defining the CTCAE grade/severity)		− Patients should be thoroughly evaluated to rule out any alternative aetiology. − Consider Gastroenterology referral.
	Grade 2	**Consider holding study drug/regimen**	**Grade 2** − Consider IV hydration − Consider Gastroenterology referral
	Grade 3, or 4	**For Grade 3** Hold study drug/study regimen until resolution of elevated enzymes and no radiologic findings. If no elevation in enzymes or return to baseline values, then resume study drug/study regimen after completion of steroid taper (<10 mg prednisone, or equivalent). **For Grade 4** Permanently discontinue study drug/study regimen.	**For Grade 3, or 4** − Promptly start systemic steroids prednisone 1 to 2 mg/kg/day PO or IV equivalent. − IV hydration.
Nervous System Disorders			
Aseptic Meningitis	**Any Grade** (Refer to NCI CTCAE in	**General Guidance** - Symptoms may include headache, photophobia, and neck stiffness, nausea/	**For Any Grade** − Consider neurology consult.

	Appendix 1 for defining the CTCAE grade/severity)	vomiting which may resemble an infectious meningitis. − Patients may be febrile. − Mental status should be normal	- Consider MRI brain with and without contrast with pituitary protocol and a lumbar puncture for diagnosis. − Exclude bacterial and viral infections. (ie HSV) - Consider antibiotic for bacterial coverage until cultures/panel results are back - Consider IV acyclovir until polymerase chain reactions are available
	Any Grade	Permanently discontinue study drug/study regimen	**For Any Grade** − Consider neurology consult. − Consider MRI brain with and without contrast with pituitary protocol and a lumbar puncture for diagnosis. − Exclude bacterial and viral infections. (ie HSV) − Consider IV acyclovir until polymerase chain reactions are available. − Consider, as necessary, discussing with GUSTO Chief Investigator or Trial Physicians. − Consider hospitalization. − Once infection has been ruled out promptly initiate empiric IV methylprednisolone 1 to 2 mg/kg/day or equivalent.
Encephalitis	**Any Grade** (Refer to NCI CTCAE in	**General Guidance**	**For Any Grade** − Consider neurology consult.

	Appendix 1 for defining the CTCAE grade/severity)	- Symptoms may include Confusion, altered behaviour, headaches, seizures, short-term memory loss, depressed level of consciousness, focal weakness, and speech abnormality.	− Consider testing including MRI of the brain with and without contrast, lumbar puncture, electroencephalogram (EEG) to evaluate for subclinical seizures, ESR, CRP, antineutrophil cytoplasmic antibody (ANCA) (if vasculitic process suspected), thyroid panel including TPO and thyroglobulin and additional autoantibodies to rule out paraneoplastic disorders. − Exclude bacterial and viral infections. (ie HSV). Consider IV acyclovir until polymerase chain reactions are available.
	Grade 2	**For Grade 2** Permanently discontinue study drug/study regimen.	**For Grade 2** − Consider, as necessary, discussing with the GUSTO Chief Investigator or Trial Physicians. − Once infection has been ruled out methylprednisolone 1–2 mg/kg/day − For progressive symptoms or if oligoclonal bands are present consider methylprednisolone 1 g IV daily for 3–5 days plus IVIG or plasmapheresis
	For Grade 3 or 4	**For Grade 3 or 4** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** − Consider, as necessary, discussing with GUSTO Chief Investigator or Trial Physicians. − Consider hospitalization. − Once infection is ruled out, start methylprednisolone 1 g IV daily for 3–5 days for progressive symptoms consider adding IVIG or plasmapheresis

Demyelinating Disease (optic neuritis, transverse myelitis, acute demyelinating encephalomyelitis (ADEM)	**Any Grade**	**General Guidance** − Permanently discontinue immunotherapy − Consider MRI of the spine and brain - Once imaging is complete, consider lumbar puncture Consider testing to rule out additional aetiologies: B12, copper, HIV, rapid plasma reagin (RPR), ANA, anti-Ro/La antibodies, aquaporin-4 IgG, myelin oligodendrocyte glycoprotein (MOG) IgG, paraneoplastic panel for anti-Hu and anti-CRMP5/CV2	**For Any Grade** − Consider neurology consult − Inpatient care – Consider prompt initiation of high methylprednisolone pulse dosing − Strongly consider IVIG or plasmapheresis
Peripheral neuropathy	**Any Grade** (Refer to NCI CTCAE in Appendix 1 for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** − Patients should be evaluated to rule out any alternative aetiology for neuropathy (e.g., disease progression, infections, metabolic syndromes or medications). It should be noted that the diagnosis of immune-mediated peripheral neuromotor syndromes can be particularly challenging in patients with underlying cancer, due to the multiple potential confounding effects of cancer (and its treatments) throughout the neuraxis. Given the importance of prompt and accurate diagnosis, it is essential to have a low threshold to obtain a neurological consult. − Neurophysiologic diagnostic testing (e.g., electromyogram and nerve conduction investigations) are routinely indicated upon suspicion of such

