Windows trial of INsulin-like Growth factor neutralising antibody Xentuzumab in MEN scheduled for radical prostatectomy

Statistical Analysis Plan

Version 1.0_24Jun2024

Linked to SAP - Data definitions and Tables
Version 1.0_24Jun2024

Based on Protocol version 2.0_21Nov2022

Trial registration: ClinicalTrials.gov NCT05110495

Oxford Clinical Trials Research Unit (OCTRU)

CONTENTS

1 INTRODUCTION

This document details the proposed data presentation and analysis for the main paper(s) and final study reports from the WINGMEN Phase 0 Windows trial of INsulin-like Growth factor neutralising antibody Xentuzumab in MEN scheduled for radical prostatectomy. The results reported in these papers should follow the strategy set out here. Subsequent analyses of a more exploratory nature will not be bound by this strategy, though they are expected to follow the broad principles laid down here. The principles are not intended to curtail exploratory analysis (for example, to decide cut-points for categorisation of continuous variables), nor to prohibit accepted practices (for example, data transformation prior to analysis), but they are intended to establish the rules that will be followed, as closely as possible, when analysing and reporting the trial. This document follows published guidelines regarding the content of statistical analysis plans for clinical trial [1].

The analysis strategy will be available on request when the principal papers are submitted for publication in a journal. Suggestions for subsequent analyses by journal editors or referees, will be considered carefully, and carried out as far as possible in line with the principles of this analysis strategy. If reported, the analyses will be marked as post-hoc; the source of the suggestion will be acknowledged, and the reader will be advised to rely on the pre-specified analysis for the interpretation of the results.

Any deviations from the statistical analysis plan will be described and justified in the final report of the trial. The analysis should be carried out by an identified, appropriately qualified and experienced statistician, who should ensure the integrity of the data during their processing. Examples of such procedures include quality control and evaluation procedures.

Integral to this Statistical Analysis Plan (SAP) is the SAP – Data Definitions and Tables document which will include full detailed descriptions of all key outcomes, including their definition, generation and how they will be reported at the end of the study. These two documents should be read in tandem.

1.1 Key personnel

List of key people involved in the drafting and reviewing this SAP, together with their role in the trial and their contact details.

Author(s) (Trial statisticians)

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1.2 Changes from previous version of SAP

A summary of key changes from earlier versions of SAP, with particular relevance to protocol changes that have an impact on the design, definition, sample size, data quality/collection and analysis of the outcomes will be provided. Include protocol version number and date.

Version number Issue date	Author of this issue	Protocol Version & Issue date	Significant changes from previous version, with reasons
V1.0_24Jun2024	See section 1.1	Protocol_2.0_21Nov2022	Not applicable as this is the 1st issue
			Add to or delete as required

2 BACKGROUND AND OBJECTIVES

WINGMEN is a windows trial of IGF blockade pre-prostatectomy. The aim is to identify key IGF-regulated genes/proteins that mediate effects of high serum IGF-1 in driving prostate cancer risk and progression.

The estimand for the primary objective (including the analysis of the primary outcome) is described in Table 1. This trial is unusual in that it lacks a control arm. Patients serve as their own control through collection of samples before xentuzumab treatment. This affects the way intercurrent events are handled.

Table 1: Estimand-to-analysis table template

Primary Objective: Assess the amount of IGF pathway inhibition induced by xentuzumab	
Estimand: The amount of IGF-1 blockade induced by xentuzumab measured through automated immunohistochemistry (IHC) analysis of IGF-1 receptor activation (phospho-IGF-1R) and downstream activation marker phospho-S6.	
Treatment: Xentuzumab infusion (1000mg), once weekly for a minimum of 4 weeks	
Estimand	**Analysis**
Target population Men ≥ 18 years old with prostate cancer who are scheduled for radical prostatectomy.	**Analysis set** All participants who meet trial eligibility criteria and initiate treatment.
Variable H-Score or % of positively stained cells for phospho-IGF-1R and phospho-S6 as measured by IHC.	**Outcome measure** Statistical significance of the difference between IGF1 axis activity in PRE treatment and POST treatment samples. All participants who have sufficient material in the diagnostic (PRE-treatment) and in theatre (POST-treatment) biopsy cores for IHC analysis will be included, provided intercurrent events have not affected their ability to meet trial eligibility criteria.
Handling of intercurrent events Intercurrent events which prevent the patient from having in theatre (POST-treatment) biopsy cores available for IHC analysis after at least 4 doses of xentuzumab will preclude the patient from analysis of the primary endpoint. This does not fall under the	**Handling of missing data** Missing data will not be imputed. Patients whose samples are not sufficient to provide a score at baseline and after treatment will be excluded from the primary endpoint analysis. For other endpoints/analyses, Chief Investigator Sensitivity analysis: NA, data will not be imputed.

typical handling of intercurrent events due to the single arm design of this trial. Intercurrent events that do not prevent the patient from having in theatre (POST-treatment) biopsy cores available for IHC analysis, and would not affect the patient's trial eligibility, will be assessed by the Chief Investigator.	
Population-level summary measure	**Analysis approach**
Statistical significance of the difference between IGF1 axis activity in PRE and POST treatment samples.	PRE/POST HALO scores will be analysed using a paired t-test, or, if the paired differences do not follow a normal distribution, the Wilcoxon matched pairs signed rank test. Sensitivity analyses: Data will be tested for normal distribution and the appropriate test chosen accordingly (i.e. there is no assumption of normality). The appropriate sample size for 80% power was calculated as in Section 1.4.

