BMJ Open

Pharmacodynamics effects of CDK4/6 inhibitor LEE011 (ribociclib) in high-risk, localised prostate cancer: a study protocol for a randomised controlled phase II trial (LEEP study)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-033667
Article Type:	Protocol
Date Submitted by the Author:	16-Aug-2019
Complete List of Authors:	Scheinberg, Tahlia; Chris O'Brien Lifehouse; The University of Sydney Kench, James; The University of Sydney; Royal Prince Alfred Hospital Stockler, Martin; University of Sydney , NHMRC Clinical Trials Centre Mahon, Kate; Chris O'Brien Lifehouse; The University of Sydney Sebastian, Lucille; The University of Sydney Stricker, Phillip; Garvan Institute of Medical Research; University of New South Wales Joshua, Anthony; St Vincent's Hospital; University of New South Wales Woo, H; The University of Sydney; Chris O'Brien Lifehouse Thanigasalam, Ruban; Chris O'Brien Lifehouse; The University of Sydney Ahmadi, Nariman; Chris O'Brien Lifehouse Centenera, Margaret; South Australian Health and Medical Research Institute; The University of Adelaide Butler, Lisa; South Australian Health and Medical Research Institute; The University of Adelaide Horvath, Lisa; Chris O'Brien Lifehouse; The University of Sydney
Keywords:	prostatic neoplasms, neoadjuvant trial, window of opportunity trial, ribociclib, translational research

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

16 August 2019

Adrian Aldcroft
Editor in Chief
BMJ Open Editorial Office
BMA House
Tavistock Square
London, WC1H 9JRUK

Dear Adrian Aldcroft,

Please find attached a manuscript entitled: "**Pharmacodynamics effects of CDK4/6 inhibitor LEE011 (ribociclib) in high-risk, localised prostate cancer: a study protocol for a randomised controlled phase II trial (LEEP study)**", which we would like considered for publication in *BMJ Open* as a protocol manuscript. This paper has not been reviewed by any other journal.

In this paper we describe a randomised controlled phase II trial of ribociclib in high-risk, localised, hormone sensitive prostate cancer. This novel trial aims to characterise the pharmacodynamics effects of an oral CDK4/6 inhibitor in prostate cancer.

Prostate cancer is the most common cause of cancer in men. Despite recent advances in its treatment, it remains the second leading cause of cancer death, and novel agents are required. CDK4/6 inhibitors have proven efficacy in breast cancer, and appear promising in preclinical models in prostate cancer.

The time between diagnosis and surgery for localised prostate cancer offers an ideal opportunity to examine pharmacodynamics effects of novel agents. This allows for a rational approach to decisions regarding which agents should be taken into phase II/III trials. This is the first study to investigate the pharmacodynamic effects of a CDK4/6 inhibitor in high-risk localised prostate cancer. It will also provide a model for similar trials with alternate novel agents.

We believe this protocol manuscript of a novel trial design investigating a novel agent, is ideally suited for BMJ open. This material is original research, has not been published previously and has not been submitted for publication elsewhere while under consideration.

Thank you for your consideration. I look forward to receiving your comments.

Regards,

Dr Tahlia Scheinberg on behalf of the authors

Pharmacodynamics effects of CDK4/6 inhibitor LEE011 (ribociclib) in high-risk, localised prostate cancer: a study protocol for a randomised controlled phase II trial (LEEP study)

Scheinberg T[1,2,3], Kench J[2,3,4], Stockler M[1,2,3,4,5], Mahon KL[1,2,3,4], Sebastian L[2], Stricker P[3,6,7], Joshua AM[3,7,8], Woo H[1,2], Thanigasalam R[1,2,5], Ahmadi N[1], Centenera MM[9,10], Butler LM[9,10]*, Horvath LG[1,2,3,4,7]*.

1. **Chris O'Brien Lifehouse, Sydney, Australia.**
2. **University of Sydney, Sydney, Australia**
3. **Garvan Institute of Medical Research, Sydney, Australia**
4. **Royal Prince Alfred Hospital, Sydney, Australia**
5. **Concord Hospital, Sydney, Australia**
6. **St Vincent's Clinic, Sydney, Australia**
7. **University of NSW, Sydney, Australia**
8. **St Vincent's Hospital, Sydney, Australia**
9. **South Australian Health and Medical Research Institute, Adelaide, Australia**
10. **University of Adelaide, Adelaide, Australia**

***Authors contributed equally**

Authors:
Dr Tahlia Scheinberg
Professor James Kench
Professor Martin Stockler
Dr Kate L Mahon
Lucille Sebastian
Professor Phillip Stricker
Associate Professor Anthony M Joshua
Professor Henry Woo
Associate Professor Ruban Thanigasalam
Dr Nariman Ahmadi
Dr Margaret M Centenera
Professor Lisa M Butler
Professor Lisa G Horvath

Corresponding author:
Professor Lisa Horvath
Chris O'Brien Lifehouse
119 – 146 Missenden Road
Camperdown NSW 2050
Australia
+61 2 8514 0000
lisa.horvath@lh.org.au

Word count: 2965 words

Keywords: neoadjuvant trial, window of opportunity trial, prostatic neoplasms, ribociclib, translational research

ABSTRACT

Introduction Despite the development of new therapies for advanced prostate cancer, it remains the most common cause of cancer and the second leading cause of cancer death in men. It is critical to develop novel agents for the treatment of prostate cancer, particularly those that target aspects of androgen receptor (AR) signalling or prostate biology other than inhibition of androgen synthesis or AR binding. Neoadjuvant pharmacodynamic studies allow for a rational approach to the decisions regarding which targeted therapies should progress to phase II/III trials. CDK4/6 inhibitors have evidence of efficacy in breast cancer, and have been shown to have activity in pre-clinical models of hormone sensitive and castrate resistant prostate cancer. The LEEP trial aims to assess the pharmacodynamic effects of LEE011 (ribociclib), an orally bioavailable and highly selective CDK4/6 inhibitor, in men undergoing radical prostatectomy for high-risk, localised prostate cancer.

Methods and analysis The multicentre randomised, controlled 4:1 two arm, phase II, open label pharmacodynamic study will recruit 47 men with high risk, localised prostate cancer who are planned to undergo radical prostatectomy. Participants who are randomised to receive the study treatment will be treated with LEE011 400mg daily for 21 days for 1 cycle. The primary endpoint is the frequency of a 50% reduction in Ki-67 proliferation index from the pre-treatment prostate biopsy compared to that present in prostate cancer tissue from radical prostatectomy. Secondary and tertiary endpoints include pharmacodynamic assessment of CDK4/6 cell cycle progression via E2F levels, apoptotic cell death by cleaved caspase-3, changes in serum and tumour levels of PSA, pathological regression, safety via incidence of adverse events and exploratory biomarker analysis.

Ethics and dissemination The protocol was approved by a central ethics review committee for all participating sites. Results will be disseminated in peer-reviewed journals and at scientific conferences.

Drug Supply Novartis

Protocol Version 2.0, 30 May 2019

Trial Registration Number ACTRN12618000354280 (Australian New Zealand Clinical Trials Registry)

ARTICLE SUMMARY

Strengths and limitations of this study

- This is the first trial evaluating the pharmacodynamic effects of CDK4/6 inhibitors in hormone sensitive prostate cancer.
- This study will explore potential biomarkers for treatment response
- This trial is designed to examine anti-tumour pharmacodynamics effects of single agent ribociclib
- This trial is not designed to determine whether a short course of neoadjuvant treatment could alter oncological outcomes or recurrence rates. These will be the next steps if the trial is positive.
- By utilising paired samples, this neoadjuvant proof of concept trial allows us to use relatively small sample sizes, through examining dynamic changes in the biomarkers of interest

INTRODUCTION

Despite advances in the detection and treatment of prostate cancer, it remains the most common cause of cancer in men in the developed world and the second leading cause of cancer death[1]. Over the last decade, the treatment of advanced prostate cancer has changed dramatically with new therapies including novel anti-androgens[2 3], novel taxanes[4], radioisotope therapy[5] and more recently PARP inhibitors[6]. However these agents are not curative, and it is recognised that in order to improve survival from prostate cancer, it is critical to develop novel agents, particularly those that target aspects of androgen receptor (AR) signalling or prostate biology other than inhibition of androgen synthesis or AR binding[7].

