Circulating tumour DNA analyses reveal novel resistance mechanisms to CDK inhibition in metastatic breast cancer

Cyclin-dependent kinase (CDK) 4/6 inhibition has been demonstrated to improve progression-free survival (PFS) in patients with human epidermal growth factor receptor 2 (HER2−), hormone receptor positive (HR+) in advanced breast cancer [1–3]. Palbociclib, ribociclib and abemaciclib are orally bioavailable selective CDK 4/6 inhibitors. These small molecules likely bind the ATP-binding pocket within the CDK4/6 protein kinases thereby inhibiting phosphorylation of retinoblastoma tumour suppressor protein (Rb). In its hypophosphorylated state Rb function as a potential resistance mechanism to CDK4/6 inhibition. They provide a case-series of three patients treated at different institutions, by separate investigators, who developed resistance to CDK4/6 inhibitors. In each case evidence of somatic alteration at the point of disease progression. In the first patient a fragile Rb chromosome was detected in a liver biopsy acquired before CDK4/6 inhibition. In the second patient a new RB1 mutation was detected in circulating tumour DNA (ctDNA) in plasma that originated from a tumour biopsy obtained before treatment. In the third patient a heterozygous RB1 mutation (c.1877G>A, p.R626H) was identified in tumour ctDNA from a biopsy obtained before treatment, which was confirmed in a subsequent biopsy obtained after progressing on CDK4/6 inhibition. In each case, the new RB1 mutation was not detected in ctDNA from the plasma of the same patient before treatment. The observed efficacy of CDK inhibition in metastatic breast cancer may relate to a dependence of HR+/HER2− breast cancer on CDK4/6 activity to override Rb mediated repression of cell cycle progression (Figure 1) [5].

CDK4/6 inhibitors have been approved by the US Food and Drug Administration (FDA) for initial endocrine therapy in postmenopausal women with metastatic or advanced HR+/HER2− breast cancer in combination with an aromatase inhibitor and for the treatment of endocrine therapy-resistant HR+/HER2− advanced or metastatic breast cancer in combination with Fulvestrant (a selective estrogen receptor degrader) [6]. In December 2017 the National Institute for Health and Care Excellence (NICE) has recommended CDK4/6 inhibitors in combination with aromatase inhibition as a first-line option for treating locally advanced or metastatic HR+/HER2− breast cancer [7]. Despite the success of the clinical studies that led to these recommendations, not all patients with HR+ breast cancer respond to CDK inhibition and a significant fraction progress within 2 years of initiation of treatment [1–3]. This underscores the need to identify mechanism of resistance to these targeted therapies to anticipate and target novel or subclonal resistance mechanisms driving breast cancer progression in these patients.

Circulating tumour DNA (ctDNA) describes molecules of cell-free DNA circulating in plasma that originate from a patient’s tumour. ctDNA analyses by next-generation sequencing are demonstrating translational utility within clinical contexts ranging from non-invasive screening [8], tracking cancer burden and identifying residual disease in patients undergoing treatment of their disease [9–11] and identifying cancer associated mutations with therapeutic implications [12, 13]. In this edition of Annals of Oncology Condorelli et al. [14] leverage the ability of ctDNA analysis to interrogate the mutational landscape of progressive metastatic cancer to highlight loss of Rb function as a potential resistance mechanism to CDK4/6 inhibition. They provide a case-series of three patients treated at different institutions, by separate investigators, who developed progressive metastatic breast cancer following treatment with CDK4/6 inhibitors. In each case evidence of somatic alteration involving the RBB1 gene was noted through plasma ctDNA analyses at the point of disease progression. In the first patient a frameshift event involving exon 8 of RBB1 was observed that was predicted to result in a non-functioning truncated version of the protein. This event was not observed through NGS analysis of a liver biopsy acquired before CDK4/6 inhibition. In the second patient of the case-series four RBB1 alterations were noted at progression on palbociclib that were not detectable before initiation of therapy. The variant with the highest allele frequency in
plasma at progression (Chr13(GRCh37): g.48937094G>A) has been previously shown in lung cancer to result in loss of the Rb protein region responsible for the binding of Rb to E2F-transcription factor complexes [15]. The final patient was observed to have a p.His483Tyr RB1 variant following ribociclib that is predicted to be deleterious.

