Phase Ib Trial of the PI3K Inhibitor Copanlisib Combined with the Allosteric MEK Inhibitor Refametinib in Patients with Advanced Cancer

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Supplementary Material

Methods

Assessments

Safety assessments included adverse events and toxicities, vital signs, medical condition, laboratory parameters, Eastern Cooperative Oncology Group performance status, multiple-gated acquisition scans and echocardiograms, 12-lead electrocardiogram, and ophthalmologic examinations. Adverse events were documented and graded by severity using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

Plasma samples for pharmacokinetic assessments were collected for copanlisib on days 1 and 15 (in combination with refametinib) at pre-dose and 0.5, 1, 1.5, 2, 4, 6, 8, 12, 25, 49 (± 2), and 73 (± 2) hours post-dose, and for refametinib on days 14 and 15 at pre-dose and 0.5, 1, 2, 4, 6, 8, and 12 hours post-dose. Pharmacokinetic parameters were calculated using WinNonlin® version 5.3 (Pharsight Corporation, Princeton, NJ, USA).

Pharmacodynamic assessments included changes in [^{18}F]-fluorodeoxyglucose positron emission tomography uptake for patients in cohorts 2A, 2B, and 2C and the expansion cohort at screening and on cycle 1, day 22.

Biomarker assessments were performed using tumor and plasma samples. Submission of archival tumor samples was mandatory when available for patients enrolled into cohorts 2A, 2B, and 2C and the expansion cohort. Paired fresh tumor biopsies (optional in cohorts 2A, 2B, and 2C and mandatory in the expansion cohort) were collected during screening and within 48 hours after copanlisib administration on cycle 1, day 15. Plasma samples for isolation of circulating tumor DNA were collected during screening. Historical tumor mutation results (i.e., any tumor mutation result generated before enrollment into the trial)

were collected at screening where available for *KRAS*, *NRAS*, *BRAF*, and/or *PIK3CA*. Mutational analysis of *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* was performed on circulating tumor DNA using beads, emulsion, amplification, and magnetics (BEAMing) technology (Sysmex Inostics GmbH, Hamburg, Germany) [35]. Next-generation sequencing was performed on pretreatment (fresh when available, archival when not) tumor samples by Foundation Medicine (Cambridge, MA, USA) using the Foundation One® panel, which tests for mutations in 236 genes, as well as select rearrangements known to frequently occur in cancer. Fresh tumor samples were evaluated for pAKT, pERK, and Ki-67 using immunohistochemistry (IHC; Mosaic Laboratories, Lake Forest, CA, USA). PTEN IHC was also performed on pretreatment tumor samples (either fresh or archival). For each antigen, stained slides were scored by a pathologist for the percent of tumor cells staining positive at each intensity level (0, negative; 1+, low; 2+, medium; 3+, high); for each sample, the total percent of tumor cells staining positive was derived, as well as a H-score (H-score = [% of tumor cells staining at 1+ intensity] + 2 × [% of tumor cells staining at 2+ intensity] + 3 × [% of tumor cells staining at 3+ intensity]), with a maximum possible score of 300).

Statistical Analysis

Safety and efficacy analyses were performed in all patients who received at least one dose of the study medication. Summary statistics were used for demographic and safety variables, and frequency tables were used for qualitative data. Pharmacokinetic analyses, including descriptive statistics, were performed on all treated patients with available data. Biomarker analyses focused on patients treated at the maximum tolerated dose for the copanlisib/refametinib combination, which included patients from cohort 2C and the expansion cohort. Correlative biomarker analyses of best overall response compared patients with stable disease to those with progressive disease. PTEN IHC data were dichotomized as

"present" (any value >0) or "absent" (0 staining). Other IHC data were dichotomized using the median value as a cut-off, and were also analyzed as continuous variables.