			conditions and may be best facilitated by means of a neurology consultation.
	Grade 1	No dose modifications.	**For Grade 1** − Consider discussing with the GUSTO Chief Investigator or Trial Physicians, as needed. − Monitor symptoms for interference with ADLS, gait difficulties, imbalance, or autonomic dysfunction
	Grade 2	Hold study drug/study regimen dose until resolution to Grade ≤1.	**For Grade 2** − Consult a neurologist. - Consider EMG/NCS − Consider discussing with the GUSTO Chief Investigator or Trial Physicians, as needed. − Observation for additional symptoms or consider initiating prednisone 0.5–1 mg/kg orally. - If progression, initiate methylprednisolone 2–4 mg/kg/day and treat as GBS − Sensory neuropathy/neuropathic pain may be managed by appropriate medications (e.g., gabapentin or duloxetine).
	Grade 3 or 4	**For Grade 3 or 4** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** − Consider discussing with GUSTO Chief Investigator or Trial Physicians, as needed. − Recommend hospitalization.

			− Monitor symptoms and consult a neurologist. − **Treat per Guillain-Barré Syndrome recommendations**
Guillain-Barré Syndrome (GBS)		**General Guidance**	- Recommend hospitalization − Obtain neurology consult − Obtain MRI of spine to rule out compression lesion − Obtain lumbar puncture − Antibody tests for GBS variants − Pulmonary function tests − Obtain electromyography (EMG) and nerve conduction studies − Frequently monitor pulmonary function tests and neurologic evaluations − Monitor for concurrent autonomic dysfunction − Initiate medication as needed for neuropathic pain
	Grade 2 - 4	**Grade 2-4** Permanently discontinue	**Start IVIG or plasmapheresis in addition to methylprednisolone 1 gram daily for 5 days, then taper over 4 weeks.**
Myasthenia gravis		**General Guidance**	- Obtain neurology consult − Recommend hospitalization − Obtain pulmonary function tests

			− Obtain labs: ESR, CRP, creatine phosphokinase (CPK), aldolase and anti-striational antibodies − Consider cardiac exam, ECG, troponin, transthoracic echocardiogram for possible concomitant myocarditis − Obtain electromyography (EMG) and nerve conduction studies − Consider MRI of brain/spine to rule out CNS involvement by disease − Avoid medications that might exacerbate MG (e.g. beta blockers, some antibiotics, IV magnesium)
	Grade 2	Permanently discontinue	- Consider pyridostigmine 30mg three times daily and gradually increase based on symptoms (max dose 120mg four times daily) − Consider starting low dose prednisone 20mg daily and increase every 3-5 days. (Target dose 1mg/kg/day. Max dose 100mg daily)
	Grade 3-4	Permanently discontinue	- Start methylprednisolone 1-2mg/kg/day. Taper steroids based on symptom improvement − Start plasmapheresis or IVIG − Consider rituximab if refractory to plasmapheresis or IVIG − Frequent PFT assessments − Daily neurologic evaluations
Myocarditis	**Any Grade**	**General Guidance**	**For Any Grade**

	(Refer to NCI CTCAE in Appendix 1 for defining the CTCAE grade/severity)	Discontinue drug permanently if biopsy-proven immune-mediated myocarditis.	− Initial work-up should include clinical evaluation, B-type natriuretic peptide (BNP), cardiac enzymes, electrocardiogram (ECG), echocardiogram (ECHO), monitoring of oxygenation via pulse oximetry (resting and exertion), and additional laboratory work-up as indicated. Spiral CT or cardiac MRI can complement ECHO to assess wall motion abnormalities when needed. - Patients should be thoroughly evaluated to rule out any alternative aetiology (e.g., disease progression, other medications, or infections) − The prompt diagnosis of immune-mediated myocarditis is important, particularly in patients with baseline cardiopulmonary disease and reduced cardiac function. − Consider discussing with the GUSTO Chief Investigator or Trial Physicians, as needed. − Monitor patients for signs and symptoms of myocarditis (new onset or worsening chest pain, arrhythmia, shortness of breath, peripheral oedema). As some symptoms can overlap with lung toxicities, simultaneously evaluate for and rule out pulmonary toxicity as well as other causes (e.g., pulmonary embolism, congestive heart failure, malignant pericardial effusion). Consult a cardiologist early, to promptly assess whether and when to complete a cardiac biopsy, including any other diagnostic procedures.

