A Signal-seeking Phase Iia Trial of Palbociclib in Advanced Cancers With Cell Cycle Pathway Alterations – A Substudy of the Molecular Screening and Therapeutics (Most) Program

Subotheni Thavaneswaran (s.thavaneswaran@garvan.org.au)
Garvan Institute of Medical Research, University of NSW

Lucille Sebastian
The NHMRC Clinical Trials Centre, University of Sydney

Maya Kansara
Garvan Institute of Medical Research, University of NSW

Mandy Ballinger
Garvan Institute of Medical Research, University of NSW

David Espinoza
The NHMRC Clinical Trials Centre, University of Sydney

Frank Lin
Garvan Institute of Medical Research, University of NSW

Chee Lee
The NHMRC Clinical Trials Centre, University of Sydney

Katrin Sjoquist
The NHMRC Clinical Trials Centre, University of Sydney

John Grady
Garvan Institute of Medical Research, University of NSW

Mark Cowley
Children's Cancer Institute Australia

Anthony Joshua
The Kinghorn Cancer Centre, St Vincent's Hospital Sydney

Min Qiu
Sydpath, St Vincent's Hospital Sydney

Samantha Oakes
Garvan Institute of Medical Research, University of NSW

John Simes
The NHMRC Clinical Trials Centre, University of Sydney

David Thomas
Garvan Institute of Medical Research, University of NSW

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Abstract

BACKGROUND: The D-type cyclin and cyclin dependent kinase 4/6 (CDK) complex phosphorylates retinoblastoma protein, thereby driving cell cycle progression. This process is blocked by inhibitors of CDK4/6. As part of the Molecular Screening and Therapeutics program, this phase IIa trial tested the clinical activity of CDK4/6 inhibitor monotherapy in tumors with cell cycle pathway alterations.

PATIENTS AND METHODS: Eligible patients ≥ 18 years old, with advanced or metastatic solid cancers, along with amplification of *CDK4/6, CCND1/2/3,* or loss of function alterations in *CDKN2A* were recruited. The primary objective of this signal-seeking trial was to evaluate the clinical activity of palbociclib – a composite of objective responses and the ratio of time to progression (TTP) on palbociclib, to TTP on treatment preceding trial entry.

RESULTS: Ten patients had *CDK4/CDK6* or *Cyclin D1* amplifications, six patients had *CDKN2A* deletions. After a median follow-up of 35 months, there were no objective responses. Seven patients had stable disease and one had non-complete response/non-progressive disease (non-CR/non-PD) based on evaluation of a non-target lesion. Two of these seven patients maintained stable disease for at least 6 months, as did the patient with non-CR/non-PD. Median PFS and OS were 3.5 and 11.0 months respectively. No unexpected toxicities were observed. Translational correlates yielded strategies for targeting cell cycle interactions with other molecular pathways and the immune system.

CONCLUSION: Palbociclib monotherapy was not associated with any objective responses, but stable disease lasting at least 6 months was observed in 19% of patients. There was no clear relationship with alteration type or histotype. The signals of immune activation provide insights into the design of future trials, with a combination approach adding checkpoint blockade to CDK4/6 inhibition.

Background

Cyclin D-dependent kinases 4 and 6 (CDK4 and CDK6) control cell cycle transition between G1 and the S phase, where DNA synthesis is initiated, leading to cell cycle progression (1, 2). *CDK4* acts as an oncogene in many cancers, resulting in phosphorylation and inactivation of the retinoblastoma tumour suppressor (Rb) (3–5). Aberrant activation of genes encoding cyclin D1 (*CCND1*) and CDK4 (6, 7), or inactivation of the *CDKN2A* locus, whose product p16 (INK4A) normally inhibits cyclin D-CDK4/6 kinase activity and may lead to uncontrolled cell cycle progression (8–14). Beyond its cell cycle effects, there is emerging pre-clinical evidence that CDK4/6 inhibition can reverse immune evasion by cancer cells (15–18). This includes stimulation and associated infiltration of programmed death (PD-1) expressing T-cells, improved antigen processing potential and a reduction in the CD4 + FOXP3 + Treg suppressor cells (15).