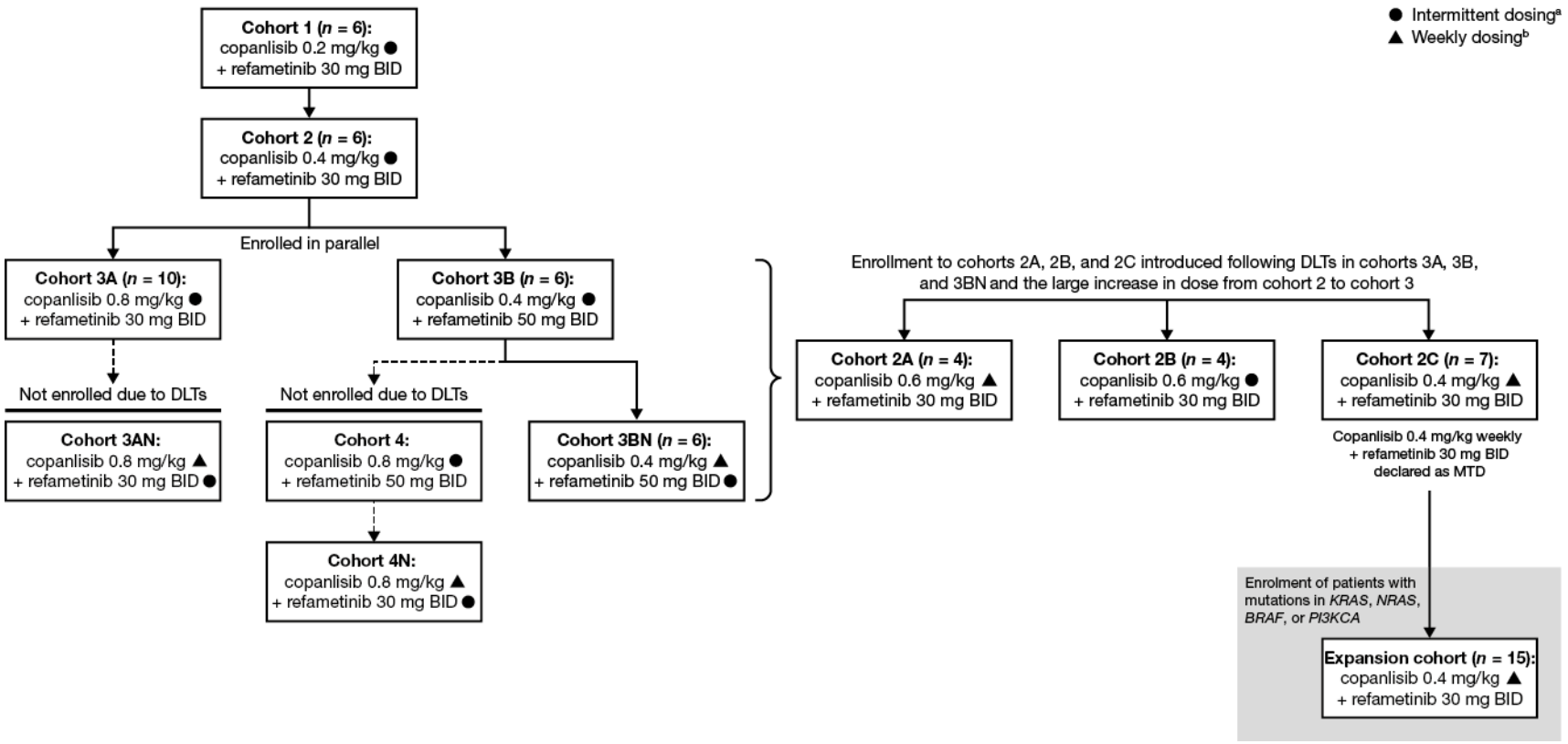
Dose-limiting Toxicities

Dose-limiting toxicities (DLTs) were assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. Non-hematological DLTs for copanlisib included any grade ≥ 3 hyperglycemia, following a fast of 8 hours, any grade 4 hyperglycemia, and any grade ≥ 3 non-hematological toxicity, with the exception of grade 3 nausea, vomiting, diarrhea, or untreated skin rash that could be adequately controlled with medical intervention, grade ≥ 3 lipase and/or amylase elevation without signs of pancreatitis, and grade 3 hypertension on the day of dosing that was manageable with medication.