CDK4/6 inhibitors for treatment of prostate cancer

One of the common driving pathways that is altered in prostate cancer, and selected for in CRPC, is aberrant cell cycle activation through the cyclin/CDK/retinoblastoma (Rb) axis, with resultant uncontrolled cellular proliferation. This axis is critically important in controlling the G1-S transition of the cell cycle. There is evidence that androgens can stimulate the increased expression of G1 cyclins and cyclin-dependent kinases and decrease the expression of CDK inhibitors. The AR may also directly contribute to the transcription of some cell cycle regulatory genes, including cyclin D3[8].

By binding to CDK4 and CDK6, selective CDK inhibitors inhibit Rb phosphorylation to prevent G1-S phase transition and induce cell cycle arrest. CDK4/6 inhibitors palbociclib (PD0332991; Pfizer), ribociclib (LEE011; Novartis) and abemaciclib (LY2835219; Eli Lilly) are oral and reversible small molecule inhibitors with high selectivity for CDK4 and CDK6, with evidence of efficacy in breast cancer[9-11].

In preclinical models of hormone-sensitive and castration-resistant prostate cancer, palbociclib has exhibited single agent activity, by limiting cellular proliferation and growth[12]. The potential therapeutic effect was determined in both *in vivo* mouse xenografts and a novel *ex vivo* assay using primary human tumours obtained from radical prostatectomy. This *ex vivo* model has also shown that LEE011 significantly inhibits prostate tumour cell proliferation in a dose dependent manner (unpublished, Butler LM, 2019). This preclinical data provides evidence that CDK4/6 inhibitors achieve clinically relevant biological responses in human prostate tumours, and supports the evaluation of CDK4/6 inhibitors for treatment of prostate cancer.

Towards more rapid assessment of new therapies

Clinical trials of new drugs in the hormone sensitive phase of prostate cancer (high-risk localised prostate cancer, or at relapse after localised treatment) require long follow-up due to the natural history of the disease. The interval between biopsy and surgery offers an ideal opportunity for *in vivo* assessment of anti-tumour activity and selection of optimal novel agents for further investigation. A recent systematic review identified that a lack of a biomarker-driven strategy and failure to achieve “proof of concept” in Phase 2 trials were significantly associated with failure of cancer drugs to achieve late-stage clinical success such as FDA approval[13]. Neoadjuvant pharmacodynamic studies, such as the one described in this project, will allow for a more rational approach to the decisions regarding which targeted therapies should go forward into phase II/III trials.

Biomarkers for treatment response

The identification of informative biomarkers in the pre-clinical phase, which can be incorporated into clinical studies, is pivotal to accelerating the drug development process,

and when incorporated into clinical decision-making, can maximise patient benefit and minimise harm, with judicious drug administration. Candidate biomarkers will be identified/assessed in this clinical trial and potentially validated in future trials with CDK4/6 inhibitors.

Absent or decreased staining of nuclear Rb proteins is commonly found in prostate cancer specimens, and it has been suggested that inactivation of the retinoblastoma gene may be an important event in prostate tumour progression[14]. In an *ex vivo* model, functional Rb is required for optimal CDK4/6 inhibitor efficacy[12]. There is evidence that cyclin D1 overexpression is implicated in tumourigenesis and tumour progression, and may be related to the evolution to castration resistance in prostate cancer[15]. The product of the INK4A gene inactivates the G1-phase cyclin dependent kinases CDK4 and CDK6. Overexpression of p16INK4A in high-grade prostatic epithelial neoplasia is associated with early relapse in prostate cancer patients treated with radical prostatectomy[16]. Given the role of Cyclin D1, Rb proteins and p16INK4A in cell cycle progression, there is interest in reviewing these as biomarkers of response to CDK4/6 inhibitors. Further, induction of cyclin D1 and p16INK4A have been identified as possible pharmacodynamic endpoints on pre-clinical models, and can be validated in a clinical setting[12 17].

HYPOTHESIS

Primary hypothesis

We hypothesise that administration of LEE011 (Ribociclib) to men prior to undergoing radical prostatectomy will lead to a 50% reduction in Ki-67 index in 30% or more of participants treated with LEE011, compared with a 50% reduction in Ki-67 index in 10% or fewer in the control group.

Secondary hypothesis

We hypothesise that treatment with LEE011 will be associated with inhibition of CDK4/6 cell cycle progression by a decrease in the level of E2F in prostate cancer tissue, and an increased level of cleaved caspase-3 in prostate tissue indicating increased apoptotic activity.

METHODS AND ANALYSIS

The LEEP study is an Australian-based, multicentre, randomised controlled, phase II, open label pharmacodynamic study. The primary aim is to determine the pharmacodynamic activity of the CDK4/6 inhibitor LEE011 in men with high-risk localised prostate cancer undergoing radical prostatectomy.

Study objectives

The primary objective of this study is to determine the effect of LEE011 on tumour cell proliferation, as determined by:

- The frequency of a 50% reduction in the Ki-67 proliferation index from the pre-treatment prostate biopsy compared to that present in prostate cancer tissue from radical prostatectomy.

The secondary objectives are to determine:

- The effect of LEE011 on CDK4/6 cell cycle progression, by measuring E2F expression in prostate tissue by immunohistochemistry and peripheral blood mononuclear cells by ELISA

- The effect of LEE011 on apoptotic cell death, by examining the frequency of a 50% increase in cleaved caspase-3 expression levels in tumour cells, measured by immunohistochemistry
- Changes in serum and tumour levels of PSA, by immunoassay
- Rates of pathological regression, assessed by histopathology, as defined by cancer cell atrophy, decreased nuclear size, increased chromatin density and pale cytoplasm.
- The incidence of adverse events (CTCAE v 4.03)

The tertiary objectives are to evaluate exploratory biomarkers as predictors of response to therapy. These include Rb status, cyclin D1 amplification, p16INK4a expression, PTEN loss, AR amplifications/mutations and aberrations of PI3K signalling pathways (assessed by reverse transcription-PCR and FISH analysis of cancer tissue from radical prostatectomy). These will also be tested in free plasma DNA, through a novel technique that has identified these aberrations in circulating tumour DNA [18]. Effects on immune system such as circulating T-cell profiling will also be assessed.

Trial oversight and monitoring

The LEEP study is a collaboration between the Chris O'Brien Lifehouse, Sydney; Royal Prince Alfred Hospital, Sydney; the University of Adelaide, Adelaide; St Vincent's Hospital, Sydney; and the National Health and Medical Research Council (NHMRC) Clinical Trials Centre (CTC), University of Sydney.

The University of Sydney is the study sponsor. The NHMRC CTC will be responsible for study co-ordination, monitoring, data acquisition, management and statistical analysis.

Safety and efficacy endpoints will be assessed when evaluable tissue is available from 22 participants treated with LEE011 and at study completion.

Protocol amendments can only be made by the trial management committee, and must be approved by the central institutional Human Research Ethics Committee (HREC) prior to implementation.

Patient and public involvement

This research was funded through a granting process that included a consumer representative from Cancer Voices NSW. The grant, study protocol and patient information sheet/consent form were all discussed, reviewed and edited by our consumer representative. A consumer representative is a member of the trial steering committee. Following completion, a plain-English version of the results will be made available to patients via their study doctor. Results of this study will be disseminated to study participants through peer-reviewed journals, at scientific conferences, and on the NHMRC CTC website.

Trial design

The protocol consists of a randomised, controlled 4:1 two arm, phase II, open label pharmacodynamic study (figure 1). The trial is currently being conducted at 2 tertiary referral centres (Chris O'Brien Lifehouse, Sydney and St Vincent's Health Network, Sydney) in New South Wales (NSW), Australia. There is a plan to open further sites during 2019.

Inclusion Criteria

Patients who fulfil all of the following characteristics will be considered eligible for enrolments:

- Males ≥ 18 years with localised prostate cancer and at least clinical stage T3a **Or** Gleason score of between 8 and 10 **Or** Preoperative PSA ≥ 20 ng/mL **AND** planned for radical prostatectomy;
- Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1;
- Histological confirmation of prostate cancer via a pre-treatment diagnostic transrectal ultrasound (TRUS) biopsy;
- Adequate bone marrow, hepatic and renal function;
- Serum calcium, potassium, phosphate and magnesium within normal range or corrected with supplements.