			− as indicated. Spiral CT or cardiac MRI can complement ECHO to assess wall motion abnormalities when needed.
	Grade 2, 3 or 4	• If Grade 2-4, permanently discontinue study drug/study regimen.	**For Grade 2-4** − Monitor symptoms daily, hospitalize. − Consider cardiology consultation and a prompt start of high-dose/pulse corticosteroid therapy. − Supportive care (e.g., oxygen). − If no improvement consider additional immunosuppressive therapy such as TNF inhibitors (e.g., infliximab), IVIG or or plasmapheresis or other therapies depending on the clinical condition of the patient, based on the discretion of the treating specialist consultant or relevant practice guidelines. **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab. Infliximab is contraindicated for patients who have heart failure.**
Myositis/ Polymyositis	**Any Grade** (Refer to NCI CTCAE in Appendix 1 for defining the CTCAE grade/severity)	**General Guidance**	**For Any Grade** − Patients should be thoroughly evaluated to rule out any alternative aetiology (e.g., disease progression, other medications, or infections). − Monitor patients for signs and symptoms of poly/myositis. Typically, muscle weakness/pain occurs in proximal muscles including upper arms, thighs, shoulders, hips, neck and back, and also difficulty breathing and/or trouble swallowing can occur and progress rapidly. Increased general feelings of tiredness and fatigue may occur, and there can be

			new onset falling, difficulty getting up from a fall, and trouble climbing stairs, standing up from a seated position, and/or reaching up. − If poly/myositis is suspected, a Neurology consultation should be obtained early, with prompt guidance on diagnostic procedures. Myocarditis may co-occur with poly/myositis; refer to guidance under Myocarditis. Given breathing complications, refer to guidance under Pneumonitis/ILD. Given possibility of an existent (but previously unknown) autoimmune disorder, consider Rheumatology consultation. − Consider, as necessary, discussing with the GUSTO Chief Investigator or Trial Physicians. - Consider that patients may present with or progress to rhabdomyolysis. Treat signs and symptoms as per institutional protocol or local clinical practice. − Initial work-up should include clinical evaluation, creatine kinase, aldolase, lactate dehydrogenase (LDH), blood urea nitrogen (BUN)/creatinine, erythrocyte sedimentation rate or C-reactive protein (CRP) level, urine myoglobin, and additional laboratory workup as indicated, including a number of possible rheumatological/antibody tests (i.e., consider whether a rheumatologist consultation is indicated and could guide need for rheumatoid factor, antinuclear antibody, anti-smooth muscle, antisynthetase [such as anti-Jo-1], and/or signal-recognition particle antibodies). Confirmatory testing may include electromyography, nerve conduction studies, MRI of the muscles, and/or a muscle biopsy.

			Consider Barium swallow for evaluation of dysphagia or dysphonia.
	Grade 1	• No dose modifications.	**For Grade 1** − Monitor and closely follow up in 2 to 4 days for clinical symptoms and initiate evaluation as clinically indicated. − Consider Neurology consult. − Consider, as necessary, discussing with the GUSTO Chief Investigator or Trial Physicians.
	Grade 2	• Hold study drug/study regimen dose until resolution to Grade ≤1. • Permanently discontinue study drug/study regimen if it does not resolve to Grade ≤1 within 30 days or if there are signs of respiratory insufficiency.	**For Grade 2** − Monitor symptoms daily and consider hospitalization. − Consider Rheumatology or Neurology consult, and initiate evaluation. − Consider, as necessary, discussing with the GUSTO Chief Investigator or Trial Physicians. − If clinical course is rapidly progressive (particularly if difficulty breathing and/or trouble swallowing), promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant − If clinical course is *not* rapidly progressive, start systemic steroids (e.g., prednisone 1 to 2 mg/kg/day PO or IV equivalent); if no improvement within 2 to 3 days, continue additional work up and start treatment with IV methylprednisolone 2 to 4 mg/kg/day