Non-hematological DLTs for refametinib included missing >7 daily doses of refametinib due to drug-related toxicity, and grade ≥ 3 non-hematological toxicity, with the exception of grade 3 nausea, vomiting, diarrhea, or untreated skin rash that could be adequately controlled with medical intervention, grade ≥ 3 lipase and/or amylase elevation without signs of pancreatitis, grade 3 elevation of creatine phosphokinase, and some instances of grade 4 elevation of creatine phosphokinase.

Hematological DLTs for both copanlisib and refametinib included grade 4 neutropenia lasting ≥ 7 days, febrile neutropenia (absolute neutrophil count <1000 with fever ≥ 38.5˚C), grade 4 anemia, platelets <25,000/mm^3 or platelets <50,000/mm^3 associated with bleeding, international normalized ratio or partial thromboplastin time elevation of grade ≥ 3 with associated bleeding, and grade ≥ 3 hemorrhage or bleeding events.

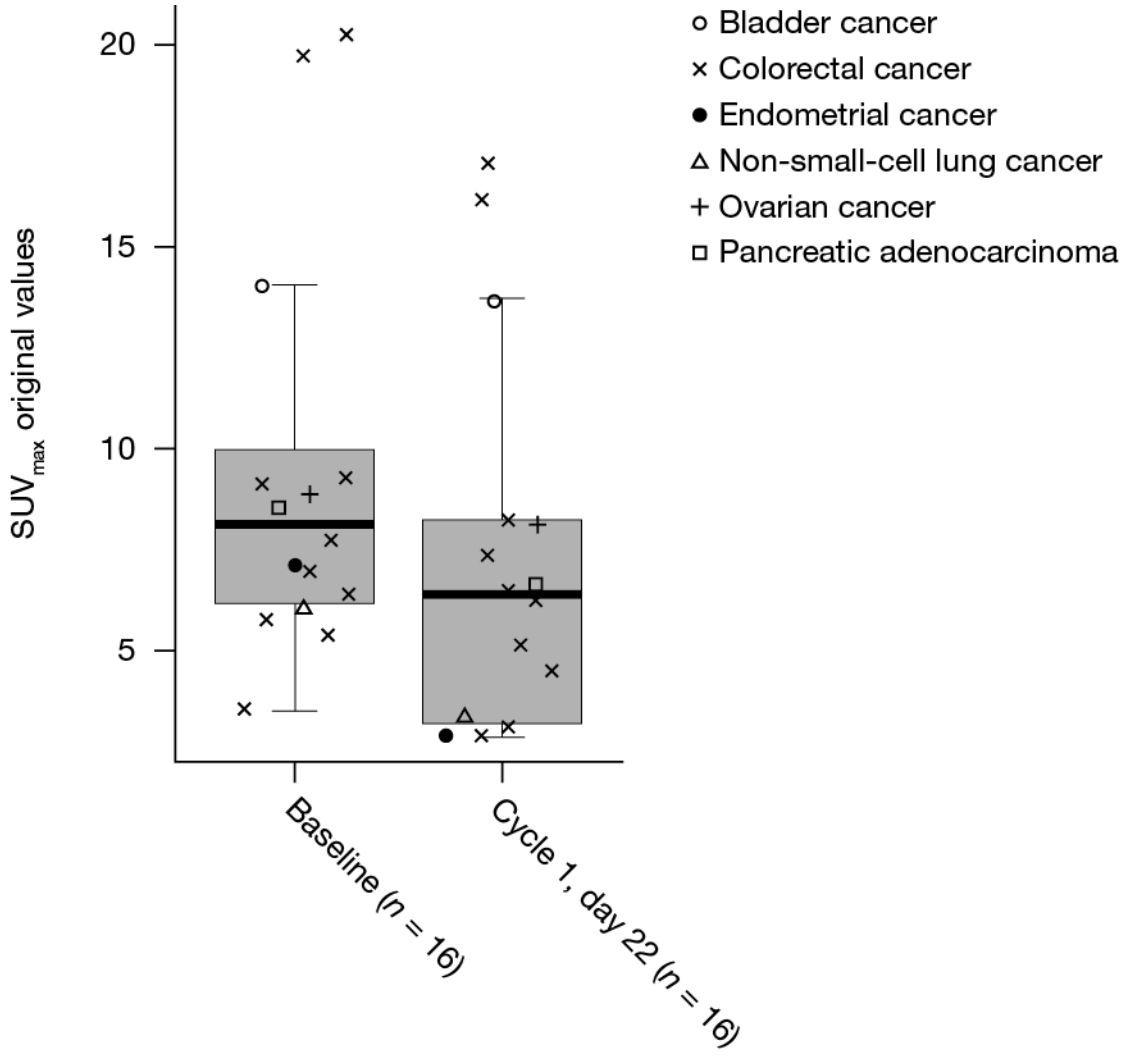
Supplementary Fig. S1 Dose-escalation schedule and number of enrolled patients