Exclusion criteria

Patients with the following characteristics will be excluded from study enrolment:

- Major surgery ≤ 2 weeks prior to enrolment or who have not recovered from side effects of such therapy. TRUS biopsy is not considered major surgery in this study;
- Known hypersensitivity to the study drug or its excipients;
- Patients with known disorders due to a deficiency in bilirubin glucuronidation (e.g. Gilbert's syndrome);
- Diarrhoea ≥ CTCAE grade 2;
- Impaired cardiac function, including any one of the following:
    - History (or family history) of long QT syndrome.
    - Those who already have, or who are at significant risk of developing QTc prolongation, including patients with:
        - Long QT syndrome,
        - Mean QTcF ≥ 450msec on baseline ECG,
        - Uncontrolled or significant cardiac disease including recent myocardial infarction,
        - Congestive heart failure, unstable angina or bradyarrhythmias,
        - Electrolyte abnormalities,
        - Clinically significant ECG abnormalities at clinician discretion
    - Other clinically significant heart disease (e.g. uncontrolled hypertension, history of labile hypertension, or history of poor compliance with an anti-hypertensive regimen);
    - Clinically significant resting bradycardia (< 50 beats per minute);
    - Patients who are currently receiving treatment with any medication that has a relative risk of prolonging QTcF interval or inducing Torsades de Pointes and cannot be discontinued or switched to an alternative treatment prior to commencing study treatment;
    - Obligate use of a cardiac pacemaker;
- Patients who have received prior antineoplastic therapy for advanced disease;
- Prior treatment with a CDK4/6 inhibitor;
- Patients who are currently receiving treatment with strong CYP3A4 inhibitors and cannot be discontinued or switched to an alternative treatment prior to commencing study treatment
- Patients receiving chronic or high-dose corticosteroid therapy;
- Significant infection, including chronic active hepatitis B, hepatitis C or HIV;
- Serious medical or psychiatric conditions that might limit the ability of the patient to comply with the protocol.

Investigational medical product – LEE011 (ribociclib)

This study will use LEE011, an orally bioavailable, highly selective, small-molecule inhibitor of CDK4/6 that blocks the phosphorylation of retinoblastoma protein, thereby preventing cell-cycle progression and inducing G1 phase arrest. Based on the results of preclinical toxicology studies and available clinical safety data, the main adverse reactions for LEE011 are bone marrow suppression including leukopenia, neutropenia, anaemia and thrombocytopaenia, dyspnoea, hepatic toxicity, renal toxicity, fatigue, nausea, vomiting, diarrhoea and prolongation of the QT interval. The risk of these toxicities may be amplified by concomitant administration of strong inhibitors of CYP3A4 or other combination treatments.

Randomisation

The method of randomisation with be minimisation with stratification by site. Participants will be allocated to the study treatment in a ratio of 4:1 (LEE011:control).

Recruitment and consent

Patient screening and enrolment will be overseen by the site principal investigator and performed by trained study personnel. Patients will provide written informed consent prior to study enrolment. Treatment will be planned to start within 7 days after randomisation.

Study procedures

The randomised, controlled, phase II, open label, pharmacodynamic study will assess the pharmacodynamic activity of the CDK4/6 inhibitor LEE011, in men with high-risk, localised prostate cancer.

Participants randomised to receive the study treatment will have a pre-treatment MUGA. Participants will receive LEE011 400mg daily taken orally for 21 days treatment for 1 cycle. The scheduled surgery will occur 22 days after the first dose of LEE011 (if randomised to study drug treatment) or 22 days after randomisation (if randomised to the control group). Dose modifications are not permitted in this study. Patients who need to come off the study due to toxicity will discontinue and proceed to surgery as planned.

Data acquisition

Tumour tissue samples will be collected at transrectal biopsy and at radical prostatectomy. Blood samples for biomarker analysis and PSA will be collected within 7 days prior to randomisation, weekly during treatment and at the time of radical prostatectomy.

Ki-67 expression will be assessed by pathologist review. Where possible, for all analyses, comparisons will be made between similar areas in the needle biopsy and radical prostatectomy specimens. Scoring for protein expression will be performed by two independent pathologist researchers, both blinded to the treatment groups and pairings of tissue from the same patient. Discrepancies will be resolved by consensus.

Cell cycle arrest will be measured by E2F expression as determined by immunohistochemistry and scored by manual counting. Apoptotic cell death will be determined by examining the cleaved caspase-3 staining in tumour cells by immunohistochemistry and scored by manual counting. PSA levels in tumour and blood will be assessed by immunoassay, with immunohistochemistry or ELISA.

Trial data will be monitored by clinical trials program staff from the NHMRC CTC.

STATISTICAL CONSIDERATIONS

Sample size estimation

Using the Simon's two-stage design, an uninteresting rate for the true response is 10% and a clinically interesting rate, which would warrant further investigation, is 30%. Based on this design, a sample size of 37 patients will have at least 90% power with 95% confidence to exclude the uninteresting rate in favour of the more clinically meaningful rate. A response is defined as ≥50% decrease in Ki-67 expression in the paired prostate biopsy baseline sample compared with the radical prostatectomy sample.

A futility analysis will be performed after 22 patients have completed 1 cycle of LEE011 and are evaluable for pharmacodynamic response. If there are 2 or fewer responses, consideration will be given to either reassessing the study design or stopping the study due to futility. When these 22 patients are assessed it is expected that there will be at least 6 patients in the control group, which will allow for assessment of the response to be performed in light of what is seen in the control arm. The study will then recruit an additional 15 patients in the treatment arm and 4 controls. This sample size allows for a modest number of drop-outs/loss to follow up. It is anticipated that at least 33 patients in the treated cohort will be evaluable for response at study completion.

10 untreated men will be enrolled in the control group to provide estimates of PD biomarkers as a basis for biological comparison, giving a total sample size of 47 patients.

It is expected that none of the control group men will have a ≥50% decrease in Ki-67. For the secondary objectives, the sample size of 10 control participants to 37 treated patients provides adequate power to detect large differences only.

Patients who do not receive study treatment, withdraw their consent or are not evaluable will be replaced.

Statistical analysis

Analysis of efficacy endpoints (i.e. response, biomarkers) will include only evaluable patients. Analysis of safety endpoints (i.e. toxicity) will be according to treatment received, including only patients who received at least 1 dose of the experimental treatment.

The response in each treatment arm will be summarised by the number and proportion of patients experiencing at least a 50% decrease in Ki-67 expression, with a two-tailed p-value significance level of 0.05. Ki-67 levels pre- and post-treatment will also be summarised for each treatment arm using standard descriptive statistics.

Analyses of secondary endpoints will include descriptive summaries. Continuous data will be compared using t-tests where appropriate and categorical data using chi-squared tests.

ETHICS AND DISSEMINATION

The study will be conducted according to the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with Therapeutic Goods Administration Drug Safety and Evaluation Branch comments (July 2000) and in compliance with applicable laws and regulations. The study will be performed in accordance with the NHMRC Statement on Ethical Conduct in Research Involving Humans 2007 – updated May 2015, the NHMRC Australian Code for the Responsible Conduct of Research 2007 and the principles laid down by the World Medical Assembly in the Declaration of Helsinki 2008.

To this end, no patient will be recruited to the study until all the necessary approvals have been obtained and the patient has provided written informed consent. Further, the investigator shall comply with the protocol, except when a protocol deviation is required to eliminate immediate hazard to a subject. In this circumstance the NHMRC CTC, principal investigator and HREC must be advised immediately.

The protocol was approved by a central ethics review committee (St Vincent's Hospital HREC) for all participating sites (HREC/17/SVH/294).

TRIAL STATUS

Patient enrolment for the study commenced in November 2018 at the Chris O'Brien Lifehouse in NSW, Australia. To date, 5 patients have been enrolled, with anticipated enrolment to allow for the futility assessment by the first quarter in 2020.

AUTHOR CONTRIBUTIONS

TS, LB and LH were responsible for manuscript writing. LH, LB, MM, KM, JK, MS, PS and LS were responsible for concept and protocol development. PS, AMJ, HW, RT, NA and LH are responsible for recruitment of study patients. All authors were responsible for final approval of the manuscript and are accountable for all aspects of the work.

FUNDING

This work was supported by Cancer Australia/Prostate Cancer Foundation of Australia grant number [APP1050880], Cancer Institute NSW and the Australian Prostate Cancer Research centre, NSW.

ACKNOWLEDGEMENTS

We would like to thank Novartis for supply of LEE011 (ribociclib).

COMPETING INTERESTS

Professor Henry Woo: lecturer and advisory boards for all of the following over the past 48 months: Astra Zeneca, Mundipharma, Janssen, Astellas, Ipsen, GSK and Boston Scientific. No shares, salary or conference travel support.

ETHICS APPROVAL: The protocol was approved at the St Vincent's Hospital Human Research Ethics Committee and ethics review committees for all participating sites.