[1]Strategies defined in E9 (R1) include treatment policy, while on treatment, principal stratum and hypothetical

3 STUDY METHODS

3.1 Trial Design/framework

This is a phase 0 'window of opportunity' study testing whether IGF neutralising antibody xentuzumab blocks IGF signalling and markers of aggressive tumour growth in men with localised prostate cancer scheduled for radical prostatectomy. Three factors influenced the trial design, and in particular the absence of a control arm. Firstly, trial design was informed by views of patients scheduled for prostatectomy. Men showing interest in the trial expressed the view that they would be less likely to participate in a trial randomising between treatment and control arms, because if they did take part, given the inconvenience of assessments and additional blood tests, they would want to receive the treatment. Other factors included the increased cost to Prostate Cancer UK of adding a control arm, doubling the trial size and cost, and the fact that the strength of a pre-operative windows study is the intra-patient pre/post treatment comparison.

3.2 Sample Size

Details of the sample size for primary outcomes and any co-primary or key secondary outcomes (if applicable), including treatment effect, power, levels of statistical significance (one-tailed or two-tailed), clinical relevance and justification.

Trial size calculations were initially based on analysis of phospho-AKT immunohistochemistry in tumours of mice following 3 weeks treatment with xentuzumab (Figure 6G in [2]). Compared with control treatment, the reduction in phospho-AKT signal induced by xentuzumab indicates an effect size of 2.35. In anticipation of a reduced effect in a heterogeneous clinical population, calculations are based on a more conservative estimate of an effect size of 0.6. Recruitment of 24 patients, giving 24 matched pairs of tissue (diagnostic biopsy and in-theatre biopsy), will enable reduction in phospho-AKT (or downstream target) (measured by H-Score or

percentage of positively stained cells) to be significant at the 5% level with 80% power. We will recruit 30 patients in case some prostate biopsies are inadequate or unsuitable for translational studies.

These calculations were checked against preliminary clinical data suggesting association of high serum IGF-1 with increased IGF-1R content of malignant but not benign prostatic epithelium in a radical prostatectomy cohort of 139 patients with T1-T3a prostate cancer (Table 2). Supporting the proposed sample size calculated above, recruitment of 25 patients will enable a 2-point reduction in IGF-1R (measured by H-Score or percentage of positively stained cells) to be significant at the 5% level with 80% power (with within-person standard deviation of 3.5). Simulations from clinical data indicate that weekly dosing of 1000mg xentuzumab reduces mean free IGF-1 by >90% and mean free IGF-2 by 64% relative to pre-treatment. This suggests that the proposed intervention will have a biological effect at least as great as the difference in circulating IGF-1 levels in the radical prostatectomy cohort.

Sample size calculations were repeated in October 2022 based on newly acquired preclinical data quantifying the amount of IGF axis pathway inhibition achieved by xentuzumab. This equates to the WINGMEN trial primary endpoint: the amount of IGF axis pathway inhibition. In a preclinical prostate cancer model, xentuzumab suppressed IGF-1R phosphorylation (pIGF-1R) with an effect size of 0.68 (G*Power v3.1). Using Wilcoxon signed rank test (matched pairs, 2-tailed), recruitment of 20 patients (20 matched pre/post-treatment pairs) will be significant at the 5% level with 80% power. Using a one-tailed test, based on the hypothesis that xentuzumab will reduce pIGF-1R, we will need 16 patients (16 matched pairs of tissue). Therefore, we aimed to recruit 20-30 patients in case some prostate biopsies are inadequate or unsuitable for translational studies.

	n	Median serum IGF-1 nmol/l		n	Median serum IGF-1 nmol/l	p value
Benign epithelium						
IGF-1R score ≤ 10	77	16.54	IGF-1R score > 10	60	16.68	p=0.871
Malignant epithelium						
IGF-1R score ≤ 14	79	15.58	IGF-1R score > 14	60	17.07	p=0.038

Table 2. Serum IGF-1 associates with IGF-1R immunoreactive score in malignant prostate epithelium. Immunoreactive scores (calculated as described in [3] were divided by the median values. IGF-1R scores were generally higher in malignant epithelium, consistent with our earlier report of IGF-1R overexpression in prostate cancer [4], hence cut point of 14 in malignant epithelium vs 10 in benign epithelium. NB Two samples had no benign epithelium in the scored sections (Aleksic, Verrill, Macaulay, unpublished).

3.3 Statistical Interim Analysis, Data Review and Stopping guidelines

State if there is a Data and Safety Monitoring Committee referencing the trial DSMC Charter (OCTRU GEN-011) for full details, but include the planned timing of meetings and of any formal comparative interim analysis. The latter should be outlined in the protocol and should be included here either in full, or summarised here with cross-reference to the full details in a separate document such as the DSMC_Report_Template (OCTRU Template OST-005, OCTRU SOP STATS-004 Interim Analysis).

If no DSMC is reviewing accruing data, state this together with justification.

The roles of the Trial Steering Committee and DSMC will be fulfilled by the Independent Early Phase Trial Oversight Committee (IEPTOC). The IEPTOC (at least 3 members including an independent chair) will provide overall supervision of the safe and effective conduct of the study according to its terms of reference. At least annually it will review study progress against agreed milestones, adherence to protocol, patient safety and consider new information. The IEPTOC has the authority to recommend study closure where appropriate.

3.4 Timing of Final Analysis

Provide details of the final analysis time-points – clarifying if the outcomes will all be assessed at the same time or are there different time-points for analysis, for example short term and long-term outcomes, or dose escalation and dose expansion phase.

To assess feasibility of tissue processing, two trial cases identified by Professor Clare Verrill, Consultant Prostate Pathologist, will be selected to check the tissue analysis pipeline. Sections of formalin-fixed paraffin-embedded (FFPE) diagnostic (PRE) and in theatre (POST) biopsy cores will be cut for primary endpoint immunohistochemistry (IHC) (2 sections), multiplex immunofluorescence (mIF, 1 section), RNA extraction (3 sections) and up to 4 additional sections depending on tumour content of diagnostic biopsies, which must not be cut to exhaustion.