[a]Intermittent dosing (copanlisib 3 weeks on/1 week off or refametinib 4 days on/3 days off); [b]Weekly dosing of copanlisib

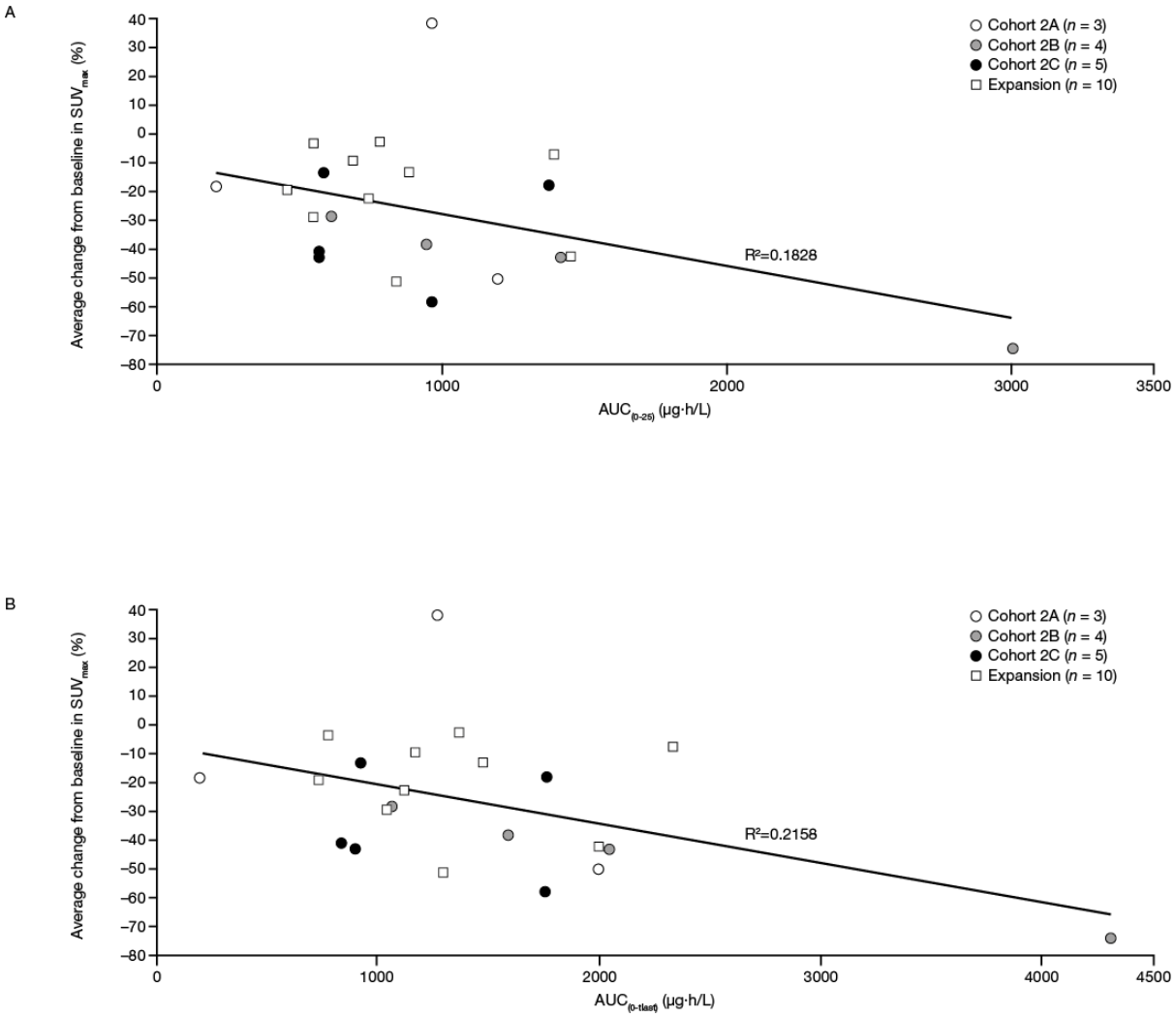
BID twice daily, *DLT* dose-limiting toxicity, *MTD* maximum tolerated dose

