

Title: Pharmacodynamic effects of ribociclib in non-metastatic prostate cancer: the LEEP randomised trial

Authors: Tahlia Scheinberg^{1,2,3,4}, James G Kench^{2,3,4,5}, Peter Ferguson^{2,4,5}, Sarah Sutherland^{1,2,4}, Nariman Ahmadi^{1,2,6}, Andrea Alimonti^{7,8,9,10,11}, Georgina Clark¹², Manuel Colucci^{7,8}, Ian D Davis^{13,14}, Andrew Fuller^{15,16,17}, Anthony M Joshua^{3,18,19}, Scott Leslie^{1,2,4}, Kate L Mahon^{1,2,3,4}, Gavin Marx^{20,21}, Tony Maxwell, Rhiannon Mellor^{1,3,19,22}, Rachel L O'Connell²³, Shomik Sengupta^{13,14}, Martin R Stockler^{1,2,3,4,6,23}, Phillip Stricker^{3,18,19}, Ruban Thanigasalam^{1,2,6}, Henry H Woo^{1,24}, Alison Y Zhang¹, Margaret M Centenera^{16,25}, Lisa M Butler^{16,25*}, Lisa G Horvath^{1,2,3,4,19*}.

Affiliations:

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. Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, NSW Health Pathology, Sydney, Australia
6. Concord Hospital, Sydney, Australia
7. Institute of Oncology Research, Bellinzona, Switzerland
8. Faculty of Biomedical Sciences, Università della Svizzera italiana, Lugano, Switzerland
9. Department of Health Sciences and Technology, ETH Zurich, Switzerland
10. Department of Medicine, University of Padova, Padova, Italy
11. Oncology Institute of Southern Switzerland, Ente Ospedaliero Cantonale, Bellinzona, Switzerland
12. Charles Perkins Centre, School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia
13. Eastern Health Clinical School, Monash University, Box Hill, VIC, Australia
14. Eastern Health, Box Hill, VIC, Australia
15. Department of Surgery, Royal Adelaide Hospital, Adelaide, SA, Australia
16. The University of Adelaide, Adelaide, Australia
17. South Terrace Urology, Adelaide, Australia
18. St Vincent's Hospital, Sydney, Australia
19. University of NSW, Sydney, Australia
20. SAN Clinical Trial Unit, Sydney Adventist Hospital, Integrated Cancer Centre, Wahroonga, Australia
21. ANU Medical School, Australian National University, Canberra, Australia
22. Westmead Hospital, Sydney, Australia
23. NHMRC Clinical Trials Centre, University of Sydney, Sydney, Australia
24. Blacktown Mount Druitt Clinical School, Western Sydney University, Sydney, Australia
25. South Australian Health and Medical Research Institute, Adelaide, Australia

*Authors contributed equally (joint corresponding authors)

Background: Neoadjuvant pharmacodynamic studies facilitate rational decisions about which therapies should progress to phase 2/3 trials. CDK4/6 inhibitors are standard care in advanced breast cancer, but have poor efficacy in metastatic prostate cancer (PCa). In preclinical patient-derived prostate-tissue explants (PDEs), 22/30 (77%) PDEs showed >50% reduction in proliferation (Ki-67) with 500nM ribociclib compared to vehicle. We sought to evaluate the pharmacodynamic effects of the CDK4/6 inhibitor ribociclib, in high-risk non-metastatic hormone-sensitive PCa.

Methods: In this open-label, window-of-opportunity trial, participants at 3 Australian sites awaiting radical prostatectomy (RP) were randomised 4:1 to treatment with or without ribociclib 400mg daily for 21 days prior to RP. The primary endpoint was a 50% reduction in Ki-67 expression from pre-treatment biopsy to RP specimen. Immune cell changes in peripheral blood and tissue were also examined.

Results: Between 2018 and 2022, 33 participants planned for radical prostatectomy (RP) were randomized to ribociclib 400mg daily for 21 days or control (24 evaluable for response). The primary endpoint was met. When examining Ki-67, ≥50% reduction in Ki-67 was observed in 10/17 (58%)

assigned ribociclib versus 2/7 (29%) assigned controls. Reduced E2F-1 expression ($p=0.008$) indicated on-target inhibition of CDK4/6 cell cycle progression.

In a post-hoc exploratory analysis, an alternative Ki-67 scoring method, the "hotspot" method demonstrated $\geq 50\%$ reduction in 5/17 (29%) assigned ribociclib versus 0/7 (0%) controls.

When analysing circulating immune cells, there were significant reductions in CD1c dendritic cells ($p=0.009$), CD141 dendritic cells ($p=0.009$) and myeloid derived suppressor cells ($p=0.012$) following treatment with ribociclib compared with controls. At a cancer tissue level, in the tumour as a whole, ribociclib treatment resulted in fewer cytotoxic T cells ($p=0.049$), and MHC class I high expressing CD3+ cells ($p=0.028$) compared to those on the control arm, indicating an immune tolerant phenotype. We explored differences in immune infiltrates between the peak proliferative areas and the cancer globally. There was a less pronounced reduction in B cells and macrophages in the lower proliferative areas, compared to the peak Ki-67 areas. There was no difference in the M1:M2 ratio.

Conclusions: The LEEP trial met the primary endpoint with more frequent $\geq 50\%$ reductions in Ki-67 after ribociclib treatment. There were less changes in Ki-67 hotspots leading to the hypothesis that the Ki-67 proliferative hotspots are less responsive to CDK4/6 inhibitors. Ribociclib treatment was associated with an immune-tolerant tissue phenotype, with a more pronounced immune-resistant phenotype within the peak Ki-67 ribociclib resistant areas. This differential immune cell infiltrate provides a hypothesis for the heterogeneous response within the tumour to ribociclib, and suggests that the tumour immune microenvironment in PCa may be contributing to the disappointing results in advanced PCa.

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Conflicts of Interest Disclosure Statement:

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