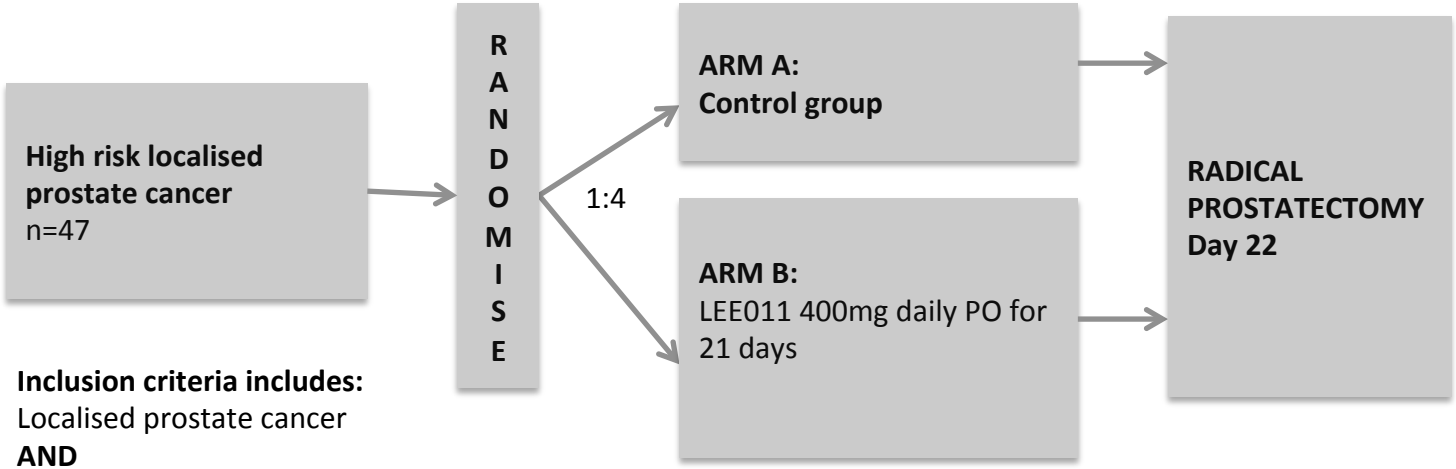
REFERENCES

1. Cancer-Institute-NSW. Cancer in New South Wales Incidence and Mortality 2018 [
2. de Bono JS, Logothetis CJ, Molina A, et al. Abiraterone and Increased Survival in Metastatic Prostate Cancer. *New England Journal of Medicine* 2011;364(21):1995-2005. doi: 10.1056/NEJMoa1014618
3. Scher HI, Fizazi K, Saad F, et al. Increased Survival with Enzalutamide in Prostate Cancer after Chemotherapy. *New England Journal of Medicine* 2012;367(13):1187-97. doi: 10.1056/NEJMoa1207506
4. de Bono JS, Oudard S, Ozguroglu M, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *The Lancet* 2010;376(9747):1147-54. doi: https://doi.org/10.1016/S0140-6736(10)61389-X
5. Parker C, Nilsson S, Heinrich D, et al. Alpha Emitter Radium-223 and Survival in Metastatic Prostate Cancer. *New England Journal of Medicine* 2013;369(3):213-23. doi: 10.1056/NEJMoa1213755
6. Mateo J, Carreira S, Sandhu S, et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. *New England Journal of Medicine* 2015;373(18):1697-708. doi: 10.1056/NEJMoa1506859
7. Scher HI, Buchanan G, Gerald W, et al. Targeting the androgen receptor: improving outcomes for castration-resistant prostate cancer. *Endocrine-Related Cancer* 2004;11(3):459-76. doi: 10.1677/erc.1.00525
8. Xu Y, Chen S-Y, Ross KN, et al. Androgens Induce Prostate Cancer Cell Proliferation through Mammalian Target of Rapamycin Activation and Post-transcriptional Increases in Cyclin D Proteins. *Cancer Research* 2006;66(15):7783. doi: 10.1158/0008-5472.CAN-05-4472
9. Hortobagyi GN, Stemmer SM, Burris HA, et al. Ribociclib as First-Line Therapy for HR-Positive, Advanced Breast Cancer. *New England Journal of Medicine* 2016;375(18):1738-48. doi: 10.1056/NEJMoa1609709
10. Turner NC, Ro J, André F, et al. Palbociclib in Hormone-Receptor–Positive Advanced Breast Cancer. *New England Journal of Medicine* 2015;373(3):209-19. doi: 10.1056/NEJMoa1505270
11. Goetz MP, Toi M, Campone M, et al. MONARCH 3: Abemaciclib As Initial Therapy for Advanced Breast Cancer. *Journal of Clinical Oncology* 2017;35(32):3638-46. doi: 10.1200/JCO.2017.75.6155
12. Comstock CES, Augello MA, Goodwin JF, et al. Targeting cell cycle and hormone receptor pathways in cancer. *Oncogene* 2013;32:5481. doi: 10.1038/onc.2013.83

https://www.nature.com/articles/onc201383 - supplementary-information

13. Jardim DL, Groves ES, Breitfeld PP, et al. Factors associated with failure of oncology drugs in late-stage clinical development: A systematic review. *Cancer Treatment Reviews* 2017;52:12-21. doi: 10.1016/j.ctrv.2016.10.009
14. Theodorescu D, Broder SR, Boyd JC, et al. p53, bcl-2 and Retinoblastoma Proteins as Long-Term Prognostic Markers in Localized Carcinoma of the Prostate. *The Journal of Urology* 1997;158(1):131-37. doi: https://doi.org/10.1097/00005392-199707000-00040

15. Drobnjak M, Osman I, Scher HI, et al. Overexpression of Cyclin D1 Is Associated with Metastatic Prostate Cancer to Bone. *Clinical Cancer Research* 2000;6(5):1891.
16. Henshall SM, Quinn DI, Lee CS, et al. Overexpression of the Cell Cycle Inhibitor p16INK4A in High-grade Prostatic Intraepithelial Neoplasia Predicts Early Relapse in Prostate Cancer Patients. *Clinical Cancer Research* 2001;7(3):544.
17. Dean JL, McClendon AK, Hickey TE, et al. Therapeutic response to CDK4/6 inhibition in breast cancer defined by ex vivo analyses of human tumors. *Cell cycle (Georgetown, Tex)* 2012;11(14):2756-61. doi: 10.4161/cc.21195
18. Azad AA, Volik SV, Wyatt AW, et al. Androgen Receptor Gene Aberrations in Circulating Cell-Free DNA: Biomarkers of Therapeutic Resistance in Castration-Resistant Prostate Cancer. *Clinical Cancer Research* 2015;21(10):2315. doi: 10.1158/1078-0432.CCR-14-2666



Inclusion criteria includes:
Localised prostate cancer
AND

- At least clinical stage T3a **or**
- Gleason score of 8-10 **or**
- Pre-operative PSA ≥ 20ng/mL

Planned for radical prostatectomy
Age ≥ 18years
ECOG PS 0-1

Exclusion criteria includes
Contra-indications to treatment with LEE011 including

- Prolonged QT syndrome or at risk for prolonged QTc
- Inadequate bone marrow, hepatic or renal function

- Tumour tissue will be collected at transrectal biopsy and at radical prostatectomy
- Blood samples for biomarker analysis and PSA will be collected within 7 days prior to randomisation, weekly during treatment and at the time of surgery

Figure 1: Study schema for randomised controlled, phase II trial of CDK4/6 inhibitor LEE011 (ribociclib) in high-risk, localised prostate cancer

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	2
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	3
Funding	#4	Sources and types of financial, material, and other support	11
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	2, 11

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	6
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	6
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	6
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	9
Objectives	#7	Specific objectives or hypotheses	5-6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	7

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	8
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5-6
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8, 10
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	8-9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8-9
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	6
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	8
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	6
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	6
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	9-10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	10
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9-10

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	11
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	6
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	2
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	6, 8

BMJ Open

Pharmacodynamics effects of CDK4/6 inhibitor LEE011 (ribociclib) in high-risk, localised prostate cancer: a study protocol for a randomised controlled phase II trial (LEEP study)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-033667.R1
Article Type:	Protocol
Date Submitted by the Author:	19-Dec-2019
Complete List of Authors:	Scheinberg, Tahlia; Chris O'Brien Lifehouse; The University of Sydney Kench, James; The University of Sydney; Royal Prince Alfred Hospital Stockler, Martin; University of Sydney , NHMRC Clinical Trials Centre Mahon, Kate; Chris O'Brien Lifehouse; The University of Sydney Sebastian, Lucille; The University of Sydney Stricker, Phillip; Garvan Institute of Medical Research; University of New South Wales Joshua, Anthony; St Vincent's Hospital; University of New South Wales Woo, H; The University of Sydney; Chris O'Brien Lifehouse Thanigasalam, Ruban; Chris O'Brien Lifehouse; The University of Sydney Ahmadi, Nariman; Chris O'Brien Lifehouse Centenera, Margaret; South Australian Health and Medical Research Institute; The University of Adelaide Butler, Lisa; South Australian Health and Medical Research Institute; The University of Adelaide Horvath, Lisa; Chris O'Brien Lifehouse; The University of Sydney
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Pathology, Urology
Keywords:	prostatic neoplasms, neoadjuvant trial, window of opportunity trial, ribociclib, translational research

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Pharmacodynamics effects of CDK4/6 inhibitor LEE011 (ribociclib) in high-risk, localised prostate cancer: a study protocol for a randomised controlled phase II trial (LEEP study)

Scheinberg T[1,2,3], Kench J[2,3,4], Stockler M[1,2,3,4,5], Mahon KL[1,2,3,4], Sebastian L[2], Stricker P[3,6,7], Joshua AM[3,7,8], Woo H[1,2], Thanigasalam R[1,2,5], Ahmadi N[1], Centenera MM[9,10], Butler LM[9,10]*, Horvath LG[1,2,3,4,7]*.