Supplementary Fig. S2 Tumor FDG-PET SUV_{max} values at baseline and cycle 1, day 22 in patients in cohort 2C and the expansion cohort



FDG-PET fluorodeoxyglucose positron emission tomography, *SUV_{max}* maximum standardized uptake value

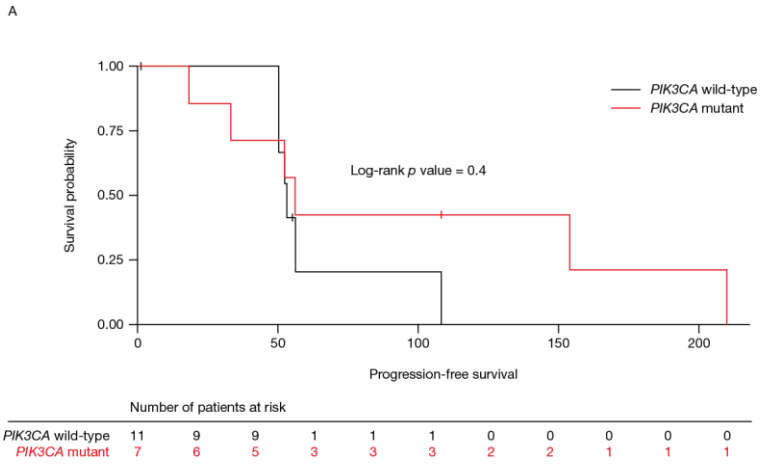
Supplementary Fig. S3 Plot of average percent change from baseline in FDG SUV_{max} versus copanlisib $AUC_{(0–25)}$ (A) and $AUC_{(0–tlast)}$ (B)



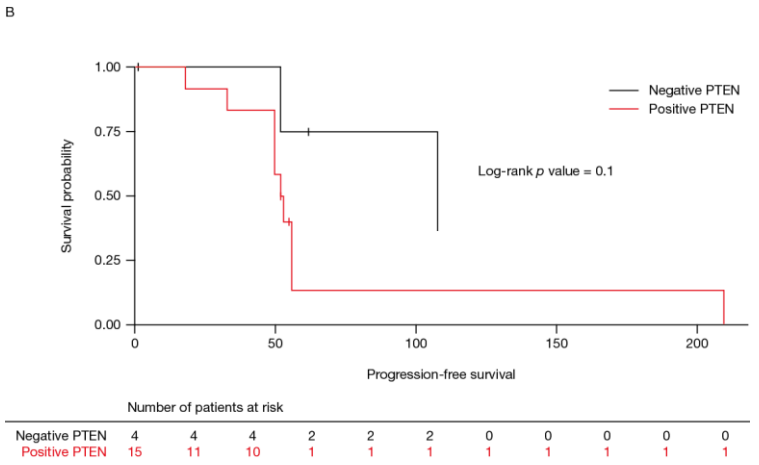
FDG-PET results are from cycle 1, day 22, while AUC results are from cycle 1, day 15