Palbociclib is a highly selective pyrido[2,3-d]pyrimidine derivative that acts as a reversible oral inhibitor of both CDK4 and CDK6 (19). Palbociclib arrests the proliferation of tumor cells that retain functional Rb, blocking its phosphorylation on CDK4/6-specific sites. It has demonstrated primarily cytostatic effects on a range of cancer cell lines (20–25), as well as in clinical trials. A phase I trial (N = 33) of palbociclib

monotherapy in Rb protein-positive advanced cancers demonstrated 3% partial response and 29% stable disease rates (26). In a phase II study (27) of advanced well-differentiated/dedifferentiated liposarcomas, characterized by *CDK4* amplification (28–30), the 12-week PFS rate was 66% (27). A phase 2 trial in Rb protein-positive advanced estrogen receptor positive breast cancer yielded 5% partial responses and 14% stable disease for at least 6 months (31). A subsequent phase 3 trial in advanced, estrogen-receptor positive breast cancer provides strong clinical evidence for the addition of palbociclib to anti-estrogen therapies as an effective method of overcoming endocrine resistance (32–34).

The Molecular Screening and Therapeutics (MoST) Program is a precision medicine platform, in which patients are assigned to treatment baskets on the basis of genomic alterations identified through genomic profiling of an archival tumor sample, irrespective of histotype, or pan-cancer (35). The MoST1 trial evaluated palbociclib monotherapy in patients whose tumors harboured cyclin-D–CDK4/6 pathway alterations. Here, we report the clinical efficacy and safety amongst patients who participated in this study.

Materials And Methods

Study Design and Population

The MoST Program is designed to evaluate signals of clinical activity for treatments targeting actionable genomic alterations found in any tumor type. A more detailed study overview has been published previously (35). In brief, patients with treatment-refractory, advanced cancers of any histotype underwent tumor profiling to identify eligible patients for the therapeutic studies. These studies were phase Ib/IIa open-label trials with the flexibility for individual trials to open and close whilst other baskets continued accrual.

In the MoST1 trial, we enrolled patients with solid tumors harboring *CDK4/6 or CCND1/2/3*- amplification with six or more copies, or *CDKN2A* biallelic deletion or loss of function mutations. Patients with breast cancer, mantel cell lymphoma, myeloma and germ cell tumors were excluded. Patients were required to have an Eastern Cooperative Oncology Group performance status (ECOG PS) 0–2; evaluable disease by Response Evaluation Criteria in Solid Tumors (RECIST v1.1) (36) and adequate hepatic, renal and bone marrow function. Patients were required to have failed (or be intolerant of) standard therapies for their tumor type, and not previously received treatment with CDK inhibitors.

The study was performed in accordance with the Declaration of Helsinki, with central or institutional ethics and local research governance approval.
An independent data and safety monitoring committee provided independent assessments of patient safety and trial progress.

Study Treatment and Assessments

Palbociclib was administered orally once daily on a 28-day cycle at 125mg for days 1 to 21 and no treatment on days 22 to 28. Treatment continued until disease progression, unmanageable toxicity, or a decision by the patient or clinician to cease. Up to three dose reductions were permitted. Treatment toxicities were evaluated using the National Cancer Institute Common Terminology Criteria, version 4.03 (37). Response assessment was performed every 8 weeks.

Study Endpoints

The primary endpoint of the trial was to evaluate clinical activity, using a composite endpoint of objective response rate (ORR), and the ratio of time to progression (TTP) on trial (TTP2), to TTP on the last line of therapy (TTP1). In a pan-cancer setting, using patients as their own control informs the rate of change in disease trajectory for that individual, with a TTP2/TTP1 ratio of 1.3 suggesting clinical activity (38, 39). If TTP1 was not evaluable, TTP2 > 6 months was pre-specified to indicate clinical activity. Secondary endpoints included progression-free survival (PFS), overall survival (OS), safety, health-related quality of life measured by the EORTC QLQ-C30 (40), and Brief Pain Inventory (BPI) assessment (41).