This study is of interest for the following reasons. Firstly, it provides observational evidence of deleterious RB1 alterations potentially being selected at disease progression following intervention with CDK4/6 inhibitors in patients with metastatic breast cancer. These observations build on a previous in vivo investigation of CDK4/6 inhibitor resistance using patient-derived tumour xenograft models that suggested Rb1 inactivation as a resistance mechanism to chronic CDK4/6 inhibition [16]. Secondly, this study provides an early glimpse into the potential of ctDNA panels to detect acquisition of actionable alterations in patients who experience disease progression on anticancer therapy. Such a resource could inform mechanisms underlying resistance across a range of systemic therapies. There are advantages to ctDNA analyses as a research tool to understand the biology of heavily treated metastatic disease. The non-invasive nature of ctDNA examination overcomes barriers to tissue acquisition in late stage disease that include poor patient health, increased risk from biopsy procedures and cost.

There are however caveats to consider regarding this case-series. The number of patients described within the manuscript is small and there is no indication as to the frequency by which Rb1 alterations are detected at progression on CDK4/6 inhibition in this patient population. Additionally, patients 1 and 3 in the case-series were treated with two lines of therapy in between the biopsies showing lack of RB1 alterations and ctDNA analyses demonstrating acquired RB1 alterations—patient 1 received everolimus and exemstane before palbociclib and patient 2 received capcitabine and paclitaxel following ribociclib. Therefore, we cannot be certain that the acquisition of Rb1 alterations solely associate with selective pressure induced by CDK4/6 inhibition. Advancing the findings reported in this case-series will require a larger cohort to determine the incidence of Rb1 alterations as resistance mechanisms in patients with metastatic breast cancer on CDK4/6 inhibitors. Furthermore, more frequent ctDNA monitoring is necessary to follow the dynamics by which RB1 alterations emerge and ascertain the association of their emergence with disease progression.

Given this work, it is notable that CDK4/6 inhibition has recently been associated with increasing tumour cell antigen presentation through a mechanism involving downregulation of Rb1-E2F induced DNA methyltransferase 1 (DNMT1) activity, increased expression of endogenous retroviral elements and type III interferon production [17]. This response to CDK4/6
inhibition was ameliorated by silencing of RB1 and therefore could conceivably underlie an immune predatory selection pressure toward selection of Rb1 altered populations whilst undergoing treatment with CDK4/6 inhibitors. The fact that CDK4/6 inhibition has recently been shown to increase PD-L1 expression in mouse models of breast cancer provides a clear rationale for anti-PD1 treatment as a combination therapy with CDK4/6 inhibition before the emergence of Rb1 loss of function [18].

C. Abbosh1, C. Swanton1,2 & N. J. Birkbak1,2*
1Cancer Research UK Lung Cancer Centre of Excellence, London and Manchester; University College London Lung Cancer Institute, London; 2Translational Cancer Therapeutics Laboratory, The Francis Crick Institute, London, UK (*E-mail: nicolai.birkbak@crick.ac.uk)

Funding
This work is supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001169, FC001202), the UK Medical Research Council (FC001169, FC001202) and the Wellcome Trust (FC001169, FC001202). CS is funded by Cancer Research UK (TRACERx and CRUK Cancer Immunotherapy Catalyst Network), the CRUK Lung Cancer Centre of Excellence, Stand Up 2 Cancer (SU2C), the Rosetrees Trust, NovoNordisk Foundation (ID 16584), the Prostate Cancer Centre of Excellence, Stand Up 2 Cancer (SU2C), the Rosetrees Immunotherapy Catalyst Network), the CRUK Lung Cancer Centre of Excellence, London and Manchester; University College London Cancer Institute, London; and the Cancer Research UK University College London Experimental Cancer Medicine Centre.