$AUC_{(0–25)}$ area under the curve from time 0 to 25 hours after the start of infusion, *$AUC_{(0–tlast)}$* area under the curve from time 0 to the last data point greater than the lower limit of quantification, *FDG-PET* fluorodeoxyglucose positron emission tomography, *SUV_{max}* maximum standardized uptake value

Supplementary Fig. S4 Analyses of PFS in patients treated at the copanlisib/refametinib maximum tolerated dose by pretreatment *PIK3CA* mutational status (A), PTEN protein loss (B), pERK % cells positive (C) and Ki-67 H-score (D)

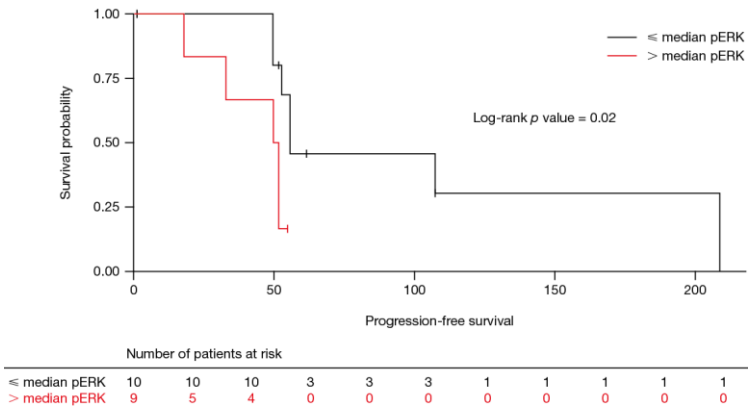


Median PFS: *PIK3CA* mutant, 56 days; *PIK3CA* WT, 53 days. Long rank test $p = 0.4$. Cox model HR (95% CI): 0.568 (0.159–2.037), $p = 0.385$. HR for mutation is expressed as mutant/WT



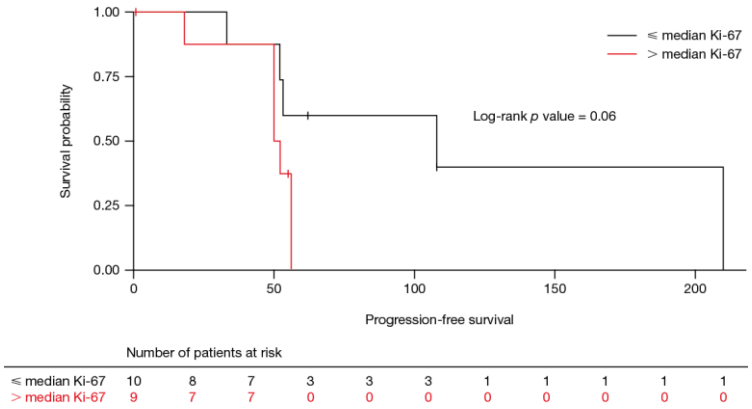
Median PFS: PTEN loss, 108 days; no PTEN loss, 53 days. Log rank test $p = 0.1$. Cox model HR (95% CI): 2.90 (0.60–13.99), $p = 0.185$. HR for PTEN is expressed as PTEN present/PTEN loss

C



Median PFS: low pERK, 56 days; high pERK, 51 days. Log rank test $p = 0.02$. Cox model HR (95% CI): 4.506 (1.047–19.387), $p = 0.043$. HR for pERK is expressed as high pERK/low pERK

