PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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)
AUTHORS	Scheinberg, Tahlia; Kench, James; Stockler, Martin; Mahon, Kate L; Sebastian, Lucille; Stricker, Phillip; Joshua, Anthony M; Woo, H; Thanigasalam, Ruban; Ahmadi, Nariman; Centenera, Margaret M; Butler, Lisa M; Horvath, Lisa G

VERSION 1 – REVIEW

REVIEWER	Francesco Schettini University of Naples Federico II, Italy and Institut D'Investigacions Biomèdiques August P i Sunyer (IDIBAPS), Spain.
REVIEW RETURNED	23-Sep-2019

GENERAL COMMENTS	Overall the study protocol provides a good rationale for the trial and explains quite well how the study will proceed. However I have some considerations: 1) I would suggest to wait one week more prior to surgery to let the blood cell count increase, do to a highly probable risk of bone marrow toxicity from Ribociclib. 2) The protocol lacks of informed consent material. 3) Check carefully the pages reported in SPIRIT checklist. For example, for point #33 the pages are incorrect. 4) I can't understand where the blinded pathologists work, please specify. Which is their affiliation? 5) The protocol must provide a planning for biospecimen collection, processing, and storage. 6) Will the biospecimen analyses be centralised or not? 7) Page 9, line 17, correct "with be" with "will be". I would suggest to re-check the whole protocol carefully for other typos. 8) Are you planning to use the McNemar test? Since it might be more appropriate than a chi square test for comparing proportion in pre/post studies. Include it in the statistical plan, if it's the case.

REVIEWER	Susan Slovin Memorial Sloan Kettering Cancer Center, USA
REVIEW RETURNED	27-Nov-2019

GENERAL COMMENTS	The authors present the plan of a multicenter randomized phase II trial using the CDK4/6 inhibitor (LEE 011; ribociclib) (LEEP study) in patients with high-risk, hormone sensitive prostate cancer with focus on the pharmacodynamic effects of the drug. The trial is to recruit 47 men with high risk localized prostate cancer who are planned to undergo radical prostatectomy. Patient will be randomized 4:1 in favor of LEE011 400mg on a 21 day cycle.

	Primary endpoint is the frequency of a 50% reduction in tumor cell proliferation index (Ki-67) comparing post with pre-treatment tumor tissue. Secondary endpoints include pharmacodynamic assessment of CDK4/6 cell cycle progression via E2F levels, apoptotic death, PSA changes in tumor and serum and pathologic response. This is a well-written essentially neoadjuvant clinical trial examining the potential pharmacodynamic and biologic impact of LEE001 in patients with high risk prostate cancer undergoing prostatectomy. It should be addressed what is defined as “high risk” patients. It is unclear why a 4:1 randomization is required despite what is written in the biostatistical section. Ki67 is not always deemed as a reliable biomarker; please indicate whether there is a particular reason that this marker was selected given that other measures of activity could be used. Not every “high risk” cancer behaves biologically as “high risk”; therefore some measure of concern about the ability of this biomarker to really capture biologic behavior. It is surprising that only 5 patients have been enrolled within one year. Might there be impediments to accrual? Some mention should be made for dose reduction of ribociclib as this drug can cause neutropenia.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: **Francesco Schettini**

Institution and Country: University of Naples Federico II, Italy and Institut D’Investigacions Biomèdiques August P i Sunyer (IDIBAPS), Spain.

Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below
Overall the study protocol provides a good rationale for the trial and explains quite well how the study will proceed. However I have some considerations:

Thank you very much for your considered appraisal of this trial. We found your critique valuable and have attempted to address your specific comments.