Several translational correlatives were also evaluated. We examined the co-occurrence of genomic alterations – both by histotype and by molecular signatures with potential collateral effects on cell cycle pathway inhibition (42, 43). We investigated the immune effects of CDK4/6 inhibition by assessing PD-L1 status, tumor infiltrating lymphocytes (TILs) and gene expression signatures corresponding to T-cell inflammation (44) in tissue samples obtained following palbociclib treatment, compared with baseline pre-treatment/archival samples. PD-L1 expression on tumor cells was determined by immunohistochemistry (IHC) using Ventana SP263 rabbit monoclonal antibody assay. TILs were assessed using a hematoxylin and eosin-stained slide which permitted morphological discrimination of lymphocytes in the tumor and its immediate periphery, as previously described (45–49). The TILs level was calculated using the proportion TILs, of total cells on the slide. Evaluated gene expression signatures comprised of a set of 128 genes, including 5 housekeeping genes and 123 genes relating to T-cell biology, inflammation and immune response, and employing published methods (Appendix 1) (44, 50–52).

Statistical Considerations

A response rate of 40% supports the molecular hypothesis for this trial. Using the method of Metha-Cain, boundaries for declaring similar activity was determined based on a one-sided 95% confidence interval for ORR at 6 months which would include the hypothesized rate of 40% (53). Thus, for a sample size of 16 patients, objective responses in 3 or more patients constitutes a promising signal of activity.

Results

From November 2016 to December 2017, 16 patients were enrolled on the trial. A range of tumor types were included, with the most common histotype being bone and soft tissue sarcomas (N = 12, 75%) (Table 1). The median age was 54 years (range 19 to 75 years), and 56% were male and 56% had an ECOG PS of 0. Tumors from eight patients had *CDK4* amplification identified through tumor profiling and seven of these were orthogonally validated using fluorescent in situ hybridisation (FISH). Another six

patients had biallelic loss of *CDKN2A*, also orthogonally validated by FISH or IHC. The remaining two patients had more than one pathway alteration (Table 1).

Table 1
Baseline characteristics including molecular eligibility and validation (n = 16)

Characteristic	**No.**	**%**		
Median age, years (range)	**54 (19 to 75)**			
Male sex	**9**	**56**		
ECOG status				
0	**9**	**56**		
1	**6**	**38**		
2	**1**	**6**		
Median lines of systemic therapy (range)	**2 (0–5)**			
Cancer type			**Molecular eligibility**	**Orthogonal validation status and method**
Bone and soft tissue sarcomas	**12**	**75**		
Alveolar rhabdomyosarcoma	1	6	*CDK4* amplification	Validated by FISH
Chordoma	2	13	Biallelic loss of *CDKN2A*	Validated by FISH
Liposarcoma	6	38	*CDK4* amplification	5 validated by FISH, 1 unvalidated
Osteosarcoma	2	13	*CDK4; CDK6* and *CCND3* amplification	*CDK4* amplification validated by FISH, *CDK6* amplification by PCR
Solitary fibrous tumor	1	6	Biallelic loss of *CDKN2A*	Validated by FISH
Carcinomas	**4**	**25**		
Adrenal cortical carcinoma	1	6	Biallelic loss of *CDKN2A*	Validated by IHC
Pancreatic adenocarcinoma	2	13	Biallelic loss of *CDKN2A*	1 validated by FISH, 1 unvalidated
Small cell carcinoma	1	6	Biallelic loss of *CDKN2A* of *CCND1* amplification	*CCND1* amplification validated by FISH

Abbreviations: FISH - fluorescent in situ hybridization, IHC – immunohistochemistry.

Amongst enrolled patients, only 15 had RECIST measurable disease at baseline. After a median follow-up of 35 months, no patients achieved a complete or partial response. Seven patients achieved stable disease, with two patients maintaining stable disease for at least 6 months. All patients achieving stable disease had tumors with *CDK4* or *CDKN2A* alterations. One additional patient with no target lesion at baseline achieved a non-complete response/non-progressive disease by evaluation of the non-target lesion, which was also maintained for > 6 months. Eight patients had progressive disease as their best response, four confirmed radiologically and the remainder based on clinical progression.

Information on TTP from a previous line of therapy before study enrolment was available for only five patients (Appendix 2). Two of these five patients achieved a ratio of ≥1.3 for TTP on study compared with a previous line of treatment. The swimmer plot details TTP2 data, end of treatment, best response and death date by individual patient (Fig. 1).

Abbreviations: EOT – end of treatment, PD – progressive disease

The median PFS was 3.5 months (95% CI 1.8 to 5.4 months), and the 6-month PFS-rate was 16% (95% CI 3 to 38%). The median OS was 11.0 months (95% CI 4.3 to 32.0 months). The 6-month and 12-month OS rates were 63% (95% CI 35 to 81) and 44% (95% CI 20–66%) respectively (Fig. 2).