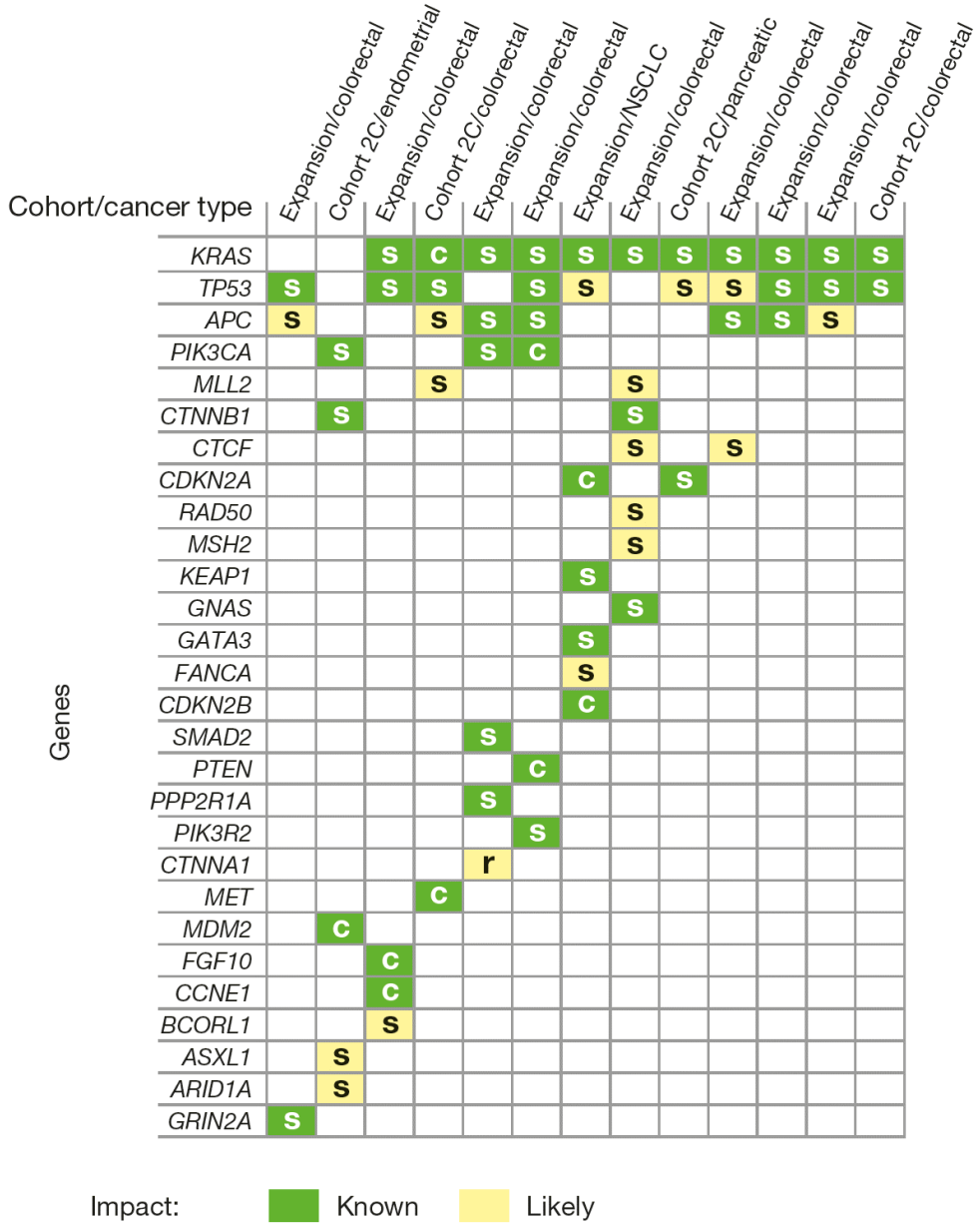
D



Median PFS: low Ki-67, 108 days; high Ki-67, 51 days. Log rank test $p = 0.06$. Cox model HR (95% CI): 3.254 (0.831–12.738), $p = 0.090$. HR for Ki-67 is expressed as high Ki-67/low Ki-67