Reviewer comment	**Response**	**Page**
1) I would suggest to wait one week more prior to surgery to let the blood cell count increase, do to a highly	**Response:** The mean plasma half-life of Ribociclib is 32 hours. It is unknown how long the in vivo pharmacodynamic effects persist following cessation of	

probable risk of bone marrow toxicity from Ribociclib.	Ribociclib treatment. In order to optimise the evaluable effects, we have chosen to have a short interval between final drug dosing and operation date. While on study, patients will have weekly FBE examination, and Ribociclib will be stopped if toxicity occurs (e.g. thrombocytopaenia and neutropenia). These toxicities are dose dependent, and the incidence is expected to be less than that observed among patient with breast cancer who are treated with a higher dose (600mg daily rather than 400mg daily on this study). A similar study design with a short-interval between final drug dosing and date of surgery has been performed in studies of neoadjuvant CDK4/6 inhibitors in breast cancer[1,2].	
2) The protocol lacks of informed consent material.	**Response:** This has now been added to supplementary material	Supplementary material
3) Check carefully the pages reported in SPIRIT checklist. For example, for point #33 the pages are incorrect.	**Response:** The SPIRIT checklist page numbers have been updated to reflect the changes made in response to reviewer comments.	Supplementary material – SPIRIT checklist
4) I can't understand where the blinded pathologists work, please specify. Which is their affiliation?	The blinded pathologists work at Royal Prince Alfred Hospital **Previous wording:** 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 **New wording:** 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	8

5) The protocol must provide a planning for biospecimen collection, processing, and storage.	**Response:** This has been updated in the study procedures. **New wording:** Study samples will be stored at the Garvan Institute for Medical Research.	7
6) Will the biospecimen analyses be centralised or not?	**Response:** Yes, the biospecimen analysis will be centralised and will be performed by the blinded pathologists. This has been updated in the protocol. **Previous wording:** Ki-67 expression will be assessed by pathologist review. **New wording:** Ki-67 expression will be assessed by central pathologist review.	7
7) Page 9, line 17, correct "with be" with "will be". I would suggest to re-check the whole protocol carefully for other typos.	**Response:** This has now been adjusted.	7
8) Are you planning to use the McNemar test? Since it might be more appropriate than a chi square test for comparing proportion in pre/post studies. Include it in the statistical plan, if it's the case.	**Response:** We agree that the McNemar test is appropriate for use in this paired data. The statistical plan has been updated. **Previous wording:** 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.	8

	New wording: 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 test.	

Reviewer: 2
Reviewer Name: **Susan Slovin**

Institution and Country: Memorial Sloan Kettering Cancer Center, USA

Please state any competing interests or state 'None declared': None

Please leave your comments for the authors below

The authors present the plan of a multicenter randomized phase II trial using the CDK4/6 inhibitor (LEE 011; ribociclib) (LEEP study) in patients with high-risk, hormone sensitive prostate cancer with focus on the pharmacodynamic effects of the drug. The trial is to recruit 47 men with high risk localized prostate cancer who are planned to undergo radical prostatectomy. Patient will be randomized 4:1 in favor of LEE011 400mg on a 21 day cycle. Primary endpoint is the frequency of a 50% reduction in tumor cell proliferation index (Ki-67) comparing post with pre-treatment tumor tissue. Secondary endpoints include pharmacodynamic assessment of CDK4/6 cell cycle progression via E2F levels, apoptotic death, PSA changes in tumor and serum and pathologic response.

This is a well-written essentially neoadjuvant clinical trial examining the potential pharmacodynamic and biologic impact of LEE001 in patients with high risk prostate cancer undergoing prostatectomy.

Thank you very much for your considered appraisal of this trial. We found your critique valuable and have attempted to address your specific comments.