			− If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 3 days, consider additional immunosuppressive therapy such as TNF inhibitors (e.g., infliximab), IVIG or plasmapheresis, or other therapies based on the discretion of the treating specialist consultant or relevant practice guideline. **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab.**
	Grade 3	**For Grade 3** • Hold study drug/study regimen dose until resolution to Grade ≤1. • Permanently discontinue study drug/study regimen if Grade 3 imAE does not resolve to Grade ≤1 within 30 days or if there are signs of respiratory insufficiency.	**For Grade 3** − Monitor symptoms closely; recommend hospitalization. − Consider Rheumatology and/or Neurology consult − Consider discussing with the GUSTO Chief Investigator or Trial Physicians, as needed. − Promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant. − If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 2 to 3 days, consider starting another immunosuppressive therapy such as a TNF inhibitor (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab.** − Consider whether patient may require IV IG, plasmapheresis.

	Grade 4	**For Grade 4** • Permanently discontinue study drug/study regimen.	**Grade 4** − Monitor symptoms closely; recommend hospitalization. − Consider Rheumatology and/or Neurology consult − Consider discussing with the GUSTO Chief Investigator or Trial Physicians, as needed. − Promptly start IV methylprednisolone 2 to 4 mg/kg/day systemic steroids along with receiving input from Neurology consultant. − If after start of IV methylprednisolone at 2 to 4 mg/kg/day there is no improvement within 2 to 3 days, consider starting another immunosuppressive therapy such as a TNF inhibitor (e.g., infliximab at 5 mg/kg IV, may be repeated at 2 and 6 weeks after initial dose at the discretion of the treating provider or relevant practice guidelines). **Caution: It is important to rule out sepsis and refer to infliximab label for general guidance before using infliximab.**
Other–Immune-Mediated Reactions			
Severity Grade of the Event **(Refer to NCI CTCAE in Appendix 1 for**	**Dose Modifications**		**Toxicity Management**

defining the CTCAE grade/severity)		
Any Grade	Note: It is possible that events with an inflammatory or immune mediated mechanism could occur in nearly all organs, some of them are not noted specifically in these guidelines (e.g. immune thrombocytopenia, haemolytic anaemia, uveitis, vasculitis).	− Patients should be thoroughly evaluated to rule out any alternative aetiology (e.g., disease progression, other medications, or infections). − The GUSTO Chief Investigator or Trial Physicians may be contacted for immune-mediated reactions not listed in the "specific immune-mediated reactions" section − Consultation with relevant specialist − Treat accordingly, as per institutional standard.
Grade 1	No dose modifications.	Monitor as clinically indicated
Grade 2	• Hold study drug/study regimen until resolution to ≤Grade 1 or baseline. • If toxicity worsens, then treat as Grade 3 or Grade 4. • Study drug/study regimen can be resumed once event stabilizes to Grade ≤1 after completion of steroid taper. • Consider whether study drug/study regimen should be permanently discontinued in Grade 2 events with high likelihood for morbidity and/or mortality when they do not rapidly improve to Grade <1 upon treatment with systemic steroids and following full taper	**For Grade 2, 3, or 4** Treat accordingly, as per institutional standard, appropriate clinical practice guidelines, and society guidelines. (See page 104).
Grade 3	Hold study drug/study regimen until resolution to Grade ≤1 or baseline	

Grade 4	Permanently discontinue study drug/study regimen	
Infusion-Related Reactions		
Severity Grade of the Event **(Refer to NCI CTCAE in Appendix 1 for defining the CTCAE grade/severity)**	**Dose Modifications**	**Toxicity Management**
Any Grade	**General Guidance**	**For Any Grade** − Manage per institutional standard at the discretion of investigator. − Monitor patients for signs and symptoms of infusion-related reactions (e.g., fever and/or shaking chills, flushing and/or itching, alterations in heart rate and blood pressure, dyspnoea or chest discomfort, or skin rashes) and anaphylaxis (e.g., generalized urticaria, angioedema, wheezing, hypotension, or tachycardia).
Grade 1 or 2	**For Grade 1** The infusion rate of study drug/study regimen may be decreased by 50% or temporarily interrupted until resolution of the event. **For Grade 2**	**For Grade 1 or 2** − Acetaminophen and/or antihistamines may be administered per institutional standard at the discretion of the investigator.