Disclosure
The authors have declared no conflicts of interest.

References
1. Finn RS, Martin M, Rugo HS et al. Palbociclib and letrozole in advanced breast cancer. N Engl J Med 2016; 375(20): 1925–1936.
2. Finn RS, Crown JP, Lang I et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol 2015; 16(1): 25–35.
3. Cristofanilli M, Turner NC, Bondarenko I et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. Lancet Oncol 2016; 17(4): 425–439.
4. Ashgar U, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov 2015; 14(2): 130–146.
5. Thangavel C, Dean JL, Ertel A et al. Therapeutically activating RB: re-establishing cell cycle control in endocrine therapy-resistant breast cancer. Endocrine Relat Cancer 2011; 18(3): 333–345.
6. Walker AJ, Wedam S, Amiril-Kordestani L, Bloomquist E, Tang S, Sridhar RA et al. FDA approval of palbociclib in combination with fulvestrant for the treatment of hormone receptor-positive, HER2-negative metastatic breast cancer. Clin Cancer Res 2016; 22(20): 4968–4972.
7. National Institute for Health and Care Excellence. Palbociclib with an aromatase inhibitor for previously untreated, hormone receptor-positive, HER2-negative, locally advanced or metastatic breast cancer. Technology Appraisal Guidance [TA495], December 2017.
8. Aravanis AM, Lee M, Klauser RD. Next-generation sequencing of circulating tumor DNA for early cancer detection. Cell 2017; 168(4): 571–574.
9. Chaudhuri AA, Chabon JJ, Lovejoy AF et al. Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. Cancer Discov 2017; 7(12): 1394–1403.
10. Garcia-Murillas I, Schiavon G, Weigelt B et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med 2015; 7(302): 302ra133.
11. Tie J, Wang Y, Tomasetti C et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med 2016; 8(346): 346ra92.
12. Zheng D, Ye X, Zhang MZ et al. Plasma EGFR T790M ctDNA status is associated with clinical outcome in advanced NSCLC patients with acquired EGFR-TKI resistance. Sci Rep. 2016; 6(1): 20913.
13. Thierry AR, El Messaoudi S, Mollevi C et al. Clinical utility of circulating DNA analysis for rapid detection of actionable mutations to select metastatic colorectal patients for anti-EGFR treatment. Ann Oncol 2017; 28(9): 2149–2159.
14. Condorelli R, Spring L, O’Shaughnessy J et al. Polyclonal RB1 mutations and acquired resistance to CDK 4/6 inhibitors in patients with metastatic breast cancer. Ann Oncol 2018; 29(3): 640–645.
15. Liu J, Lee W, Jiang Z et al. Genome and transcriptome sequencing of lung cancers reveal diverse mutational and splicing events. Genome Res 2012; 22(12): 2315–2327.
16. Herrera-Abreu MT, Palafox M, Asghar U et al. Early adaptation and acquired resistance to CDK4/6 inhibition in estrogen receptor–positive breast cancer. Cancer Res 2016; 76(8): 2301–2313.
17. Goel S, DeCristo MJ, Watt AC et al. CDK4/6 inhibition triggers anti-tumour immunity. Nature 2017; 548(7668): 471.
18. Zhang J, Bu X, Wang H et al. Cyclin D–CDK4 kinase destabilizes PD-L1 and therefore
Published online 17 January 2018

Uncovering the links between systemic hormones and oncogenic signaling in the pathogenesis of meningioma
A number of risk factors have been associated with meningioma development including radiation exposure (radiation-induced meningioma), female gender, germline mutations, high body mass index and hormone exposure (Figure 1). The relationship between meningioma risk and sex hormones has been of keen interest for decades, sparked by several observations. The most important of these has been the finding of estrogen receptor and progesterone receptor (PR) expression in a substantial portion of meningiomas [1, 2]. In addition, a link between meningiomas and hormones has been supported by the skewed gender distribution of meningiomas [3]. Low-grade meningiomas develop two times more often in women than in men, and three times more