Outcomes will be assessed at different time points as follows:

3.4.1 Primary endpoint

The primary endpoint, the amount of IGF axis pathway inhibition, will be assessed by batch analysis using automated IHC for phospho-IGF-1R and downstream marker phospho-S6. Multiple rounds of validation have confirmed that the selected antibodies are stable, providing reproducible signal in multiple prostate samples and analytical runs. Analysis of freshly cut paired sections of diagnostic biopsy and in-theatre prostate biopsy cores from a non-trial subject confirmed that the selected phospho-epitopes are stable between these two time-points. The paired diagnostic (PRE-treatment) and in theatre (POST-treatment) biopsy cores of the two trial patients identified above will be analysed in the same staining run, alongside a negative IHC control. If the results are satisfactory, sections will be cut from the remaining PRE/POST biopsy core pairs and freshly stained for phospho-IGF-1R and phospho-S6. Again, each pair of tissues will be analysed in the same staining run, alongside a negative control.

3.4.2 Secondary endpoints

Feasibility of recruitment and safety data will be accrued in real time, the latter during trial treatment and up to and including the End of Study visit. These data will be analysed after data lock.

3.4.3 Research/tertiary endpoints

There are several tertiary and exploratory endpoints for which data will be collected in this trial.

- IGF-1, IGFBP3, insulin and PSA levels will be collected on a per patient basis in real time and analysed after data lock.
- Serum IGF bioactivity will be batch analysed by phospho-IGF-1R ELISA after data lock, ensuring pairs of PRE/POST serum samples from individual subjects are analysed in the same batch.
- For metabolomics profiling, samples of sodium heparin plasma will be molecular weight filtered then transferred on ice to the McCullagh laboratory in a single batch on completion of recruitment. Pairs of PRE/POST plasma will be analysed by mass spectrometry in the same analytical run.
- PBMC immunophenotyping will be done by mass cytometry (CyTOF) using 36 markers of diverse immune cell classes and their functional states. We will use non-trial samples to test the viability of cryopreserved cells post-thawing and to determine the number PBMC cells required for acquisition to identify low frequency but functionally important immune subsets. Pairs of PRE/POST PBMCs from individual subjects will be barcoded with a CD45 based approach, allowing all samples to be batch analysed in a single run. This significantly reduced batch effects).

- ctDNA: currently there are no funds to sequence these samples. Opportunities will be explored to perform this analysis in collaboration, for example using a next generation sequencing assay that identifies prostate-cancer relevant genomic alterations as in [5].
- Exploratory IHC markers (see WINGMEN_DataDefinitions&Tables Section 5.2.1) will be batch analysed as for primary endpoint markers (Section 3.4.1 above) after completion of primary endpoint IHC.
- RNA will be extracted from unstained FFPE sections of diagnostic biopsy and in-theatre prostate biopsy cores, initially using freshly cut sections of the two first trial patients identified in Section 3.4, for transcriptional profiling. RNA will be stored in a designated -80°C freezer. If RNA yield and quality are sufficient for sequencing, RNA will be extracted from FFPE sections of the remaining cases and stored at -80°C as above. If RNA yield is insufficient, we will re-review each biopsy with Prof Clare Verrill to decide whether we can cut additional sections from some/all of the cases. RNA will be sent on dry ice to Azenta for quality control and sequening as described in WINGMEN_DataDefinitions&Tables Section 5.2.2. Final analysis will be performed when RNA-seq data have been accrued from all evaluable trial subjects (i.e. those with sufficient tumour in the diagnostic biopsy to allow within patient comparison with RNA extracted from prostate cancer cores post-xentuzumab).
- Mutation panel screening and TPMRSS2-ERG FISH will be performed on sections of radical prostatectomies as single batches after the samples from the last patient have been collected.
- Phospho-proteomic profiling: fresh frozen punch biopsies taken from unfixed prostatectomies are stored in the Oxford Radcliffe Biobank. Given that we have no matched pre-treatment fresh frozen biopsies, we plan to hold analysis of these samples until the other endpoint analyses have been completed. If sufficient funds remain, and if we can access fresh biopsies from Gleason-matched prostatectomies in non-trial patients, we will analyse tumour lysates by Reverse Phase Protein Array (e.g. Institute of Genetics and Cancer in Edinburgh: www.ed.ac.uk/cancer-centre/facilities/htpu-microarray-services/reverse-phase-protein-arrays). The analysis will be performed in a single batch after samples from the last patient have been collected.

3.4.4 Final integration of translational research data

Final data integration will be performed when all results have been obtained from the primary, secondary and research/tertiary endpoint markers, as described in WINGMEN_DataDefinitions&Tables Section 5.3. Set-up of a private cBioPortal will allow us to study associations and correlations between different data types. Exploratory analyses will be conducted using the cBioPortal and followed up using independent analyses where relevant. Recruitment was completed by Q1 2023 and the study is funded until April 2025 to allow time for analysis of these datasets.

3.5 Blinded analysis

There will be no blinding for treatment: all trial subjects will be treated with xentuzumab. Research staff of the Translational Histopathology Lab will be blinded. Macaulay lab members will not be blinded as to whether the samples are PRE or POST xentuzumab.

3.6 Statistical Analysis Outline

Statistical analysis will follow the principles and consensus criteria set out in [1], including the minimum set of items that should be addressed and excluding items not relevant to this trial design (e.g. procedures for performing randomisation). The statistical tests that will be used are described in detail in Section 4.