The most common adverse events (AEs) included general disorders (n = 14, 88% of patients) – comprised mainly of fatigue and pain; gastrointestinal disorders (n = 12, 75%) and cytopenias (n = 11, 69%), including anemia (n = 7, 44%), neutropenia (n = 7, 44%), and thrombocytopenia (n = 4, 25%). Grade 3 or worse adverse events were reported in 10 patients, and were comprised mainly of neutropenia, anemia, fever and sepsis (Appendix 3).

The median relative dose intensity (the ratio of administered doses to planned doses) was 97%. The dose of palbociclib was reduced in three patients (19%), with two of these patients also requiring treatment delays. An additional three patients (19%) required dose delays without dose reductions. There were no permanent treatment discontinuations due to toxicity.

The mean global EORTC QLQ-C30 was 15 points poorer on study (range − 41.7 points worse, to 4.2 points better) compared with baseline. The mean BPI score improved on study by 0.25 points (range − 5.25 points better to 1.96 points worse) compared with baseline, while the mean pain severity score was 0.29 points worse (range − 1.5 points better to 1.75 points worse) compared with baseline.

Translational correlates

The most common co-occurring molecular alterations were in *MDM2* (n = 6 liposarcomas, n = 1 osteosarcoma), *TP53* (n = 2 pancreatic adenocarcinomas, n = 1 osteosarcoma), KRAS (n = 2 pancreatic adenocarcinomas, n = 1 osteosarcoma) and NF1 (n = 1 pancreatic adenocarcinoma, n = 1 adenoid cystic carcinoma) (Fig. 3).

Three patients who had tumor samples obtained post-progression were evaluated in detail. One patient (P003) progressed after only two cycles of palbociclib with new brain metastases. A resected brain metastasis demonstrated no PD-L1 expression and very few TILs. Despite this, radiotherapy to the resection cavity followed by dual checkpoint blockade resulted in a partial RECIST response within the extensive lung metastases. Another patient (P009) received five cycles of palbociclib, with SD as best response. Tissue obtained at progression within the vertebral column displayed no PD-L1 expression and a 2-fold decline in TILs compared to the archival sample. On the other hand, one tumor sample (P001) obtained after exposure to four cycles of palbociclib and nine months of dual checkpoint blockade demonstrated a 30-fold increase in PD-L1 expression, 1.25-fold increase in immune cell PD-L1 expression and a 2-fold decline in TILs. There was also an upregulation of genes associated with immune activation and T-cell activation based on gene expression signatures (Appendix 4). This patient's best response on both treatments was SD.

Discussion

In this study there were no objective responses observed, however three patients (19%) maintained SD or non-CR/ non-PD for at least 6 months and two additional patients (13%) achieved a TTP2 to TTP1 ratio of > 1.3. Despite the absence of any objective responses, the median PFS and OS were 3.5 months and 7.5 months respectively. Taken together, these constitute weak evidence for clinical activity. Palbociclib was well-tolerated and AEs reported in this trial were consistent with other studies (27, 31). This study also reports supplementary translational correlates that can improve our understanding of the totality of effects of CDK4/6 inhibition.

A comparison to other studies that employed molecular selection demonstrates similar outcomes as our trial, with a histologically diverse patient population. The TAPUR study which evaluated palbociclib in a range of advanced cancer types, selected on the basis of *CDKN2A* alterations reported a disease control rate of 31% and 0% at 16 weeks in the non-small cell lung cancer (NSCLC) and pancreatic/biliary cancer cohorts, respectively (54, 55). The median PFS and OS of our trial (14 weeks and 30 weeks respectively) were also consistent with the TAPUR study for the pancreas (7.2 and 12.4 weeks), biliary (7.3 weeks and 11.1 weeks) and NSCLC (8.1 and 21.6 weeks) cohorts. Additionally, the 12-week PFS rate of 56% in our trial is consistent with the phase 2 liposarcoma trial with 12-week PFS rate of 66% (27). However, given the histological diversity and small representation of each tumor type in our study, comparisons according to histotype specific PFS or OS are limited. In the advanced breast cancer setting, the addition of palbociclib to endocrine therapy has demonstrated significant improvements in PFS (32). The rationale for its addition is founded on a notable emergence of cell cycle pathway alterations associated with estrogen resistance (56). An analysis performed within a subset of patients with *PIK3CA* mutations, indicating an alternative mechanism of endocrine resistance, demonstrated a comparable improvement in PFS (HR 0.45 and 0.48) to their *PIK3CA* wildtype counterparts, despite the absence of improved objective response rates (57). Taken together, these data support a predominantly cytostatic, rather than cytotoxic effect of palbociclib and highlight the potential importance of examining co-occurring mutations.