	• The infusion rate of study drug/study regimen may be decreased 50% or temporarily interrupted until resolution of the event. • Subsequent infusions may be given at 50% of the initial infusion rate.	− Consider premedication per institutional standard prior to subsequent doses. − Consider steroids for patients who have previously experienced infusion reaction; use of steroid premedication may be permitted in these situations.
Grade 3 or 4	**For Grade 3 or 4** Permanently discontinue study drug/study regimen.	**For Grade 3 or 4** − Manage severe infusion-related reactions per institutional standard, appropriate clinical practice guidelines, and society guidelines.

Non-Immune-Mediated Reactions		
Severity Grade of the Event **(Refer to NCI CTCAE in Appendix 1 for defining the CTCAE grade/severity)**	**Dose Modifications**	**Toxicity Management**
Any Grade	Note: Dose modifications are not required for AEs not deemed to be related to study treatment (i.e., events due to underlying disease) or for laboratory abnormalities not deemed to be clinically significant.	Treat accordingly, as per institutional standard.
Grade 1	No dose modifications.	Treat accordingly, as per institutional standard.
Grade 2-3	Hold study drug/study regimen until resolution to ≤Grade 1 or baseline.	Treat accordingly, as per institutional standard.

Grade 4	Discontinue study drug/study regimen (Note: For Grade 4 labs, decision to discontinue should be based on accompanying clinical signs/symptoms, the Investigator's clinical judgment, and consultation with the GUSTO Chief Investigator or Trial Physicians).	Treat accordingly, as per institutional standard.

[1] SCAR terms include Stevens-Johnson Syndrome (SJS), Toxic Epidermal Necrolysis (TEN), Erythema Multiforme, Acute Generalized Exanthematous Pustulosis, Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) and Drug-induced hypersensitivity syndrome.

Appendix 5 ClassIntra v1.0 classification of intraoperative Adverse Events

ClassIntra® v1.0 classification of intraoperative adverse events (iAE). The classification defines iAE as any deviation from the ideal intraoperative course occurring between skin incision and skin closure. Any surgery- and anaesthesia-related event during the index-surgery must be considered and should be rated directly after surgery.[1] A prerequisite is that the indication for surgery and the interventions conform to current guidelines. (BMJ, 2020, author Salome Dell-Kuster et al.)

Grade	Definition	Examples
Grade 0	No deviation from the ideal intraoperative course	
Grade I	Any deviation from the ideal intraoperative course • Without the need for any additional treatment or intervention • Patient asymptomatic or mild symptoms	• **Bleeding**: Bleeding above average from small-calibre vessel: self-limiting or definitively manageable without additional treatment than routine coagulation • **Injury**: Minimal serosal intestinal lesion, not requiring any additional treatment • **Cautery**: Small burn of the skin, no treatment necessary • **Arrhythmia**: arrhythmia (e.g. extrasystoles) without relevance
Grade II	Any deviation from the ideal intraoperative course • With the need for any additional minor treatment or intervention • Patient with moderate symptoms, not life-threatening and not leading to permanent disability	• **Bleeding**: Bleeding from medium-calibre artery or vein, ligation: use of tranexamic acid • **Injury**: Non-transmural intestinal lesion requiring suture(s) • **Cautery**: Moderate burn requiring non-invasive wound care • **Arrhythmia**: arrhythmia requiring administration of antiarrhythmic drug, no hemodynamic effect
Grade III	Any deviation from the ideal intraoperative course • With the need for any additional moderate treatment or intervention • Patient with severe symptoms, potentially life-threatening and/or potentially leading to permanent disability	• **Bleeding**: Bleeding from large-calibre artery or vein with transient hemodynamic instability, ligation or suture: blood transfusion • **Injury**: Transmural intestinal lesion requiring segmental resection • **Cautery**: Severe burn requiring surgical debridement • **Arrhythmia**: arrhythmia requiring administration of antiarrhythmic drug, transient hemodynamic effect
Grade IV	Any deviation from the ideal intraoperative course	• **Bleeding**: Life-threatening bleeding with splenectomy; massive blood transfusion; ICU stay

	• With the need for any additional major and urgent treatment or intervention • Patient with life-threatening symptoms and/or leading to permanent disability	• **Injury**: Injury of central artery or vein requiring extended intestinal resection • **Cautery**: Life-threatening bum injury by cautery leading to fire requiring ICU treatment • **Arrhythmia**: arrhythmia requiring electroconversion, defibrillation or admission to ICU
Grade V	Any deviation from the ideal intraoperative course • With intraoperative death of the patient	

[1] The following events are not defined as intraoperative adverse events: sequelae, failures of cure, events related to the underlying disease, wrong-site or wrong-patient surgery or errors in indication