4 STATISTICAL PRINCIPLES

4.1 Statistical Significance and Multiple Testing

If the analysis is under a conventional (statistical hypothesis testing approach), state the level of statistical significance and the confidence intervals to be reported. Acknowledgement of the issue of multiple testing (if applicable), together with the rationale of the intention to formally or informally adjust for multiplicity. If no adjustment planned this will be stated. State which outcomes will be adjusted for multiplicity (if applicable).

Initial analysis will assess variables for normal distribution using the Shapiro-Wilk test. All tests will be 2-tailed and $p \leq 0.05$ will be accepted as statistically significant.

4.1.1 Primary endpoint analysis

PRE/POST FFPE tissue biopsy sections stained for phospho-IGF-1R and phospho-S6 will be scanned and analysed on the HALO platform. This is a deep-learning convolutional neural network that generates H-Scores, representing the sum of the % tissues staining 0 (negative), 1+, 2+ and 3+ to generate a score range of 0-300. If the staining intensity is not sufficient to define a scoring range for H-Scores, percentage of positively stained cells will be used as output instead. To assess the significance of differences before and after xentuzumab, the PRE/POST HALO scores will be analysed using a paired t-test, or, if the paired differences do not follow a normal distribution, the Wilcoxon matched pairs signed rank test. Phospho-IGF-1R staining patterns will also be assessed by Prof Clare Verrill, to semi-quantitatively score signal in the plasma membrane, cytoplasm and nucleus. Existing data from our group suggest that nuclear IGF-1R associates with higher stage prostate cancer [3] .

4.1.2 Secondary endpoints

The feasibility of treatment in a pre-operative setting will be assessed as the number (percent) of patients whose radical prostatectomy was performed on schedule after 4 doses of xentuzumab. Any reasons for delay post-4 cycles will be tabulated.

The safety and tolerability of xentuzumab administered in the pre-prostatectomy setting will be examined through treatment-related adverse events scored using NCI-CTCAE v5.0. These results will be tabulated.

4.1.3 Research/translational endpoints producing single values

Exploratory analyses will be conducted using various statistical tests as appropriate for the data type. These include, but are not limited to, the following.

For endpoints which produce a single value PRE/POST xentuzumab (e.g. circulating biomarkers such as insulin, PSA, PMBC immunotypes; IGF bioactivity; exploratory tissue biomarkers) paired t-tests or Wilcoxon rank sum tests will be used to assess the significance of differences pre and post treatment. Which test will be used will be determined by the results of the Shapiro-Wilk test for normal distribution. For unpaired data, either unpaired t-tests or the Mann-Whitney U test will be used, again depending on results of the Shapiro-Wilk test for normal distribution. These results will be presented and analysed as for primary endpoint markers and as shown in WINGMEN_DataDefinitions&Tables Section 5. Results will be displayed as either the mean (and standard deviation), or median (with lower and upper quartiles).

Relationships between many of these variables may be assessed for correlation. The Pearson correlation coefficient r will be used where two sets of raw data show a linear relationship with one another. If the

relationship is monotonic (i.e. non-linear) the Spearman's rank test (correlation coefficient ρ) will be used to analyse correlation between ranked data.

We had planned to assess changes in on-study serum IGF-1, previously reported to rise in patients on xentuzumab reflecting hypothalamic-pituitary axis response to blockade of pituitary IGF-1R [6]. However, on-treatment serum IGF-1 values fell in WINGMEN trial subjects, and we found this was due to interference by xentuzumab in the IGF-1R assay used in the Biochemistry laboratory of the John Radcliffe Hospital.

Other research endpoints (e.g. RNA-seq, metabolomics) will generate larger datasets and will be handled using the methods described below.

4.1.4 Metabolomics

Metabolomics profiles will be analysed in the laboratory of Prof James McCullagh (Department of Chemistry, Oxford University) using statistical methods described in [6] and presented as in WINGMEN_DataDefinitions&Tables Figure 4. In brief, univariate statistical analysis will be used to identify significantly deregulated metabolites (defined by ≥2 fold-change and false discovery rate (FDR) adjusted p-value <0.05). Unsupervised multivariate statistical analysis will include principal component analysis (PCA) to visualise global metabolic profiles and identify outliers. Supervised multivariate statistical analysis will be used to model important compound features that differentiate PRE and POST xentuzumab samples, and pathway analysis will use MetaboAnalyst, based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolite library specific to *Homo sapiens*.

4.1.5 RNA-seq

RNA-seq data will be analysed by Dr Fiona Hartley and Dr Alessandro Barberis, Macaulay lab bioinformaticians. Analyses will use R Statistical Software and the RStudio integrated development environment. A matrix of gene counts containing all samples will be generated by the process(es) described in WINGMEN_DataDefinitions&Tables Section 5.2.2. Genes with low counts will be filtered using the filterByExpression function in the edgeR R package [8]. Differential expression analysis will be completed using widely used software packages such as DESeq2, edgeR, and limma [9, 8, 10]. All packages correct for the effect of library size. DESeq2 models genes using a negative binomial generalised linear model and tests the null hypothesis that there is no differential expression across the two sample groups using a Wald test. edgeR also tests for differential expression by fitting a negative binomial model and uses the exact test to identify differentially expressed genes. Limma fits a gene-wise linear model and uses moderated t--statistics to perform hypothesis testing. The p-values are corrected for multiple testing using the Benjamini-Hochberg (BH) method to produce an adjusted p-value. Results will be considered significant at adjusted p-value ≤0.05, meaning that the proportion of false positives expected amongst the differentially expressed genes is 5%. If the signal from differential expression analysis is not strong, an adjusted p-value ≤0.1 may be used. An unfiltered list of ranked genes will be used for gene set enrichment analysis (GSEA) to identify pathways that are activated or suppressed by xentuzumab [9]. The primary database that will be used for GSEA is the Gene Ontology (GO) terms for Biological Processes (BP) [10] and the analysis will be run using the clusterProfiler R package with BH multiple test correction. Depending on results of other analyses (e.g. PBMC immunoprofiling), we may manually select relevant pathways (e.g. immune pathways) from Gene Ontology Biological Processes [10], KEGG [11, 12], BioCarta [13] , WikiPathways [14], Reactome [15], and Hallmarks MSigDB [16]. Results of GSEA analysis will be considered significant at an adjusted p-value ≤0.05 or, if the expression signal is low, ≤0.1.

