PROVENT Statistical Analysis Plan

Study Protocol Title:

PROVENT: A randomised, double blind, placebo controlled feasibility trial to examine the clinical effectiveness of aspirin and/or Vitamin D3 to prevent disease progression in men on Active Surveillance for prostate cancer.

Version 2.0

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Author Name: Mr Kier Finnegan Digitally signed by Kier Finnegan Date: 2020.10.22 15:17:41 +01'00' Date: _____

Reviewer Name: Professor Jack Cuzick _____ Date: _____

Approver Name: Mr Greg Shaw _____ Date: _____

Distribution List

Name	Function	Contact
Mr. Greg Shaw	Chief Investigator	Queen Mary University of London
Professor Jack Cuzick	Trial Chairman	Queen Mary University of London
Mays Jawad	Representative of Sponsor (QMUL)	Queen Mary University of London
Mr. Paul Cathcart	Co-Investigator	Guy's Hospital Great Maze Pond
Professor Dan Berney	Collaborator	Queen Mary University of London
Dr. Adrian Martineau	Collaborator	Queen Mary University of London
Mrs. Caroline Moore	Collaborator	University College London Hospitals
Professor Tim Oliver	Collaborator	Queen Mary University of London
Mr Kier Finnegan	Trial Statistician	Queen Mary University of London

Introduction

The PROVENT study aims to test the feasibility of a randomised controlled trial to examine the clinical effectiveness of aspirin and/or Vitamin D3 in preventing disease progression in men on active surveillance for low- to medium-risk prostate cancer. The main objective of this randomised feasibility trial is to demonstrate the acceptability of recruitment rates and the potential numbers required for a definitive trial. The study has a 3x2 factorial design, comparing a placebo to aspirin administered at low dose (100mg) or high dose (300mg), and placebo to Vitamin D3 at 4000IU.

Sample size

Sample size calculations suggested that 800 men are needed for a future definitive randomised chemoprevention study of standard (300mg) or low dose (100mg) aspirin vs. placebo and/or Vitamin D3 vs. placebo in patients enrolled on an Active Surveillance programme for prostate cancer.

This feasibility trial aims to demonstrate the acceptability and viability of recruitment for this larger study. Data from this trial will be used to determine the number of centres required to enable the larger, 800 patient trial to fully recruit within 4 years. The sample size was chosen to examine acceptance rates and the time required to recruit 102 patients. We plan to recruit 102 patients over 18 months. If the true recruitment rate is 33.3%, approaching 300 individuals, and therefore recruiting ≥100, would achieve 89% power to show that the true recruitment rate is above 25% (using a two-sided binomial proportion test with a 0.05 significance level).

Randomisation procedure

The randomisation will be by permuted block randomisation in order to achieve approximate balance across the 6 treatment groups. This randomisation scheme consists of a sequence of blocks such that each block contains a pre-specified number of treatment assignments in random order, for example each treatment occurring twice in a block. The purpose of this is so that the randomisation scheme is balanced at the completion of each block. PROVENT has 3x2 treatment combinations (a block of 6). However, there are two sizes of placebo aspirin to match the active drug: small (100mg size) and large (300mg size) providing an added complication, as this effectively produces eight possible combinations. To maintain the correct balance of drug and placebo, the PROVENT feasibility study will use two blocks of 6 to make a 'Superblock' of 12. In one block of 6 the placebo aspirin will be first small and then large, and in the other block they will be first large and then small. This ensures the correct number and size of placebo aspirin are dispensed, and that all 8 treatment combinations are represented in each superblock. Consequently, twice as many aspirin, as placebo aspirin tablets, are required for the trial.

Primary Endpoint

The primary endpoint for this feasibility study is the rate of patient recruitment over a 12-month period which will be assessed through pre-screening logs and eligibility CRFs.

Secondary Endpoints

The secondary endpoints involve measures of disease progression from baseline to 12 months, assessed by serial multi-parametric magnetic resonance imaging (MRI) of the prostate, serum PSA measurement, and prostate biopsy.

Disease progression by MRI

Disease progression by MRI of the prostate will be defined as an increase in lesion volume of ≥33% or an upgrading of MRI stage of disease to ≥T3. Disease regression by MRI will be defined as a decrease in lesion volume of ≥33% or the absence of a lesion at 12 months where one was detected at baseline. In the case where no lesion was identified by MRI screening, disease progression will be defined as the development of a lesion of 0.2cc or greater and being assigned a PIRADS score of 4 or 5.

Biochemical disease progression

Biochemical disease progression (or regression) will be defined as a 50% increase (or decrease) in serum PSA at 12 months from baseline.