CDK4/6 activity serves as the common pathway between several extracellular signaling pathways and the cell cycle (58, 59). Prior studies have demonstrated that co-occurring molecular alterations can potentiate tumor senescence with CDK4/6 inhibition, where subsequent growth signals do not re-instate cell cycle progression (60–65). Important molecular aberrations include *MDM2* downregulation, chromatin-remodeling enzyme *ATRX* redistribution and repression of *HRAS* transcription (62, 66). In our trial, all six liposarcomas and an osteosarcoma demonstrated *MDM2* amplification, with other common alterations consisting of *TP53, KRAS* and *NF1*. Of these genes, particular alteration types in *MDM2, KRAS* and *NF1* are potentially also therapeutically actionable (67–70) and should be investigated in future studies of combinatory treatments to promote senescence. Furthermore, mechanisms beyond canonical cyclin-D–CDK4/6 pathway inhibition, involving immune detection and tumor eradication are increasingly recognized (71, 72). Previous studies have shown that CDK4/6 can enhance effector CD8 T-cell activity. Decreased overall TILS in the tumor microenvironment in two of the cases may reflect a reduction in the proliferation of suppressor regulatory T-cells, as has been observed with CDK4/6 inhibition in preclinical models (73). In the limited number of biospecimens available post-palbociclib treatment, we demonstrated a shift in immune parameters and improved disease control with checkpoint inhibitors as subsequent trial treatment. This provides further rationale for evaluating a combinatory approach – in this case, cell cycle inhibition with palbocliclib and immune checkpoint blockade, in future studies.

This study has several strengths. It recruited rapidly, collected meaningful clinical data, and discovered important hypothesis-generating molecular findings in the translational work that could inform the design of future trials. This study also has limitations. Inherent within a basket study design, the patient population consisted of a diverse range of tumor histotypes, with significant reliance on genomic biomarkers to define a common biological driver or pathway. The broad molecular eligibility may have been insufficient to optimize patient selection, and limited its signal seeking capacity. In the advanced breast cancer setting, no molecular features have emerged beyond estrogen receptor positivity (56), and the true predictive nature of *CDK4* amplification in liposarcomas is questionable, since 90% of these cancers display this particular molecular alteration (27, 74). In the absence of any objective tumor responses, quantifying benefits based on long-term outcomes using endpoints such as PFS is challenging, given the absence of appropriate historical controls to benchmark across a range of cancer histotypes. Using patients as their own control requires accurate data on TTP1 at study enrolment, which was not always available on this study. Limiting enrolment to those patients having recently progressed radiologically on their penultimate line of therapy would also facilitate interpretation of disease stabilisation.

The findings of our study have several implications. The translational correlates provide first hand insights into the logical development of future concepts. In this particular instance, the signals of immune activation provide further supportive rationale for tumor priming with CDK4/6 inhibition, followed by immune checkpoint blockade to increase the likelihood of an effective anti-tumor immune response (15, 16, 18). In fact, a MoST trial evaluating palbociclib plus avelumab, an immune checkpoint inhibitor is due to open shortly (Clinical trial registration number: ACTRN12620000568910p). Similarly, co-occurring mutations can also provide insights into worthwhile targeted therapy combinations with

potential for synergistic effects (75–77). In the presence of histological diversity, evaluating the effectiveness of a targeted therapy in the presence of a particular genomic target requires sufficient patient numbers and can be achieved more efficiently through international collaboration, particularly in rare and less common cancers.

In conclusion, palbociclib monotherapy has limited clinical activity in this study. Its proposed effects on the tumor microenvironment and immune cells is encouraging and warrants further evaluation of combination therapeutic strategies of CDK4/6 and immune checkpoint inhibition.