4.1.6 RPPA data

Normally distributed unpaired RPPA data (WINGMEN_DataDefinitions&Tables Section 5.2.4 and Figure 9) will be analysed for the most significantly deregulated proteins using the methods described in Section 4.1.3.

4.2 Definition of Analysis Populations

See WINGMEN_DataDefinitions&Tables Section 3.

5 TRIAL POPULATION AND DESCRIPTIVE ANALYSES

Summary of flow of trial participants through the trial and baseline stratification, demographic and clinical characteristics of each group.

5.1 Representativeness of Study Sample and Patient Throughput

5.1.1 Representativeness of study sample

Subjects recruited to this trial will be fit (performance status 0-1) and thus representative of men scheduled for radical prostatectomy. For inclusion, the patient must have sufficient tumour in the diagnostic biopsy to provide at least 2 tissue sections for primary endpoint assessment. This criterion will be fulfilled for the majority of men whose prostate cancer is suitable for treatment by radical surgery. Exclusion criteria include:

- Treatment with systemic corticosteroids, insulin, metformin, other oral hypoglycaemic agent, or anti-androgens in the 28 days prior to first dose of study drug
- Diabetes mellitus
- Previous prostate radiotherapy
- Current or previous treatment with xentuzumab or other IGF or GH -modifying therapy
- Patients who are known to be serologically positive for Hepatitis B, Hepatitis C or HIV
- Treatment with any other investigational agent, or treatment in another interventional clinical trial within 28 days prior to enrolment
- Other psychological, social or medical condition, physical examination finding, or laboratory abnormality that the Investigator considers would make the patient a poor trial candidate or could interfere with protocol compliance or the interpretation of trial results.

5.1.2 Patient Throughput

See WINGMEN_DataDefinitions&Tables Section 4.2, Table 5, and Appendix 1: CONSORT Flow Diagram. The CONSORT flowchart will provide an overview, but additional expansion of reasons and timing of withdrawals from the study may be required.

5.2 Withdrawal from Treatment and/or follow-up

This information will be included in WINGMEN_DataDefinitions&Tables Section 4.2, Table 5, and Appendix 1: CONSORT Flow Diagram. No statistical tests will be used here. If patients withdraw early, their research blood and tissue samples will still be used for analysis if the patient gives consent.

5.3 Baseline Characteristics

Trial subjects' baseline characteristics will be tabulated as in WINGMEN_DataDefinitions&Tables Section 4.1 and Table 4. There will be no stratification by treatment group. Numbers (with percentages) for binary and categorical variables and mean (and standard deviation), or median (with lower and upper quartiles) for continuous variables will be presented. There will be no tests of statistical significance nor confidence intervals for differences between randomised groups on any baseline variable.

5.4 Unblinding

Not applicable: all trial subjects will receive the investigational medicinal product (IMP).

5.5 Treatment Compliance with Details of Intervention

The intervention being assessed in this trial is the IMP xentuzumab (Boehringer Ingelheim), an insulin-like growth factor (IGF) neutralising antibody. The drug is administered by intravenous infusion over one hour, once weekly for 4 weeks prior to standard of care radical prostatectomy. Trial subjects whose surgery is delayed will continue to receive weekly xentuzumab for up to 10 cycles in total. The final dose must be given within 6 days of surgery.

Compliance and adherence to the protocol will be assessed by:

- Ability to attend for trial treatment on a weekly basis ± 1 day of scheduled treatment visits. Incidence of delayed cycles and reasons for delay will be recorded as in WINGMEN_DataDefinitions&Tables Section 4.2 and Table 5.
- Ability and agreement to provide samples of blood and tissue for research.
- Attendance for a post-xentuzumab blood sample (1-3 days before surgery) at the same time of day and interval after last meal (± 2-3 hr) as the pre-xentuzumab blood sample. This is important for consistency of metabolomic profiling.

5.6 Reliability

Note: *This section should detail how the reliability of any variables, particularly outcomes, will be checked, for example those derived from raw data. Calculations performed by a computer program may be checked by hand calculations or confirmed by comparison with other measures. This also applies to calculations made to replace missing data.*

***Note**: In the case of different assessors recording data, specify how inter-rater reliability will be assessed and any steps taken in analysis to allow for variations.*

5.6.1 Primary endpoint markers

Staff of the Translational Histopathology Lab (THL) have assessed and optimised the primary endpoint markers phospho-IGF-1R and phospho-S6 by IHC using control cell samples generated in the Macaulay lab and tissue samples including tissue microarrays, prostatectomy sections, and prostate biopsies. The results are summarised in the THL Validation Report and confirm stable antibody performance over triplicate independent staining runs. Tests were also carried out to check stability of phospho-IGF-1R and phospho-S6 epitopes by comparing the intensity and distribution of endpoint marker signal in diagnostic biopsy and in-theatre core of a non-trial patient. An example is shown in WINGMEN_DataDefinitions&Tables Figure 1.

5.6.2 Secondary endpoints

Feasibility of treatment in the pre-operative setting, and safety and tolerability of xentuzumab administered in the pre-prostatectomy setting, will use standard clinical metrics including safety data assessed according to NCI-CTCAE v5.0.

