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Prognostic Implications of *RAS* Alterations in Diverse Malignancies and Impact of Targeted Therapies

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RAS alterations in diverse cancers

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Abbreviations

Clinical laboratory improvement amendments (CLIA)
Confidence interval (CI)
Formalin-fixed, paraffin-embedded (FFPE)
Hazard ratio (HR)
Mitogen-activated protein kinase (MAPK)
Next-generation sequencing (NGS)
Non-small cell lung cancer (NSCLC)
Odds ratio (OR)
Overall survival (OS)
Partial response (PR)
Phosphoinositide 3-kinase (PI3K)
Progression-free survival (PFS)
University of California, San Diego (UCSD)

Novelty and Impact

RAS alterations are considered “undruggable”. To better understand the biology of *RAS*-altered cancers, comprehensive analysis was performed. Prognostically, we demonstrated that specific alterations in *KRAS* were associated with worse survival across cancers. Moreover, co-alterations in *RAS* and PI3K signaling or cell-cycle-associated genes were linked to shortest survival of all. Therapeutically, a subset of the patients with *RAS* alterations treated with MEK inhibitors plus tailored non-MAPK-targeting agents showed responses, suggesting that overcoming resistance requires tailored combinations.

ABSTRACT

RAS alterations are often found in difficult-to-treat malignancies and are considered "undruggable." To better understand the clinical correlates and co-altered genes of *RAS* alterations, we used targeted next-generation sequencing (NGS) to analyze 1,937 patients with diverse cancers. Overall, 20.9% of cancers (405/1,937) harbored *RAS* alterations. Most *RAS*-altered cases had genomic co-alterations (95.3%, median: 3, range: 0-51), often involving genes implicated in oncogenic signals: PI3K pathway (31.4% of 405 cases), cell cycle (31.1%), tyrosine kinase families (21.5%) and MAPK signaling (18.3%). Patients with *RAS*-altered versus wild-type *RAS* malignancies had significantly worse overall survival (OS) (P=0.02 [multivariate]), with *KRAS* alterations in particular showing shorter survival. Moreover, co-alterations in both *RAS* and PI3K signaling or cell-cycle-associated genes correlated with worse OS (P=0.004 and P<0.0001, respectively [multivariate]). Among *RAS*-altered patients, MEK inhibitors alone did not impact progression-free survival (PFS), while matched targeted therapy against non-MAPK pathway co-alterations alone showed a trend towards longer PFS (versus patients who received unmatched therapy) (HR: 0.79, 95% CI: 0.61-1.03, P=0.07). Three of 9 patients (33%) given tailored combination therapies targeting both MAPK and non-MAPK pathways achieved objective responses. In conclusion, *RAS* alterations correlated with poor survival across cancers. The majority of *RAS* alterations were accompanied by co-alterations impacting other oncogenic pathways. MEK inhibitors alone were ineffective against *RAS*-altered cancers while matched targeted therapy against co-alterations alone correlated with a trend toward improved PFS. A subset of the small number of patients given MEK inhibitors plus tailored non-MAPK-targeting agents showed responses, suggesting that customized combinations warrant further investigation.

INTRODUCTION

Since the discovery of activating *RAS* mutations in 1982 [1-3], further research in cancer genomics revealed that alterations in this gene family (*KRAS*, *NRAS* and *HRAS*) are among the most frequent in cancer, being discerned in about 20-30% of tumors[4-6]. Single point mutations in the *RAS* gene lock the protein in a GTP-bound state, leading to constitutive activation of the Ras protein and persistent signaling in multiple downstream pathways, including the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3K) pathways [4]. At the cellular level, oncogenic *RAS* alterations are also associated with increased anchorage-independent cell growth and implicated in cancer initiation and aggressiveness [7-10].

Among different *RAS* alterations, *KRAS* is the isoform most frequently altered (86% of all *RAS* alterations), followed by *NRAS* (11%) and *HRAS* (3%) [4, 5]. The frequency of alterations in *RAS* differs depending on the cancer type. For example, *KRAS* alterations are most commonly seen in pancreatic adenocarcinoma (71-98% of pancreatic adenocarcinoma) followed by colorectal adenocarcinoma (35-45%) and lung adenocarcinoma (19-31%). *NRAS* alterations are frequently observed in cutaneous melanoma (28%) followed by thyroid carcinoma (8-9%). *HRAS* alterations can be discerned in bladder urothelial carcinoma (6%), head and neck squamous cell carcinoma (5%) and thyroid carcinoma (3-4%) [4-6]. Moreover, the frequencies of specific codon mutations in each *RAS* gene differ from cancer to cancer [5], and different codon mutations can lead to distinct downstream signaling patterns [8]. Clinically, evaluation of *RAS* alteration status is routinely done for patients with metastatic colorectal cancer since their presence predicts lack of response to anti-EGFR therapies (cetuximab and panitumumab). Presence of *RAS* alterations have also been associated with significantly worse overall survival (OS) among lung, colorectal and pancreatic cancers when compared to patients with wild-type *RAS* [11-14].