Reviewer comment	**Response**	**Page**
It should be addressed what is defined as "high risk" patients.	**Added – previous wording** 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	6

	score of between 8 and 10 **Or** Preoperative PSA ≥ 20 ng/mL **AND** planned for radical prostatectomy; **New wording** 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;	
It is unclear why a 4:1 randomization is required despite what is written in the biostatistical section.	**Response:** A 4:1 randomisation is required to provide greater power and allow assessment of expected interpatient variability in biological responses to the study drug.	
Ki67 is not always deemed as a reliable biomarker; please indicate whether there is a particular reason that this marker was selected given that other measures of activity could be used. Not every "high risk" cancer behaves biologically as "high risk"; therefore some measure of concern about the ability of this biomarker to really capture biologic behavior.	**Response:** Yes, not all "high risk" cancers behave biologically high risk, but it is not always possible to identify this up front. Both Ki67 and Cleaved Caspase 3 has been used in previous neoadjuvant window studies in prostate and breast cancer (including a PI3 kinase pathway inhibitor [3] and an mTOR pathway inhibitor[4] in prostate cancer, and a CDK4/6 inhibitor in breast cancer[1]). Ki67 has been found in several studies to correlate with clinical response[5,6]. Although there can be inter-reader interpretation variability, Ki67 is a robust marker that is able to be performed on FFPE samples that may have been taken at the time of surgery at different sites, improving the reproducibility of our results. Biospecimen analysis will be centralised and will be performed by two independent pathologist researchers, with discrepancies resolved by consensus. In addition to Ki67, as our secondary endpoints, we plan to examine apoptosis by Cleaved caspase 3 and inhibition of CDK4/6 cell cycle progression by a	3

	decrease in E2F expression. This will allow us to examine further pharmacodynamic effects of Ribociclib in these patients. **Added:** 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].	
It is surprising that only 5 patients have been enrolled within one year. Might there be impediments to accrual?	**Response:** Since our submission a further 3 patients have been recruited. Given the slower than predicted accrual, 1 further site has opened already, and at least 3 further sites will open in 2020. We hope that this will improve the accrual. **Previous wording:** 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. **New wording:** 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	9

	futility assessment by the first quarter in 2021.	
Some mention should be made for dose reduction of ribociclib as this drug can cause neutropenia.	**Response:** We also had concerns regarding neutropenia with Ribociclib treatment. To this end, we have chosen to use a lower dose of Ribociclib (400mg daily) than that used as a starting dose in women with breast cancer (600mg daily). Neutropenia is dose dependent. Given the short duration of Ribociclib treatment and our desire not to compromise or delay the surgical date, we have chosen not to allow dose modifications of Ribociclib in the trial. Patients who need to come off the study due to toxicity will discontinue and proceed to surgery as planned. **Previous wording:** 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. **New wording:** 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.	7

1. Curigliano G, Gómez Pardo P, Meric-Bernstam F, Conte P, Lolkema MP, Beck JT *et al*. Ribociclib plus letrozole in early breast cancer: A presurgical, window-of-opportunity study. *The Breast* 2016; **28:** 191-198.

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3. Anantharaman A, Nguyen HG, Cooperberg MR, Meng MV, Carroll P, Friedlander TW *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-e14110.

4. Armstrong AJ, Netto GJ, Rudek MA, Halabi S, Wood DP, Creel PA *et al*. A Pharmacodynamic Study of Rapamycin in Men with Intermediate- to High-Risk Localized Prostate Cancer. *Clinical Cancer Research* 2010; **16**(11)**:** 3057.

5. Baselga J, Semiglazov V, Van Dam P, Manikhas A, Bellet M, Mayordomo J *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)**:** 2630-2637.

6. Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, A'Hern R *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)**:** 167-170.

VERSION 2 – REVIEW

REVIEWER	Francesco Schettini University of Naples Federico II, Naples, Italy and IDIBAPS, Barcelona, Spain
REVIEW RETURNED	20-Dec-2019

GENERAL COMMENTS	The revised version of the protocol and the authors' replies fairly address all of my concerns and provide satisfying new details or explanations for some investigators' choices. I have no more revisions to suggest.

REVIEWER	Slovin, Susan Memorial Sloan Kettering Cancer Center
REVIEW RETURNED	23-Dec-2019

GENERAL COMMENTS	authors have responded to reviewers' comments in a satisfactory manner