5.6.3 Research/tertiary endpoints

Tertiary endpoints will be assessed in the Macaulay lab, Translational Histopathology Lab, the McCullagh Research Chemistry lab, and the Azenta Sequencing Facility using tested, standardised protocols.

5.6.3.1 Tissue endpoints

The reliability of research/tertiary IHC (e.g. Ki67, IGF-1R, RRM2, CD31) and TMPRSS2-ERG FISH will be checked by including positive and negative controls in each staining run. The signal will be quantified using HALO (WINGMEN_DataDefinitions&Tables Sections 4.3.1, 5.2.1 and 5.2.3) and reviewed with trial pathologist Prof Clare Verrill to check: staining of controls (e.g. cell pellets as shown in WINGMEN_DataDefinitions&Tables Figure 1A), technical quality of tissue staining, and whether pathological appearances are accurately represented by the HALO data.

5.6.3.2 Circulating endpoints

Paired PRE/POST serum samples will be tested for IGF bioactivity using a validated protocol obtained from Boehringer Ingelheim and used to assess the effect of xentuzumab in the Boehringer Ingelheim-sponsored Phase I trials [6]. Paired samples will be tested in the same assay run, and each assay will include a dose response of recombinant IGF-1, and the results in serum samples expressed as IGF-1 equivalents.

5.6.3.3 PBMCs

Immunoprofiling of peripheral blood mononuclear cells (PBMCs) will be performed either by flow cytometry or CyTOF. The latter will use Standardised Operating Procedures developed and validated by David Aherne (Research Associate and CyTOF Facility manager, Kennedy Institute of Rheumatology).

5.6.3.4 RNA-seq data

RNA derived from the FFPE-extracted tissue sections will undergo a series of quality checks. First the RNA quantity and purity will be tested in the Macaulay lab. On receipt at Azenta, RNA quantity will be rechecked, and the RNA integrity number (RIN) measured by TapeStation (Agilent). Azenta Standard Operating Procedures (SOPs) undergo regular review and all instruments are regularly calibrated and maintained, with records recorded in calibration and maintenance logs. Reagent and materials (including lot numbers) are tracked, laboratory staff are trained to the level required for clinical research, and IT systems are regularly maintained and backed up. The data will be analysed and cross-checked by two bioinformaticians in the Macaulay group, and the analysis will include checks on the expression of specific 'indicator' genes known to be IGF-regulated.

5.6.3.5 Metabolomics

Metabolomic profiles will be assessed on paired PRE/POST sodium heparin plasma using methods described in [7].

6 ANALYSIS

Statistical methods to be used to compare groups for primary and secondary outcomes and methods for point and interval estimation. Include methods for additional analyses, such as adjusted analyses and subgroup analyses, together with which populations will be analysed.

***Note:** It may be appropriate to list the outcome definitions, analysis methods, missing data and sensitivity analyses separately for each primary and secondary outcome to be analysed.*

Section 4 above describes the methods that will be used to assess statistical significance of differences in endpoint marker signal between samples taken pre- and post- xentuzumab.

The primary endpoint will be assessed by HALO analysis of 2 immunohistochemical (IHC) markers: phospho-IGF-1R and phospho-S6 (see WINGMEN_DataDefinitions&Tables Figure 1 and Appendix 2). The scanned IHC images will be quantified using HALO by DPhil student Jinseon (Selena) Kim (Macaulay lab), and HALO data

will be reviewed with trial pathologist Prof Clare Verrill as described in Section 5.6 above. Depending on the results of this assessment, we may use mean percentage change in one or both markers to quantify the amount of IGF axis inhibition. This measure will be used in correlation analyses with research/tertiary endpoints in both the entire population and in pre-defined subgroups as described in WINGMEN_DataDefinitions&Tables Section 5.3 and 5.3.1.

6.1 Outcome Definitions

Provide a simple list of all primary and secondary outcomes, together with time-points at which they are collected, with full details of definitions, and how these outcome variables will be generated and reported as provided in SAP - Data Definitions and Tables which should be cross-referenced.

	Objectives	Endpoints
Primary endpoint	Assess the amount of IGF pathway inhibition induced by xentuzumab	Phospho-IGF-1R and phospho-S6 immunohistochemistry on tissue from biopsy and prostatectomy
Secondary endpoints	1. Feasibility of treatment in pre-operative setting 2. Assess safety and tolerability of xentuzumab administered in the pre-prostatectomy setting	1. Number of patients whose radical prostatectomy is performed within 7 days of the 4th dose of xentuzumab (patients can receive up to 10 doses of xentuzumab if surgery is delayed) 2. Treatment-related adverse events scored using NCI-CTCAE v5.0
Tertiary/exploratory endpoints	1. Assess change in PSA following administration of xentuzumab in the pre-prostatectomy setting 2. Assess changes in tissue markers following administration of xentuzumab in the pre-prostatectomy setting 3. Assess changes in circulating markers following administration of xentuzumab in the pre-prostatectomy setting 4. Correlate changes in IGF-1R expression, IGF axis activity with cancer profile which may include mutation analysis, gene expression and phosphoproteomic profile	1. Change in serum PSA 2. Changes by IHC on FFPE tissues in tissue markers (e.g. Ki67 index, IGF-1R, RRM2, CD31, immune markers: PD-L1, CD4, CD8, FoxP3) 3. Changes in circulating markers of endocrine response to IGF blockade (e.g. IGF-1, insulin, IGFBPs, circulating IGF bioactivity, plasma metabolomic profile) 4. Tumour profiling (e.g. gene mutation panel, IHC for PTEN, FISH for TMPRSS2-ERG, transcriptional and phospho-proteomic profile of index tumour)

Table 3. Definition of WINGMEN outcomes.