Histological disease progression

Change in Gleason score

Histological disease progression/regression will be defined as an increase/decrease in Gleason score or primary grade upon repeat prostate biopsy. Examples:

- Gleason score 6 to 7 (increase in overall score)
- Gleason score 8 to 7 (disease regression, decrease in overall score)
- Gleason 3+4 to 4+3 (both score 7, increase in primary grade)
- Gleason 4+3 to 3+4 (disease regression, decrease in primary grade)

Change in maximum cancer core length (MCCL)

Disease progression/regression will also be defined as a 50% increase/decrease in maximum cancer core length

Serum hormone levels

Levels of serum testosterone, Follicle Stimulating Hormone (FSH), and Luteinizing Hormone (LH) will be measured every 6 months. Analyses will focus on whether serum levels have an effect on disease progression, and whether the serum concentrations differ between treatment arms.

Cell Cycle Progression (CCP) Score

Biopsy samples taken pre-study and at 12 months will be used for CCP score analysis. Exploratory analyses will investigate how the change in CCP score differs between treatment arms and whether CCP score has an effect on disease progression.

Pathology review

A minimum 10% sample of all prostate biopsy tissue received will undergo a central pathology review by the collaborating study pathologist at Centre for Molecular Oncology, Barts Cancer Institute, to ensure consistency of reporting.

Serum vitamin D3 and calcium

Levels of serum vitamin D3 and serum calcium will be measured at baseline, 6, 12, and 18 months. Hypercalcaemia (corrected serum calcium > 2.65mmol/L) is a well-known symptom of chronic vitamin D3 overdose.

Study period and visits

The study will last for 18 months (plus ≥30 days for pharmacovigilance). Data will be collected at 3-monthly intervals. Secondary endpoint measures will be carried out at the following times:

- MRI taken at baseline and 12 months
- Prostate biopsy at baseline and 12 months for pathology review & CCP score analysis.
- Serum PSA measured at baseline and every 3 months until 18 months
- Serum testosterone, FSH, and LH at baseline and every 6 months until 18 months
- Serum vitamin D3 taken at baseline and every 6 months until 18 months
- Serum calcium at screening and 6, 12, and 18 months

Demographics and clinical variables

Summary statistics for the baseline and follow-up demographics and clinical variables will be presented, both overall and by treatment arm, focusing on:

- Age
- BMI
- Serum PSA
- Gleason score
- Cancer of the Prostate Risk Assessment (CAPRA) score

The CAPRA scoring system was developed by the University of California, San Francisco and takes into account age, PSA, Gleason score, clinical stage, and percentage of positive cancer cores.

Analysis of primary endpoint

Patient recruitment will be defined as the proportion of eligible patients recruited from the total number of eligible patients. This will be reported with 95% CI calculated using a binomial test for proportions.

Analysis of secondary endpoints

Overall disease progression

The proportion of patients whose disease has progressed will be reported, split by type of progression (MRI, serum PSA and prostate biopsy). The proportion of patients displaying no disease progression, progressing by any, and progressing by all types will also be reported. The main analysis will compare the proportion of patients that received active treatment and display disease progression to those that received placebo, stratified by the other treatment (3 levels for aspirin stratification and two for vitamin D3), presented for both aspirin (both doses combined) and vitamin D3. The proportion of patients whose disease has *regressed* will also be reported, following the same methods outlined above.

Additional analyses will compare low dose aspirin to placebo and high dose to placebo, again stratified by the other treatment (Vitamin D3). Further supplementary analyses will look at all 6 treatment groups and explore treatment interactions.

Disease progression by logistic regression

Five logistic regression models will be run to determine the effect of treatment on disease progression. For all models aspirin (0 = placebo, 1 = 100mg, 2 = 300mg) and vitamin D3 (0 = placebo, 1 = active) will be used as predictors. The main analysis will be unadjusted for covariates but secondary analyses will be adjusted for age, BMI, serum hormone levels (testosterone, FSH, LH), and CCP score. An interaction term between aspirin and vitamin D3 will be included in some secondary analyses. Also included will be a trend test p-value for the 3 aspirin treatments.

1. Change in tumour volume

An ordinal logistic regression model using proportional odds will be run to assess the progression or regression of disease as assessed by the change in tumour volume by MRI from baseline to 12 months. The dependent variable will be coded according to percentage change.

DV code	Tumour volume percentage change (%)	Description
0	$-100 \leq x \leq -33$	Greater than 33% decrease in tumour volume
1	$-33 < x \leq 33$	Absolute change less than 33% in tumour volume
2	$x > 33$	Greater than 33% increase in tumour volume

2. Change in serum PSA

An ordinal logistic regression model using proportional odds will be run to assess the progression or regression of disease as assessed by the change serum PSA. The dependent variable will be coded according to percentage change.