Declarations

Ethics approval and consent to participate

Ethics approval was provided by the St Vincent's Hospital Human Research Ethics Committee and all patients were at least 18 years of age and provided written informed consent to partake in the trial.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

Not applicable.

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Authors' contributions

LS, MB, DE, CL, KS, JS and DT were involved in the conception and design of the trial. Data collection was performed by ST, LS, MK, MB, MC and MRQ and SO. Data analysis and interpretation was performed by ST, MK, DE, FL, CL, JG, MC, AJ, JS and DT. ST, MK, DE, FL, CL and DT drafted the manuscript. All authors contributed to critical revision of the manuscript and approved the final published version.

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Figures

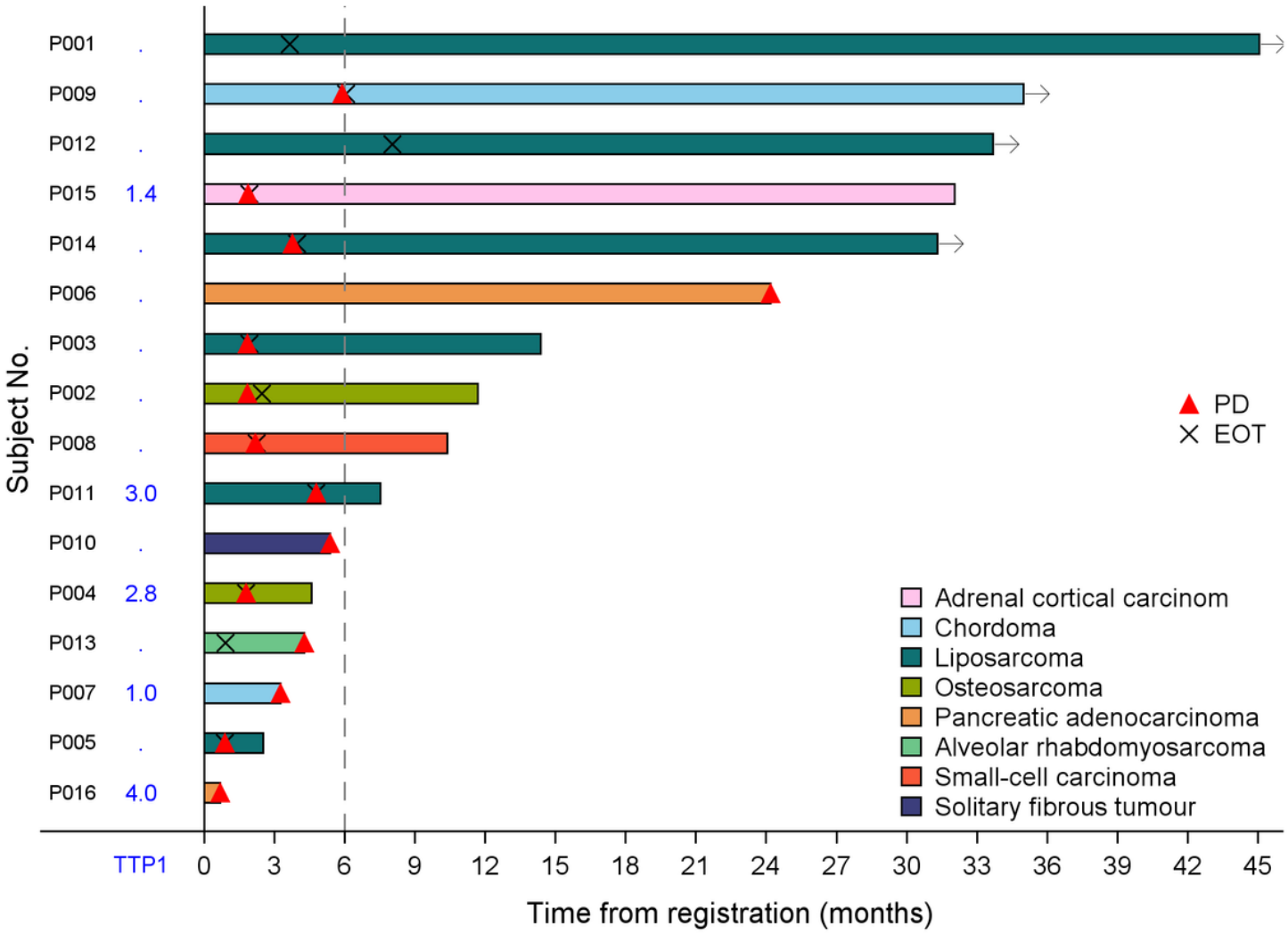

Figure 1

Swimmer plot by individual patient and cancer histotype. Abbreviations: EOT – end of treatment, PD – progressive disease