1. **Chris O'Brien Lifehouse, Sydney, Australia.**
2. **University of Sydney, Sydney, Australia**
3. **Garvan Institute of Medical Research, Sydney, Australia**
4. **Royal Prince Alfred Hospital, Sydney, Australia**
5. **Concord Hospital, Sydney, Australia**
6. **St Vincent's Clinic, Sydney, Australia**
7. **University of NSW, Sydney, Australia**
8. **St Vincent's Hospital, Sydney, Australia**
9. **South Australian Health and Medical Research Institute, Adelaide, Australia**
10. **University of Adelaide, Adelaide, Australia**

***Authors contributed equally**

Authors:
Dr Tahlia Scheinberg
Professor James Kench
Professor Martin Stockler
Dr Kate L Mahon
Lucille Sebastian
Professor Phillip Stricker
Associate Professor Anthony M Joshua
Professor Henry Woo
Associate Professor Ruban Thanigasalam
Dr Nariman Ahmadi
Dr Margaret M Centenera
Professor Lisa M Butler
Professor Lisa G Horvath

Corresponding author:
Professor Lisa Horvath
Chris O'Brien Lifehouse
119 – 146 Missenden Road
Camperdown NSW 2050
Australia
+61 2 8514 0000
lisa.horvath@lh.org.au

Study sponsor: University of Sydney, NSW, 2006

Word count: 3012 words

Keywords: neoadjuvant trial, window of opportunity trial, prostatic neoplasms, ribociclib, translational research

ABSTRACT

Introduction Despite the development of new therapies for advanced prostate cancer, it remains the most common cause of cancer and the second leading cause of cancer death in men. It is critical to develop novel agents for the treatment of prostate cancer, particularly those that target aspects of androgen receptor (AR) signalling or prostate biology other than inhibition of androgen synthesis or AR binding. Neoadjuvant pharmacodynamic studies allow for a rational approach to the decisions regarding which targeted therapies should progress to phase II/III trials. CDK4/6 inhibitors have evidence of efficacy in breast cancer, and have been shown to have activity in pre-clinical models of hormone sensitive and castrate resistant prostate cancer. The LEEP trial aims to assess the pharmacodynamic effects of LEE011 (ribociclib), an orally bioavailable and highly selective CDK4/6 inhibitor, in men undergoing radical prostatectomy for high-risk, localised prostate cancer.

Methods and analysis The multicentre randomised, controlled 4:1 two arm, phase II, open label pharmacodynamic study will recruit 47 men with high risk, localised prostate cancer who are planned to undergo radical prostatectomy. Participants who are randomised to receive the study treatment will be treated with LEE011 400mg daily for 21 days for 1 cycle. The primary endpoint is the frequency of a 50% reduction in Ki-67 proliferation index from the pre-treatment prostate biopsy compared to that present in prostate cancer tissue from radical prostatectomy. Secondary and tertiary endpoints include pharmacodynamic assessment of CDK4/6 cell cycle progression via E2F levels, apoptotic cell death by cleaved caspase-3, changes in serum and tumour levels of PSA, pathological regression, safety via incidence of adverse events and exploratory biomarker analysis.

Ethics and dissemination The protocol was approved by a central ethics review committee (St Vincent's Hospital HREC) for all participating sites (HREC/17/SVH/294). Results will be disseminated in peer-reviewed journals and at scientific conferences.

Drug Supply Novartis

Protocol Version 2.0, 30 May 2019

Trial Registration Number ACTRN12618000354280 (Australian New Zealand Clinical Trials Registry)

ARTICLE SUMMARY

Strengths and limitations of this study

- This is the first trial evaluating the pharmacodynamic effects of CDK4/6 inhibitors in hormone sensitive prostate cancer.
- This study will explore potential biomarkers for treatment response
- This trial is designed to examine anti-tumour pharmacodynamics effects of single agent ribociclib
- This trial is not designed to determine whether a short course of neoadjuvant treatment could alter oncological outcomes or recurrence rates. These will be the next steps if the trial is positive.
- By utilising paired samples, this neoadjuvant proof of concept trial allows us to use relatively small sample sizes, through examining dynamic changes in the biomarkers of interest

INTRODUCTION

Despite advances in the detection and treatment of prostate cancer, it remains the most common cause of cancer in men in the developed world and the second leading cause of cancer death[1]. Over the last decade, the treatment of advanced prostate cancer has changed dramatically with new therapies including novel anti-androgens[2 3], novel taxanes[4], radioisotope therapy[5] and more recently PARP inhibitors[6]. However these agents are not curative, and it is recognised that in order to improve survival from prostate cancer, it is critical to develop novel agents, particularly those that target aspects of androgen receptor (AR) signalling or prostate biology other than inhibition of androgen synthesis or AR binding[7].

CDK4/6 inhibitors for treatment of prostate cancer

One of the common driving pathways that is altered in prostate cancer, and selected for in CRPC, is aberrant cell cycle activation through the cyclin/CDK/retinoblastoma (Rb) axis, with resultant uncontrolled cellular proliferation. This axis is critically important in controlling the G1-S transition of the cell cycle. There is evidence that androgens can stimulate the increased expression of G1 cyclins and cyclin-dependent kinases and decrease the expression of CDK inhibitors. The AR may also directly contribute to the transcription of some cell cycle regulatory genes, including cyclin D3[8].

By binding to CDK4 and CDK6, selective CDK inhibitors inhibit Rb phosphorylation to prevent G1-S phase transition and induce cell cycle arrest. CDK4/6 inhibitors palbociclib (PD0332991; Pfizer), ribociclib (LEE011; Novartis) and abemaciclib (LY2835219; Eli Lilly) are oral and reversible small molecule inhibitors with high selectivity for CDK4 and CDK6, with evidence of efficacy in breast cancer[9-11].

In preclinical models of hormone-sensitive and castration-resistant prostate cancer, palbociclib has exhibited single agent activity, by limiting cellular proliferation and growth[12]. The potential therapeutic effect was determined in both *in vivo* mouse xenografts and a novel *ex vivo* assay using primary human tumours obtained from radical prostatectomy. This *ex vivo* model has also shown that LEE011 significantly inhibits prostate tumour cell proliferation in a dose dependent manner (unpublished, Butler LM, 2019). This preclinical data provides evidence that CDK4/6 inhibitors achieve clinically relevant biological responses in human prostate tumours, and supports the evaluation of CDK4/6 inhibitors for treatment of prostate cancer.

Towards more rapid assessment of new therapies

Clinical trials of new drugs in the hormone sensitive phase of prostate cancer (high-risk localised prostate cancer, or at relapse after localised treatment) require long follow-up due to the natural history of the disease. The interval between biopsy and surgery offers an ideal opportunity for *in vivo* assessment of anti-tumour activity and selection of optimal novel agents for further investigation. Both Ki67 and Cleaved Caspase 3 have been used to assess pharmacodynamic activity of novel therapies in neoadjuvant studies in prostate and breast cancer[13-15]. Ki67 reduction has also been found to correlate with response in neoadjuvant studies in breast cancer[16 17], and with outcome in prostate cancer[18]. However there can be significant intra-tumour Ki67 heterogeneity, particularly in high risk prostate cancer[19], as well as inter-reader variability in its measurement[20]. Where possible, centralised review of Ki67 in clinical trials is advisable [18].