DV code	Serum PSA percentage change (%)	Description
0	$-100 \leq x \leq -50$	Greater than 50% decrease in serum PSA
1	$-50 < x \leq 50$	Absolute change less than 50% in serum PSA
2	$x > 50$	Greater than 50% increase in serum PSA

3. Change in Gleason score

An ordinal logistic regression model using proportional odds will be run to assess the progression or regression of disease as assessed by the change in Gleason score or primary grade. The dependent variable will be coded according to an increase or decrease: 0 = decrease in score/grade; 1 = no change; 2 = increase in score/grade

4. Change in maximum cancer core length

This model will be identical to model 2 (serum PSA) with maximum cancer core length as the dependent variable.

*5. Disease **progression** by any measure*

A final logistic regression model will be run to assess the effect of treatment on disease progression by any of the three measures (tumour volume, serum PSA, Gleason score). Disease progression will be coded as 1 if disease progression is observed for any of the three disease measures and 0 otherwise. A regression of disease – a decrease in disease measure – will be treated as "no progression".

Survival analysis

Five proportional hazards model will also be run to look at early drop-out, with and without the disease progression measures above (including by *any* measure). Kaplan-Meier curves will be used to illustrate time to drop-out for the different treatments.

Supplementary analyses

Change in disease progression measures

The effect of treatment on the non-dichotomised change in disease measures will be explored. The differences in tumour volume, serum PSA and maximum cancer core length from baseline to 12 months will each be treated as the dependent variable. Serum PSA will be transformed to log (1+PSA). Patients without a second MRI or biopsy will be excluded from specific analyses where change was based on that measure.

Linear regression will be used to assess the effect of each treatment on disease progression, both univariate (treatment Y/N), and adjusted for the other treatment. Further analyses will adjust for serum hormone levels (testosterone, FSH, LH), and include an interaction term between aspirin and vitamin D3.

A Wilcoxon rank sum test will be performed to look at treatment differences using the change from baseline for each disease measure. For aspirin, the 100mg and 300mg doses will be combined for comparison to the placebo and also compared separately. All tests will be stratified by the other treatment and un-stratified. A Wilcoxon test for trend (Cuzick test) will also be carried out to determine whether there is an increased change in disease measure with increased aspirin dose.

Further exploratory analyses to look at time to change may be undertaken using a mixed effects model applied to log (1+PSA). As before, aspirin and vitamin D3 will be included as predictors, with an interaction effect and covariates included. Covariates may include age, BMI, or hormone levels. The interaction term will be removed if not significant. A plot of log (1 + PSA) will be generated, containing the predicted trajectories by treatment arm.

Change in serum hormone levels and CCP score

The effect of treatment on testosterone, FSH, LH, and CCP will be investigated using an ANOVA model. The difference in serum hormone concentration from baseline to 18 months for each of the hormones, or the difference in CCP score from baseline to 12 months will act as the dependent variable. Treatment will be the independent variable, and a treatment interaction term may also be added. The hormone concentrations will be log-transformed, as with PSA.

Effect of serum hormone levels and CCP on disease progression

A logistic regression model will be run to determine whether final measurements of testosterone, FSH and LH, or final CCP score has an effect on overall disease progression (progression by *any* measure). It will be adjusted by the baseline measurements.

Pathology review

Consistency of pathology reporting will be assessed by the proportion of reviewed biopsy results in agreement with the original result. Any disagreements will be presented and will include the reasons such as Gleason pattern and/or score, as well as any possible changes to diagnoses.

Serum vitamin D3 and calcium

The median corrected serum calcium level will be reported for each treatment arm, as well as the proportion of samples >2.65mmol/L (hypercalcaemic). The relationship between serum vitamin D3 and PSA level at 12 months will be quantified using a linear regression model, with log(1+PSA) as the dependent variable. Baseline PSA and serum vitamin D3 measures will be included to adjust for differences between patients.

Treatment adherence

Adherence to the dosing schedule will be assessed at 6, 12, and 18 months by a count of remaining aspirin tablets or an estimate of remaining vitamin D3 oil. Each patient will also receive a diary where they may record the date of any missed doses. The proportion of used tablets (aspirin) and oil (vitamin D3) will be recorded, as will the proportion of daily doses taken.

Safety endpoints

Toxicity data will be presented as the proportion of patients displaying either mild, moderate, or severe symptoms split by treatment *group* (aspirin: placebo, 100mg, 300mg; vitamin D3: placebo, active). Serious adverse events data will be presented as the proportion of mild, moderate and severe events recorded by treatment group.