6.2 Analysis Methods

For each primary and secondary outcome, describe what analysis methods will be used and how treatment effects will be presented (e.g. tabular and graphically- Cross-reference to the SAP – Data definitions and Tables). Detail which analysis population will be analysed. Detail any adjustment for covariates. Describe any methods to be used to check statistical assumptions and detail alternative methods if the assumptions do not hold, e.g. normality, proportional hazards. Where multiple analyses are planned, clearly distinguish which analysis will be the principal analysis, on which the outcome of the trial will be assessed, and which will be

supporting analyses to aid interpretation. Details of sensitivity analyses and multiple imputation should be detailed either under each key outcome analysis where applicable or in separate sections (5.3 and 5.4)

The primary endpoint (i.e. the amount of IGF pathway inhibition induced by xentuzumab)s will be presented as in WINGMEN_DataDefinitions&Tables Section 4.3.1, Figure 1, and Table 6 and analysed as described in Section 4 above.

The trial will be assessed on a modified intention-to-treat basis. Patients who are not evaluable for the primary endpoint may be replaced at the discretion of the Trial Management Group; including:

- Patients who receive fewer than 3 doses of xentuzumab (e.g. due to early withdrawal).
- Patients who for any reason do not have their planned standard of care prostatectomy.
- Patients whose prostatectomy reveals predominantly neuroendocrine/small cell cancer.
- Patients from whom diagnostic biopsy or surgical specimens were not taken or were of insufficient quantity or quality for analysis.
- Patients who do not receive their planned standard of care prostatectomy within 6 days of their final dose of xentuzumab.

All participants who receive one or more doses of xentuzumab will be evaluable for safety analysis, and patients withdrawing early after one or more doses of xentuzumab, irrespective of their stage in the trial, will undergo evaluations on the day before surgery.

The secondary outcomes relate to safety and feasibility of treatment and will be presented as described in Section 4.1.2 and WINGMEN_DataDefinitions&Tables Section 4.3.2 and Tables 7 and 8. The data will be tabulated and statistical analyses will not be conducted on these results.

In a recent study testing the feasibility of RNA extraction and RNA-seq on FFPE diagnostic prostate biopsies, 19/20 cases were found to provide RNA of sufficient quantity and quality for 3'RNA-seq [7]. The plan to recruit 30 patients to this trial reflects recognition that not all cases may have sufficient tumour tissue in the diagnostic biopsy to provide sections for RNA extraction and research IHC markers. Reasons for unsuitability of research blood and tumour samples are described in the legend to Table 5 in WINGMEN_DataDefinitions&Tables.

The completeness of data required for primary and secondary outcomes is described, detailing methods to limit the possibility of missing data and methods for dealing with missing data when it occurs.

A description of methods utilised for dealing with missing, spurious (outliers) and unused data during statistical analysis including the assumption for and type of missing data (Missing Completely At Random, Missing At Random, Missing Not At random). If multiple imputation is to be used the methods should be specified, including the imputation method, variables used and number of imputations generated. If multiple Imputation is to be used if appropriate, please clarify what appropriate means, e.g. Multiple imputation will be used if >5% (or >10%) of data is missing, otherwise complete (or available) case analysis will be undertaken. Methods for handling withdrawals and protocol deviations will be documented. State if no missing data adjustment will be used. Where multiple imputation is utilised the methods used to test the validity of the different assumptions will be described, either here or under the sensitivity analyses section.

6.3 Sensitivity Analysis

Describe any analysis utilised to check the robustness of the results. This may include repeating the primary analysis for different patient populations. Any sensitivity analyses checking the robustness of the results against the missing data assumptions should be included here or in the missing data section.

Primary endpoint analysis of phospho-IGF-1R and phospho-AKT or S6 IHC will be performed in parallel with controls as in WINGMEN_DataDefinitions&Tables Figure 1 and will require 2x 4 µm FFPE sections. Prof Clare Verrill advises that it is usually possible to cut maximum ~24 4-5 µm sections from a prostate biopsy, so the majority of trial subjects will have additional material if there are technical issues with initial analysis. Method optimisation indicates that IHC is capable of detecting changes in phospho-S6 phosphorylation comparable to changes detected on western blot of parallel whole cell extracts (see in WINGMEN_DataDefinitions&Tables Figure 1)

Similarly, the planned blood sample collection will provide sufficient serum and sodium heparin plasma to repeat assays for IGF bioactivity and metabolomic profiling if there are technical issues with initial assays. Both assays on circulating biomarkers will use controls in the form of recombinant IGF-1 and metabolite controls respectively. The lower detection limit for phospho-IGF-1R ELISA is 2 nmol/l IGF-1 equivalents, with approximately linear detection up to 20 nmol/l (WINGMEN_DataDefinitions&Tables Figure 3).

6.4 Pre-specified Subgroup Analysis

Describe any pre-specified subgroups for analysis, together with the justification for their relevance and importance, which outcomes they will be analysed for (it may just be the primary outcome), and include methods of analysis (reporting is dealt with in the SAP – Data Definitions and Tables document). It is recommended that subgroup-treatment interaction methods are used with presentation using forest plots. Subgroups are usually pre-specified in the protocol, but additional subgroup analyses may be added to the SAP prior to the final data lock following the blinded analysis or publication of other trials/research. Clearly specify if this analysis will be descriptive or if statistical testing will be undertaken.

Subgroup analysis will be performed to assess correlation of specific phenotypes/genotypes with response to IGF axis blockade, as described in WINGMEN_DataDefinitions&Tables Section 5.3.1.

6.5 Supplementary/ Additional Analyses and Outcomes

Describe any supplementary/additional statistical analyses for primary or secondary outcomes required (if not already detailed elsewhere), e.g. complier-average causal effect analysis.