A recent systematic review identified that a lack of a biomarker-driven strategy and failure to achieve “proof of concept” in Phase 2 trials were significantly associated with failure of

cancer drugs to achieve late-stage clinical success such as FDA approval[21]. Neoadjuvant pharmacodynamic studies, such as the one described in this project, will allow for a more rational approach to the decisions regarding which targeted therapies should go forward into phase II/III trials.

Biomarkers for treatment response

The identification of informative biomarkers in the pre-clinical phase, which can be incorporated into clinical studies, is pivotal to accelerating the drug development process, and when incorporated into clinical decision-making, can maximise patient benefit and minimise harm, with judicious drug administration. Candidate biomarkers will be identified/assessed in this clinical trial and potentially validated in future trials with CDK4/6 inhibitors.

Absent or decreased staining of nuclear Rb proteins is commonly found in prostate cancer specimens, and it has been suggested that inactivation of the retinoblastoma gene may be an important event in prostate tumour progression[22]. In an *ex vivo* model, functional Rb is required for optimal CDK4/6 inhibitor efficacy[12]. There is evidence that cyclin D1 overexpression is implicated in tumourigenesis and tumour progression, and may be related to the evolution to castration resistance in prostate cancer[23]. The product of the INK4A gene inactivates the G1-phase cyclin dependent kinases CDK4 and CDK6. Overexpression of p16INK4A in high-grade prostatic epithelial neoplasia is associated with early relapse in prostate cancer patients treated with radical prostatectomy[24]. Given the role of Cyclin D1, Rb proteins and p16INK4A in cell cycle progression, there is interest in reviewing these as biomarkers of response to CDK4/6 inhibitors. Further, induction of cyclin D1 and p16INK4A have been identified as possible pharmacodynamic endpoints on pre-clinical models, and can be validated in a clinical setting[12 25].

HYPOTHESIS

Primary hypothesis

We hypothesise that administration of LEE011 (Ribociclib) to men prior to undergoing radical prostatectomy will lead to a 50% reduction in Ki-67 index in 30% or more of participants treated with LEE011, compared with a 50% reduction in Ki-67 index in 10% or fewer in the control group.

Secondary hypothesis

We hypothesise that treatment with LEE011 will be associated with inhibition of CDK4/6 cell cycle progression by a decrease in the level of E2F in prostate cancer tissue, and an increased level of cleaved caspase-3 in prostate tissue indicating increased apoptotic activity.

METHODS AND ANALYSIS

The LEEP study is an Australian-based, multicentre, randomised controlled, phase II, open label pharmacodynamic study. The primary aim is to determine the pharmacodynamic activity of the CDK4/6 inhibitor LEE011 in men with high-risk localised prostate cancer undergoing radical prostatectomy.

Study objectives

The primary objective of this study is to determine the effect of LEE011 on tumour cell proliferation, as determined by:

- The frequency of a 50% reduction in the Ki-67 proliferation index from the pre-treatment prostate biopsy compared to that present in prostate cancer tissue from radical prostatectomy.

The secondary objectives are to determine:

- The effect of LEE011 on CDK4/6 cell cycle progression, by measuring E2F expression in prostate tissue by immunohistochemistry and peripheral blood mononuclear cells by ELISA
- The effect of LEE011 on apoptotic cell death, by examining the frequency of a 50% increase in cleaved caspase-3 expression levels in tumour cells, measured by immunohistochemistry
- Changes in serum and tumour levels of PSA, by immunoassay
- Rates of pathological regression, assessed by histopathology, as defined by cancer cell atrophy, decreased nuclear size, increased chromatin density and pale cytoplasm.
- The incidence of adverse events (CTCAE v 4.03)

The tertiary objectives are to evaluate exploratory biomarkers as predictors of response to therapy. These include Rb status, cyclin D1 amplification, p16INK4a expression, PTEN loss, AR amplifications/mutations and aberrations of PI3K signalling pathways (assessed by reverse transcription-PCR and FISH analysis of cancer tissue from radical prostatectomy). These will also be tested in free plasma DNA, through a novel technique that has identified these aberrations in circulating tumour DNA [26]. Effects on immune system such as circulating T-cell profiling will also be assessed.

Trial oversight and monitoring

The LEEP study is a collaboration between the Chris O'Brien Lifehouse, Sydney; Royal Prince Alfred Hospital, Sydney; the University of Adelaide, Adelaide; St Vincent's Hospital, Sydney; and the National Health and Medical Research Council (NHMRC) Clinical Trials Centre (CTC), University of Sydney.

The University of Sydney is the study sponsor. The NHMRC CTC will be responsible for study co-ordination, monitoring, data acquisition, management and statistical analysis.

Safety and efficacy endpoints will be assessed when evaluable tissue is available from 22 participants treated with LEE011 and at study completion.

Protocol amendments can only be made by the trial management committee, and must be approved by the central institutional Human Research Ethics Committee (HREC) prior to implementation.

Patient and public involvement

This research was funded through a granting process that included a consumer representative from Cancer Voices NSW. The grant, study protocol and patient information sheet/consent form were all discussed, reviewed and edited by our consumer representative. A consumer representative is a member of the trial steering committee. Following completion, a plain-English version of the results will be made available to patients via their study doctor. Results of this study will be disseminated to study participants through peer-reviewed journals, at scientific conferences, and on the NHMRC CTC website.

Trial design

The protocol consists of a randomised, controlled 4:1 two arm, phase II, open label pharmacodynamic study (figure 1). The trial is currently being conducted at 2 tertiary referral centres (Chris O'Brien Lifehouse, Sydney and St Vincent's Health Network, Sydney) in New South Wales (NSW), Australia. There is a plan to open further sites during 2020.

Inclusion Criteria

Patients who fulfil all of the following characteristics will be considered eligible for enrolments:

- Males ≥ 18 years with high-risk localised prostate cancer (at least clinical stage T3a **Or** Gleason score of between 8 and 10 **Or** Preoperative PSA ≥ 20 ng/mL) **AND** planned for radical prostatectomy;
- Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1;
- Histological confirmation of prostate cancer via a pre-treatment diagnostic transrectal ultrasound (TRUS) biopsy;
- Adequate bone marrow, hepatic and renal function;
- Serum calcium, potassium, phosphate and magnesium within normal range or corrected with supplements.

Exclusion criteria

Patients with the following characteristics will be excluded from study enrolment:

- Major surgery ≤ 2 weeks prior to enrolment or who have not recovered from side effects of such therapy. TRUS biopsy is not considered major surgery in this study;
- Known hypersensitivity to the study drug or its excipients;
- Patients with known disorders due to a deficiency in bilirubin glucuronidation (e.g. Gilbert's syndrome);
- Diarrhoea ≥ CTCAE grade 2;
- Impaired cardiac function, including any one of the following:
    - History (or family history) of long QT syndrome.
    - Those who already have, or who are at significant risk of developing QTc prolongation, including patients with:
        - Long QT syndrome,
        - Mean QTcF ≥ 450msec on baseline ECG,
        - Uncontrolled or significant cardiac disease including recent myocardial infarction,
        - Congestive heart failure, unstable angina or bradyarrhythmias,
        - Electrolyte abnormalities,
        - Clinically significant ECG abnormalities at clinician discretion
    - Other clinically significant heart disease (e.g. uncontrolled hypertension, history of labile hypertension, or history of poor compliance with an anti-hypertensive regimen);
    - Clinically significant resting bradycardia (< 50 beats per minute);
    - Patients who are currently receiving treatment with any medication that has a relative risk of prolonging QTcF interval or inducing Torsades de Pointes and cannot be discontinued or switched to an alternative treatment prior to commencing study treatment;
    - Obligate use of a cardiac pacemaker;
- Patients who have received prior antineoplastic therapy for advanced disease;

- Prior treatment with a CDK4/6 inhibitor;
- Patients who are currently receiving treatment with strong CYP3A4 inhibitors and cannot be discontinued or switched to an alternative treatment prior to commencing study treatment
- Patients receiving chronic or high-dose corticosteroid therapy;
- Significant infection, including chronic active hepatitis B, hepatitis C or HIV;
- Serious medical or psychiatric conditions that might limit the ability of the patient to comply with the protocol.

Investigational medical product – LEE011 (ribociclib)

This study will use LEE011, an orally bioavailable, highly selective, small-molecule inhibitor of CDK4/6 that blocks the phosphorylation of retinoblastoma protein, thereby preventing cell-cycle progression and inducing G1 phase arrest. Based on the results of preclinical toxicology studies and available clinical safety data, the main adverse reactions for LEE011 are bone marrow suppression including leukopenia, neutropenia, anaemia and thrombocytopaenia, dyspnoea, hepatic toxicity, renal toxicity, fatigue, nausea, vomiting, diarrhoea and prolongation of the QT interval. The risk of these toxicities may be amplified by concomitant administration of strong inhibitors of CYP3A4 or other combination treatments.