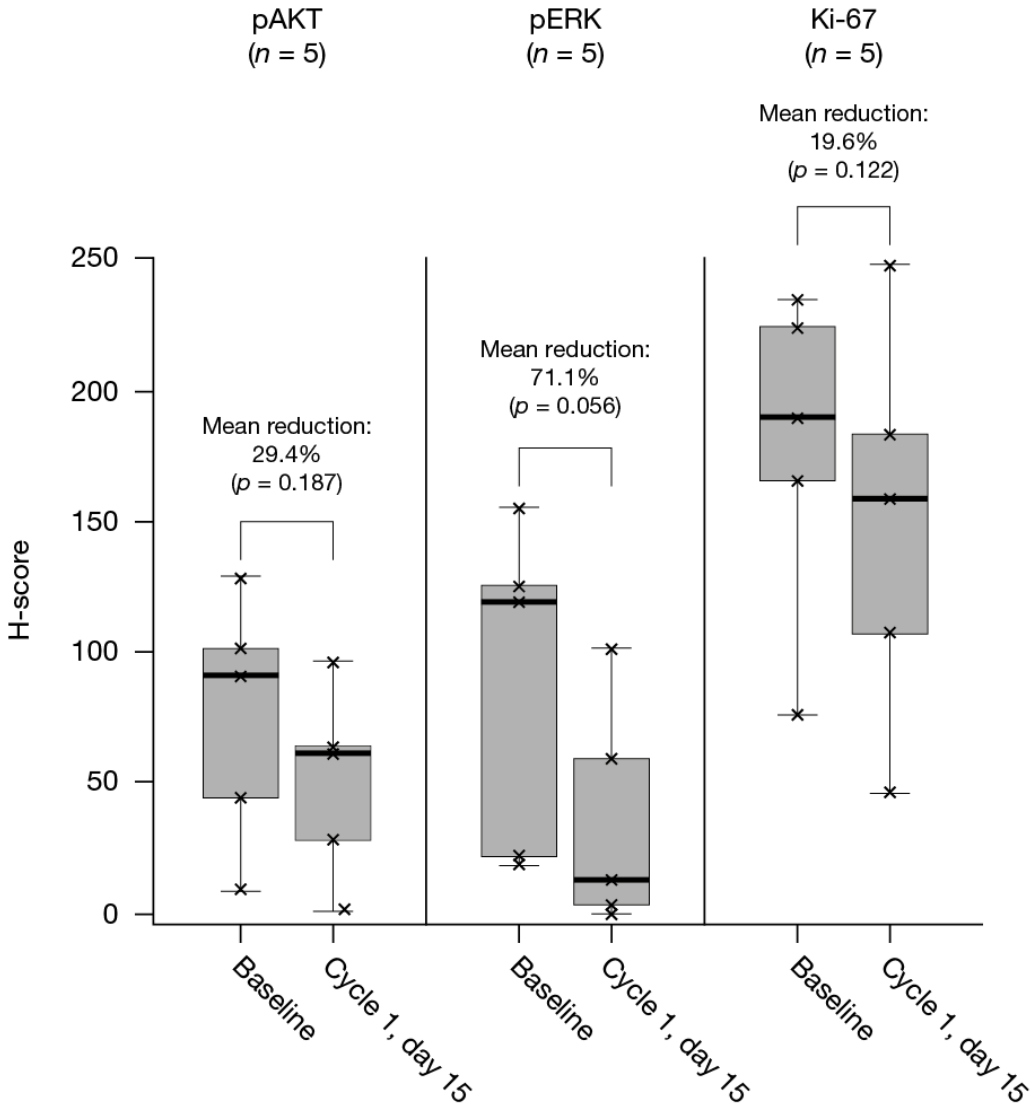
CI confidence interval, *HR* hazard ratio, *PFS* progression-free survival, *WT* wild-type

Supplementary Fig. S5 Known somatic and likely somatic mutations detected by next-generation sequencing



c copy number alteration, *NSCLC* non-small-cell lung cancer, *r* rearrangement, *s* short variant

Supplementary Fig. S6 Pharmacodynamic changes in tumor pAKT, pERK, and Ki-67 from baseline to cycle 1, day 15 by IHC H-score



H-score = [% of tumor cells staining at 1+ intensity] + 2 × [% of tumor cells staining at 2+ intensity] + 3 × [% of tumor cells staining at 3+ intensity])

IHC immunohistochemistry