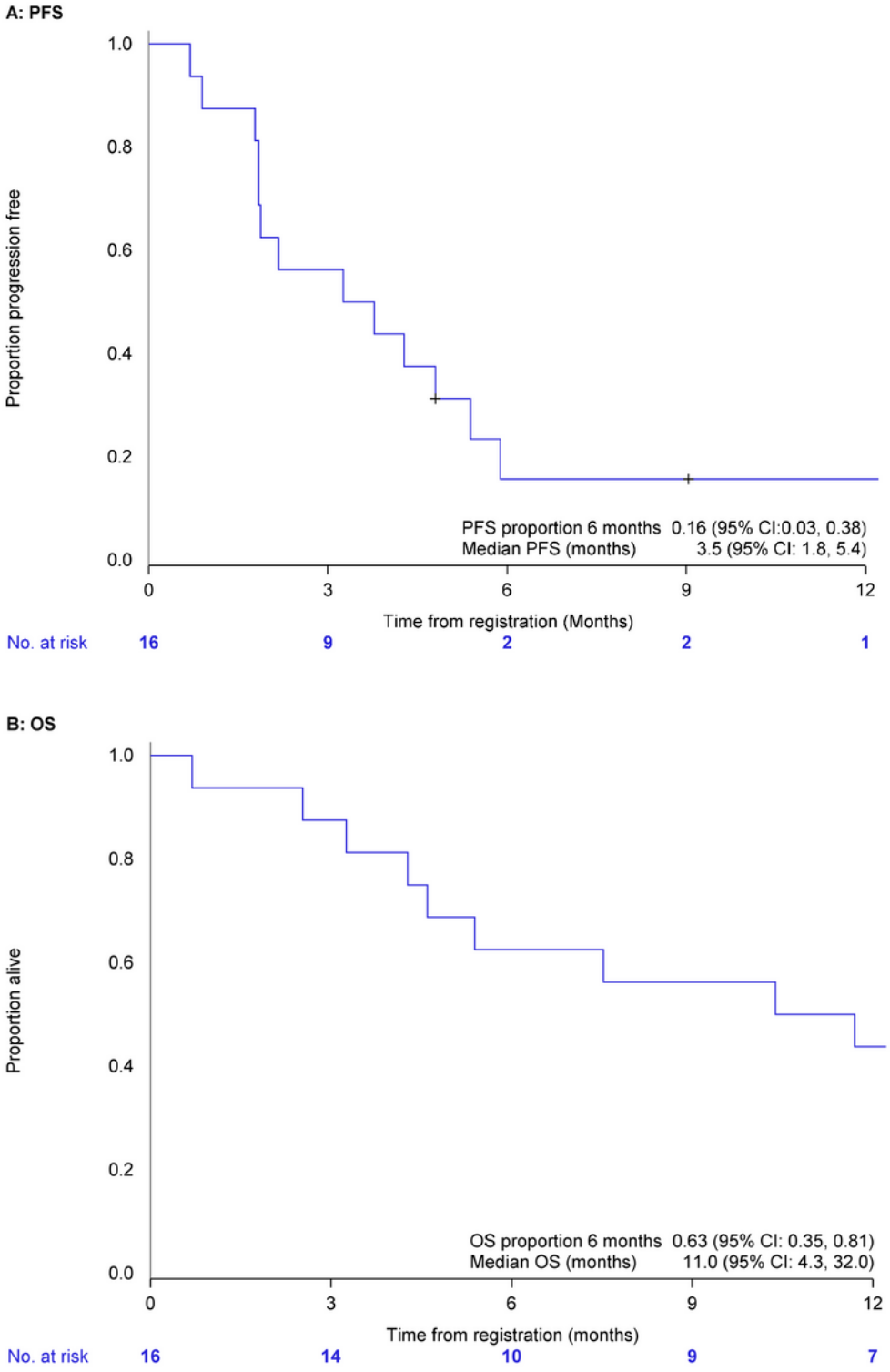

Figure 2

(A) Progression-free survival and (B) Overall survival

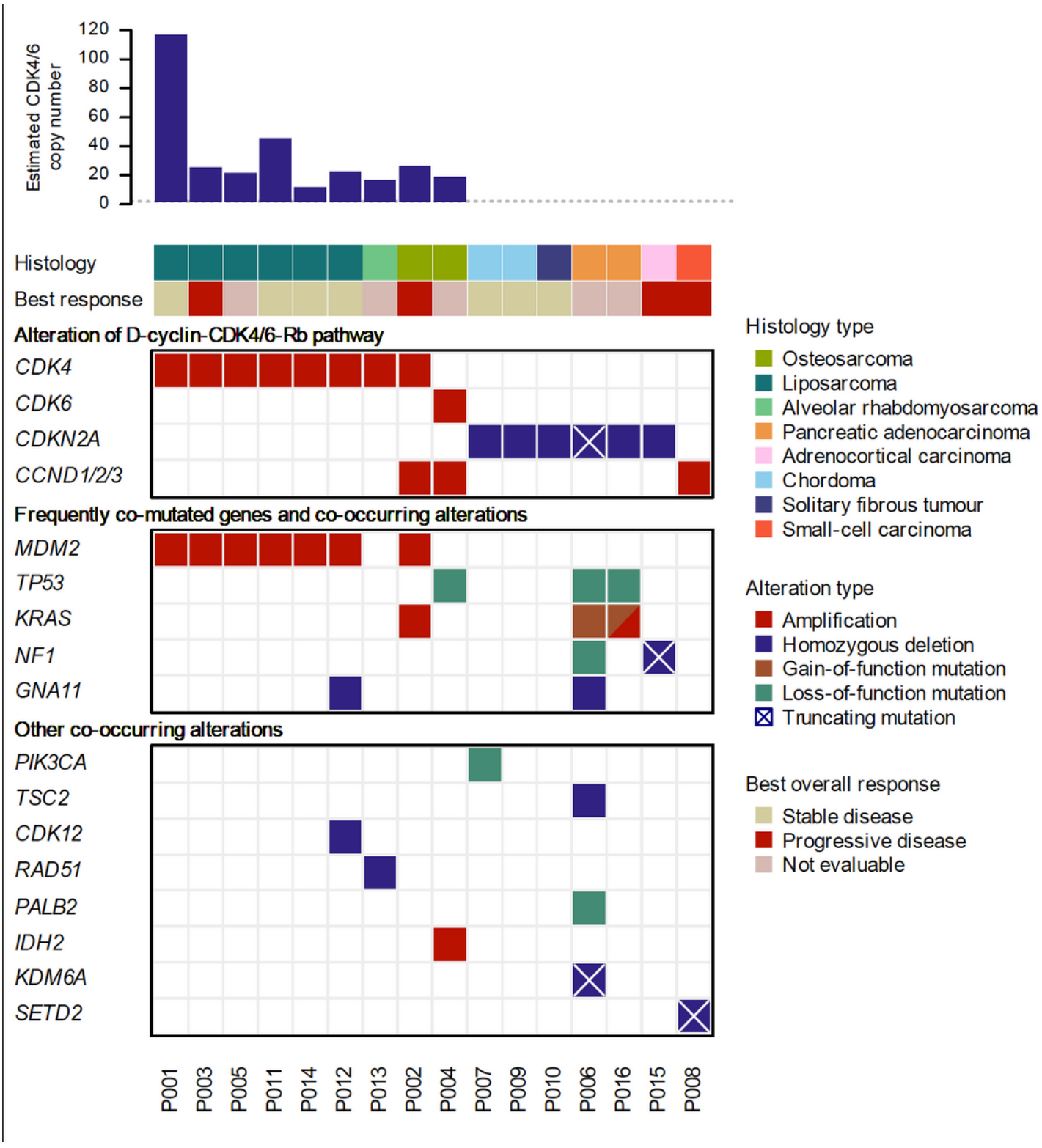

Figure 3

Co-occurring genomic alterations with cell cycle pathway alterations identified through the screening phase of the MoST program.

Supplementary Files

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- SupplementaryMaterialAppendix18Feb21.docx