Describe any outcomes and analyses pre-specified as exploratory in the protocol. Include detailed definition and analysis methodology or if not being undertaken by the statistician clearly state this and signpost to separate documents where these are detailed together with who will perform these

There will be no supplementary analyses of primary or secondary endpoints. The exploratory research/tertiary endpoints will be subject to detailed analysis as described in Section 4 above and WINGMEN_DataDefinitions&Tables Section 5.

6.6 Harms

Provide sufficient detail on summarising safety data, e.g. information on severity, expectedness, and causality; details of how adverse events are coded or categorised; how adverse event data will be analysed, e.g. incident case analysis, intervention emergent analysis. Will adverse events/complications also be recorded, summarised and analysed – full details for reporting should be specified in the SAP – Data Definitions and Tables document.

Potential harms include potential for drug toxicity and delay to standard of care surgery. Both will be captured as secondary endpoints and will be defined as described in WINGMEN_DataDefinitions&Tables Section 2.2 and Table 2.

There is a risk of COVID-19 exposure and infection from trial-specific hospital attendances, but measures have been put in place to minimise this risk as far as possible. The WINGMEN recruitment site, Churchill Hospital, is

nominally COVID-19 free, and infected or potentially infected patients are treated at a geographically distinct site (John Radcliffe Hospital). The following procedures have also been put in place on site:

- All patients (in and outpatients) are screened upon entry to the Churchill site.
- Human traffic at the Churchill site is minimal, with face to face appointments avoided and staff working from home whenever possible.
- Face masks/social distancing/level 1 PPE for patient examinations and other protective measures are mandatory for staff at the Churchill site

Furthermore, this trial requires only 5 extra patients visits: screening and 4 treatment visits (unless surgery is unexpectedly delayed). The post-treatment End-of-Study trial assessment will be held on the same day as the routine 6-week post-operative assessment, and so will not involve an extra hospital visit. There is no evidence that treatment with an IGF inhibitory drug will cause patients to be immunosuppressed. Indeed, given experimental evidence that IGFs themselves can suppress the immune system [19, 20, 21], it is possible that treatment with xentuzumab could enhance immune responses. Therefore, we accept that extra hospital visits may confer additional COVID-19 risk, but the treatment itself should not increase this risk. There is no contraindication to having a COVID-19 vaccination before, during or after the study, and participants will be encouraged to do so if not already fully vaccinated.

6.7 Health Economics and Cost Effectiveness (where applicable)

NA - this not a planned objective of the trial.

7 VALIDATION OF THE PRIMARY ANALYSIS

To validate the primary outcome and key secondary outcomes a statistician not involved in the trial will independently repeat the analyses detailed in this SAP using different statistical software (if possible). The results will be compared and any unresolved discrepancies will be reported in the Statistical Report (See OCTRU SOP STATS-005 Statistical Report). Primary and secondary outcomes are defined and tabulated in Section 6.1.

8 SPECIFICATION OF STATISTICAL PACKAGES

All analysis will be carried out using appropriate validated statistical software such as GraphPad Prism, STATA, SAS, SPLUS or R. The relevant package and version number will be recorded in the Statistical Report. Specialised analyses of metabolomic and RNA-seq data will be performed as outlined in Section 4.1.4 and 4.1.5.

9 PUBLICATION

A statement of CTU involvement will be included in any publication of the SAP. For example: This study was conducted as part of the portfolio of trials in the registered UKCRC Oxford Clinical Trials Research Unit (OCTRU) at the University of Oxford. It followed their Standard Operating Procedures ensuring compliance with the principles of Good Clinical Practice and the Declaration of Helsinki and any applicable regulatory requirements.

The trial results will be reported in an appropriate journal once analysis is complete.

10 REFERENCES

Provide references for nonstandard statistical methods
Reference to Data Management Plan
Reference to Trial Master File and Statistical Trial Master File
Reference Standard Operating Procedures or documents to be adhered to

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APPENDIX: GLOSSARY OF ABBREVIATIONS

AKT	Protein kinase B
BH	Benjamini-Hochberg
BP	Biological processes
CI	Chief investigator
CONSORT	Consolidated Standards of Reporting Trails
ctDNA	Circulating tumour DNA
CyTOF	Cytometry by time of flight
DSMC	Data and Safety Monitoring Committee
ELISA	Enzyme linked immunosorbent assay
FDR	False discovery rate
FFPE	Formalin-fixed paraffin-embedded
FISH	Fluorescence in situ hybridisation
GO	Gene ontology
GSEA	Gene set enrichment analysis
IEPTOC	Independent Early Phase Trial Oversight Committee
IGF-1	Insulin-like growth factor 1
IGF-2	Insulin-like growth factor 2
IGF-1R	IGF-1 receptor
IGFBP3	IGF binding protein 3
IHC	Immunohistochemistry
IMP	Investigational medicinal product
KEGG	Kyoto Encyclopedia of Genes and Genomes
mIF	Multiplex immunofluorescence
NCI-CTCAE	National Cancer Institute - Common Terminology Criteria for Adverse Events
OCTRU	Oxford Clinical Trials Research Unit
PCA	Principal component analysis
pIGF-1R	Phosphorylated IGF-1R
PMBC	Peripheral blood mononuclear cells
POST	Post xentuzumab treatment timepoint
PRE	Pre xentuzumab treatment timepoint
PSA	Prostate-specific antigen

RIN	RNA integrity number
RNA	Ribonucleic acid
RPPA	Reverse-phase protein array
SAP	Statistical analysis plan
SOP	Standard operating procedure
THL	Translational Histopathology Lab
TSC	Trial Steering Committee
WINGMEN	Windows trial of INsulin-like Growth factor neutralising antibody Xentuzumab in MEN