Randomisation

The method of randomisation will be minimisation with stratification by site. Participants will be allocated to the study treatment in a ratio of 4:1 (LEE011:control).

Recruitment and consent

Patient screening and enrolment will be overseen by the site principal investigator and performed by trained study personnel. Patients will provide written informed consent prior to study enrolment. Treatment will be planned to start within 7 days after randomisation.

Study procedures

The randomised, controlled, phase II, open label, pharmacodynamic study will assess the pharmacodynamic activity of the CDK4/6 inhibitor LEE011, in men with high-risk, localised prostate cancer.

Participants randomised to receive the study treatment will have a pre-treatment MUGA. Participants will receive LEE011 400mg daily taken orally for 21 days treatment for 1 cycle. The scheduled surgery will occur 22 days after the first dose of LEE011 (if randomised to study drug treatment) or 22 days after randomisation (if randomised to the control group). Dose modifications are not permitted in this study. Patients who need to come off the study due to toxicity (e.g. neutropenia or thrombocytopaenia) will discontinue and proceed to surgery as planned.

Local pharmacy departments will record drug recept including a pill count to assess compliance.

Study samples will be stored at the Garvan Institute for Medical Research.

Data acquisition

Tumour tissue samples will be collected at transrectal biopsy and at radical prostatectomy. Blood samples for biomarker analysis and PSA will be collected within 7 days prior to randomisation, weekly during treatment and at the time of radical prostatectomy.

Ki-67 expression will be assessed by central pathologist review. Where possible, for all analyses, comparisons will be made between similar areas in the needle biopsy and radical prostatectomy specimens. Scoring for protein expression will be performed by two independent pathologist researchers from Royal Prince Alfred Hospital, both blinded to the treatment groups and pairings of tissue from the same patient. Discrepancies will be resolved by consensus.

Cell cycle arrest will be measured by E2F expression as determined by immunohistochemistry and scored by manual counting. Apoptotic cell death will be determined by examining the cleaved caspase-3 staining in tumour cells by immunohistochemistry and scored by manual counting. PSA levels in tumour and blood will be assessed by immunoassay, with immunohistochemistry or ELISA.

Trial data will be monitored by clinical trials program staff from the NHMRC CTC.

STATISTICAL CONSIDERATIONS

Sample size estimation

Using the Simon's two-stage design, an uninteresting rate for the true response is 10% and a clinically interesting rate, which would warrant further investigation, is 30%. Based on this design, a sample size of 37 patients will have at least 90% power with 95% confidence to exclude the uninteresting rate in favour of the more clinically meaningful rate. A response is defined as ≥50% decrease in Ki-67 expression in the paired prostate biopsy baseline sample compared with the radical prostatectomy sample.

A futility analysis will be performed after 22 patients have completed 1 cycle of LEE011 and are evaluable for pharmacodynamic response. If there are 2 or fewer responses, consideration will be given to either reassessing the study design or stopping the study due to futility. When these 22 patients are assessed it is expected that there will be at least 6 patients in the control group, which will allow for assessment of the response to be performed in light of what is seen in the control arm. The study will then recruit an additional 15 patients in the treatment arm and 4 controls. This sample size allows for a modest number of drop-outs/loss to follow up. It is anticipated that at least 33 patients in the treated cohort will be evaluable for response at study completion.

10 untreated men will be enrolled in the control group to provide estimates of PD biomarkers as a basis for biological comparison, giving a total sample size of 47 patients.

It is expected that none of the control group men will have a ≥50% decrease in Ki-67. For the secondary objectives, the sample size of 10 control participants to 37 treated patients provides adequate power to detect large differences only.

Patients who do not receive study treatment, withdraw their consent or are not evaluable will be replaced.

Statistical analysis

Analysis of efficacy endpoints (i.e. response, biomarkers) will include only evaluable patients. Analysis of safety endpoints (i.e. toxicity) will be according to treatment received, including only patients who received at least 1 dose of the experimental treatment.

The response in each treatment arm will be summarised by the number and proportion of patients experiencing at least a 50% decrease in Ki-67 expression, with a two-tailed p-value significance level of 0.05. Ki-67 levels pre- and post-treatment will also be summarised for

each treatment arm using standard descriptive statistics. Data will be compared using McNemar's test.

Analyses of secondary endpoints will include descriptive summaries. Continuous data will be compared using t-tests where appropriate and categorical data using chi-squared tests.

ETHICS AND DISSEMINATION

The study will be conducted according to the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with Therapeutic Goods Administration Drug Safety and Evaluation Branch comments (July 2000) and in compliance with applicable laws and regulations. The study will be performed in accordance with the NHMRC Statement on Ethical Conduct in Research Involving Humans 2007 – updated May 2015, the NHMRC Australian Code for the Responsible Conduct of Research 2007 and the principles laid down by the World Medical Assembly in the Declaration of Helsinki 2008.

To this end, no patient will be recruited to the study until all the necessary approvals have been obtained and the patient has provided written informed consent. Further, the investigator shall comply with the protocol, except when a protocol deviation is required to eliminate immediate hazard to a subject. In this circumstance the NHMRC CTC, principal investigator and HREC must be advised immediately.

The protocol was approved by a central ethics review committee (St Vincent's Hospital HREC) for all participating sites (HREC/17/SVH/294).

Results will be disseminated in peer-reviewed journals and at scientific conferences.

TRIAL STATUS

Patient enrolment for the study commenced in November 2018 at the Chris O'Brien Lifehouse in NSW, Australia. St Vincent's Hospital opened in late 2019 and there are plans to open several new sites in 2020. To date, 8 patients have been enrolled, with anticipated enrolment to allow for the futility assessment by the first quarter in 2021.

AUTHOR CONTRIBUTIONS

TS, LB and LH were responsible for manuscript writing. LH, LB, KM, JK, MS, PS, MC and LS were responsible for concept and protocol development. PS, AMJ, HW, RT, NA and LH are responsible for recruitment of study patients. All authors were responsible for final approval of the manuscript and are accountable for all aspects of the work.

FUNDING

This work was supported by Cancer Australia/Prostate Cancer Foundation of Australia grant number [APP1050880], Cancer Institute NSW and the Australian Prostate Cancer Research centre, NSW.

ACKNOWLEDGEMENTS

We would like to thank Novartis for supply of LEE011 (ribociclib).

COMPETING INTERESTS

Professor Henry Woo: lecturer and advisory boards for all of the following over the past 48 months: Astra Zeneca, Mundipharma, Janssen, Astellas, Ipsen, GSK and Boston Scientific. No shares, salary or conference travel support.

ETHICS APPROVAL: The protocol was approved at the St Vincent's Hospital Human Research Ethics Committee and ethics review committees for all participating sites.

DATA AVAILABILITY

De-identified raw data will be made available upon reasonable written request.

REFERENCES

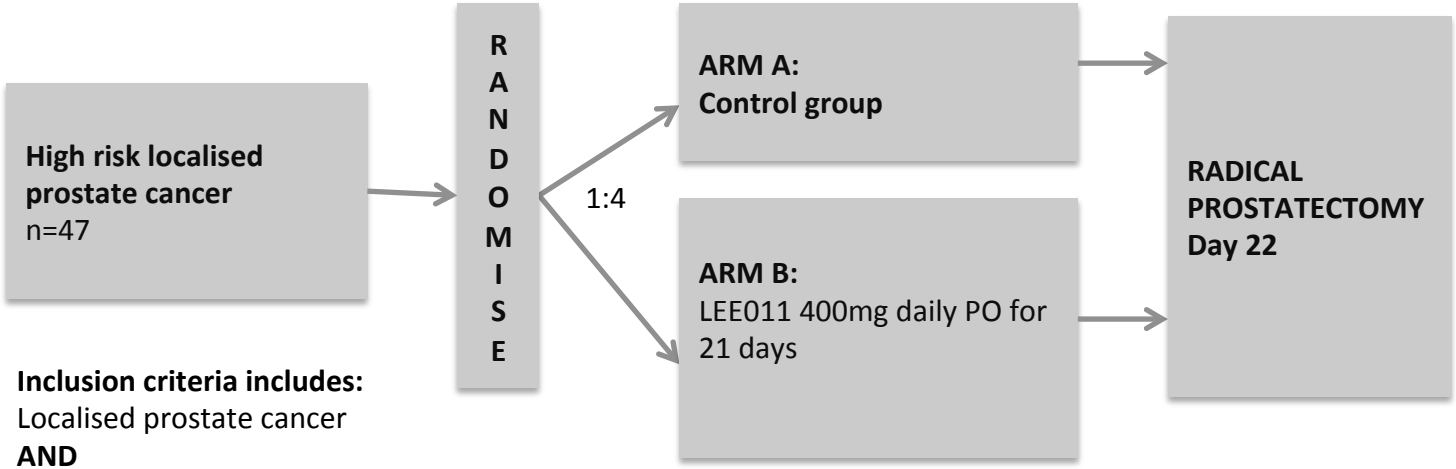
1. Cancer-Institute-NSW. Cancer in New South Wales Incidence and Mortality 2018 [
2. de Bono JS, Logothetis CJ, Molina A, et al. Abiraterone and Increased Survival in Metastatic Prostate Cancer. *New England Journal of Medicine* 2011;364(21):1995-2005. doi: 10.1056/NEJMoa1014618
3. Scher HI, Fizazi K, Saad F, et al. Increased Survival with Enzalutamide in Prostate Cancer after Chemotherapy. *New England Journal of Medicine* 2012;367(13):1187-97. doi: 10.1056/NEJMoa1207506
4. de Bono JS, Oudard S, Ozguroglu M, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *The Lancet* 2010;376(9747):1147-54. doi: https://doi.org/10.1016/S0140-6736(10)61389-X
5. Parker C, Nilsson S, Heinrich D, et al. Alpha Emitter Radium-223 and Survival in Metastatic Prostate Cancer. *New England Journal of Medicine* 2013;369(3):213-23. doi: 10.1056/NEJMoa1213755
6. Mateo J, Carreira S, Sandhu S, et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. *New England Journal of Medicine* 2015;373(18):1697-708. doi: 10.1056/NEJMoa1506859
7. Scher HI, Buchanan G, Gerald W, et al. Targeting the androgen receptor: improving outcomes for castration-resistant prostate cancer. *Endocrine-Related Cancer* 2004;11(3):459-76. doi: 10.1677/erc.1.00525
8. Xu Y, Chen S-Y, Ross KN, et al. Androgens Induce Prostate Cancer Cell Proliferation through Mammalian Target of Rapamycin Activation and Post-transcriptional Increases in Cyclin D Proteins. *Cancer Research* 2006;66(15):7783. doi: 10.1158/0008-5472.CAN-05-4472
9. Hortobagyi GN, Stemmer SM, Burris HA, et al. Ribociclib as First-Line Therapy for HR-Positive, Advanced Breast Cancer. *New England Journal of Medicine* 2016;375(18):1738-48. doi: 10.1056/NEJMoa1609709
10. Turner NC, Ro J, André F, et al. Palbociclib in Hormone-Receptor–Positive Advanced Breast Cancer. *New England Journal of Medicine* 2015;373(3):209-19. doi: 10.1056/NEJMoa1505270
11. Goetz MP, Toi M, Campone M, et al. MONARCH 3: Abemaciclib As Initial Therapy for Advanced Breast Cancer. *Journal of Clinical Oncology* 2017;35(32):3638-46. doi: 10.1200/JCO.2017.75.6155
12. Comstock CES, Augello MA, Goodwin JF, et al. Targeting cell cycle and hormone receptor pathways in cancer. *Oncogene* 2013;32:5481. doi: 10.1038/onc.2013.83

https://www.nature.com/articles/onc201383#supplementary-information
13. Curigliano G, Gómez Pardo P, Meric-Bernstam F, et al. Ribociclib plus letrozole in early breast cancer: A presurgical, window-of-opportunity study. *The Breast* 2016;28:191-98. doi: 10.1016/j.breast.2016.06.008
14. Anantharaman A, Nguyen HG, Cooperberg MR, et al. A pharmacodynamic study of pre-prostatectomy buparlisib in men with high-risk, localized prostate cancer. *Journal of Clinical Oncology* 2016;34(15_suppl):e14110-e10. doi: 10.1200/JCO.2016.34.15_suppl.e14110

15. Armstrong AJ, Netto GJ, Rudek MA, et al. A Pharmacodynamic Study of Rapamycin in Men with Intermediate- to High-Risk Localized Prostate Cancer. *Clinical Cancer Research* 2010;16(11):3057. doi: 10.1158/1078-0432.CCR-10-0124
16. Baselga J, Semiglazov V, Van Dam P, et al. Phase II randomized study of neoadjuvant everolimus plus letrozole compared with placebo plus letrozole in patients with estrogen receptor–positive breast cancer. *Journal of Clinical Oncology* 2009;27(16):2630-37.
17. Dowsett M, Smith IE, Ebbs SR, et al. Prognostic Value of Ki67 Expression After Short-Term Presurgical Endocrine Therapy for Primary Breast Cancer. *JNCI: Journal of the National Cancer Institute* 2007;99(2):167-70. doi: 10.1093/jnci/djk020
18. Richardsen E, Andersen S, Al-Saad S, et al. Evaluation of the proliferation marker Ki-67 in a large prostatectomy cohort. *PLoS ONE* 2017;12(11):e0186852. doi: 10.1371/journal.pone.0186852
19. Mesko S, Kupelian P, Demanes DJ, et al. Quantifying the Ki-67 Heterogeneity Profile in Prostate Cancer. *Prostate Cancer* 2013;2013:5. doi: 10.1155/2013/717080
20. Polley M-YC, Leung SCY, McShane LM, et al. An international Ki67 reproducibility study. *J Natl Cancer Inst* 2013;105(24):1897-906. doi: 10.1093/jnci/djt306 [published Online First: 2013/11/07]
21. Jardim DL, Groves ES, Breitfeld PP, et al. Factors associated with failure of oncology drugs in late-stage clinical development: A systematic review. *Cancer Treatment Reviews* 2017;52:12-21. doi: 10.1016/j.ctrv.2016.10.009
22. Theodorescu D, Broder SR, Boyd JC, et al. p53, bcl-2 and Retinoblastoma Proteins as Long-Term Prognostic Markers in Localized Carcinoma of the Prostate. *The Journal of Urology* 1997;158(1):131-37. doi: https://doi.org/10.1097/00005392-199707000-00040
23. Drobnjak M, Osman I, Scher HI, et al. Overexpression of Cyclin D1 Is Associated with Metastatic Prostate Cancer to Bone. *Clinical Cancer Research* 2000;6(5):1891.
24. Henshall SM, Quinn DI, Lee CS, et al. Overexpression of the Cell Cycle Inhibitor p16INK4A in High-grade Prostatic Intraepithelial Neoplasia Predicts Early Relapse in Prostate Cancer Patients. *Clinical Cancer Research* 2001;7(3):544.
25. Dean JL, McClendon AK, Hickey TE, et al. Therapeutic response to CDK4/6 inhibition in breast cancer defined by ex vivo analyses of human tumors. *Cell cycle (Georgetown, Tex)* 2012;11(14):2756-61. doi: 10.4161/cc.21195
26. Azad AA, Volik SV, Wyatt AW, et al. Androgen Receptor Gene Aberrations in Circulating Cell-Free DNA: Biomarkers of Therapeutic Resistance in Castration-Resistant Prostate Cancer. *Clinical Cancer Research* 2015;21(10):2315. doi: 10.1158/1078-0432.CCR-14-2666

Figure Legend

Figure 1: Study schema for randomised controlled, phase II trial of CDK4/6 inhibitor LEE011 (ribociclib) in high-risk, localised prostate cancer



Inclusion criteria includes:
Localised prostate cancer
AND

- At least clinical stage T3a **or**
- Gleason score of 8-10 **or**
- Pre-operative PSA ≥ 20ng/mL

Planned for radical prostatectomy
Age ≥ 18years
ECOG PS 0-1

Exclusion criteria includes
Contra-indications to treatment with LEE011 including

- Prolonged QT syndrome or at risk for prolonged QTc
- Inadequate bone marrow, hepatic or renal function

- Tumour tissue will be collected at transrectal biopsy and at radical prostatectomy
- Blood samples for biomarker analysis and PSA will be collected within 7 days prior to randomisation, weekly during treatment and at the time of surgery

Figure 1: Study schema for randomised controlled, phase II trial of CDK4/6 inhibitor LEE011 (ribociclib) in high-risk, localised prostate cancer

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	10
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 10

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1, 5
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	5
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	5
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	4-5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4-5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be	4

		collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	7
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	7
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6, 7
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	4, 5
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7, 9

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	7
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	7
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7, 8

Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7, 8
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	5, 8
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8-9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8-9
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	8
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	5
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	8
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	5, 9

Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	5
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	9, 10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	9
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8, 9
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	10
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	5

Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	1
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplementary file
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	7