A number of clinical trials have attempted to target *RAS*-altered cancers. For instance, blocking of Ras membrane association, which is an essential step for Ras activation, and targeting of Ras downstream effector signaling, have been extensively studied. Unfortunately, to date, most trials have failed to demonstrate clinical benefit among patients with *RAS* alterations (**Supplemental Table 1**). For example, tipifarnib (farnesyltransferase inhibitor [15]) and L-778,123 (dual inhibitor of farnesyltransferase and geranylgeranyltransferase type 1) (block Ras membrane association) had minimal activity among patients with *KRAS*-mutated cancers [16-18] (though recently there is preliminary evidence of activity in *HRAS*-mutated head and neck cancer [19]). Moreover, targeting of downstream signaling with a MEK inhibitor among patients with *KRAS*-mutated non-small cell lung cancer (NSCLC) and the dual inhibition with MEK and AKT inhibitors among pancreatic cancer patients also failed to demonstrate clinical benefit when compared to chemotherapy as a comparator [20, 21]. Although targeting *RAS* has been challenging, early phase clinical trial with AMG 510 (a novel small molecule inhibitor specifically for *KRAS* G12C) among *KRAS* G12C altered cancer patients demonstrated clinical responses and further enrollment is ongoing [22]. Moreover, the MEK inhibitor cobimetinib induced a remarkable response in a patient with Rosai-Dorfman syndrome, whose disease harbored a *KRAS* mutation but no other characterized alterations [23]. Therefore, factors such as genomic co-alterations might also attenuate responsiveness to agents that directly or indirectly impact Ras signaling.

In order to better understand the genotypic and phenotypic ecosystem of *RAS* [4], we used next-generation sequencing (NGS) to interrogate the molecular landscape of *RAS* alterations in 1,937 patients with diverse cancers. We also investigated the clinical characteristics, genomic co-alterations, and survival impact of *RAS* alterations, as well as the therapeutic impact of matched therapies, including illustrative patients given MEK inhibitors together with agents targeting co-alterations.

MATERIALS AND METHODS

Patients

We evaluated the genomic landscape of *RAS* alterations among 1,937 patients with diverse malignancies that were seen at the University of California, San Diego (UCSD) Moores Cancer Center from December 2012 to June 2017 (**Supplemental Figure 1**). This study was performed according to the guidelines of the UCSD Institutional Review Board (Profile Related Evidence Determining Individualized Cancer Therapy [PREDICT], NCT02478931) or I-PREDICT (NCT02534675) and for any investigational therapies for which the patients consented.

Tissue samples and mutational analysis

Tumors were provided as formalin-fixed, paraffin-embedded (FFPE) samples and evaluated by NGS in a clinical laboratory improvement amendments (CLIA)-certified lab (Foundation Medicine, Cambridge, MA). The methods used for NGS have been previously reported [24-26]. Briefly, 50-200 ng of genomic DNA was extracted and purified from the submitted FFPE tumor samples. DNA was adaptor ligated, and hybrid capture was performed for all coding exons of 182-406 cancer-related genes plus selected introns from 14-31 genes frequently rearranged in cancer (Illumina HiSeq platform). Sequencing was performed with an average sequencing depth of coverage greater than 250x, with >100x at > 99% of exons. Somatic mutations were identified with 99% specificity and >99% sensitivity for base substitutions at ≥5% mutant allele frequency, and >95% sensitivity for copy number alterations. Gene amplification was reported at ≥8 copies above ploidy, with ≥6 copies considered equivocal. The exception was *ERRB2*, for which ≥5 copies is considered equivocal amplification [25, 26]. One case underwent NGS at UCSD laboratory (N = 397 genes) (CLIA-certified). Variants of unknown significance were not curated for the analyses.

Endpoints and statistical methods

Patient characteristics, prevalence of *RAS* alterations and genomic co-alterations were summarized by descriptive statistics. The Fisher's exact test and logistics regression analysis were used for categorical variables. Progression-free survival (PFS) was defined as time interval between the start of therapy and the date of disease progression. OS was defined as time from cancer diagnosis with recurrent or metastatic disease condition to last follow up or death. Patients with ongoing therapy without progression at the last follow up date were censored for PFS at that date. Patients alive at last follow up were censored for OS. Log-rank test and Cox regression analysis were used to compare subgroups of patients. All tests were 2-sided and variables with $P < 0.1$ were included for multivariate analysis. P-values ≤ 0.05 were considered significant. Statistical analyses were performed with assistance from co-author RO using Graph-Pad Prism version 7.0 (San Diego, CA, USA) and SPSS version 24.0 (Chicago, IL, USA).

Data Availability

Data for this study will be made available by the corresponding author upon reasonable request.

RESULTS

Among 1,937 patients with diverse cancers, the most common diagnosis was lung cancer (11.8% [229/1,937]), followed by hematologic malignancies (10.7% [208/1,937]), breast (9.6% [185/1,937]) and colorectal cancers (9.6% [185/1,937]) (**Figure 1.A. and Supplemental Table 2**). Overall, *RAS* alterations were found in 20.9% of cases (405/1,937). Among different *RAS* alterations (N=405), *KRAS* was most commonly altered (80.0% [324/405]) followed by *NRAS* (16.0% [65/405]) and *HRAS* (4.9% [20/405]) alterations (N=4 had both *KRAS* and *NRAS* alterations). *RAS* alterations were significantly associated with pancreatic cancer (72.1% [44/61], odds ratio [OR]: 8.41), appendiceal (57.8% [37/64], OR: 3.37) and colorectal cancers (57.3% [106/185], OR: 4.45) (all P<0.0001 [multivariate]). *RAS* alterations were significantly less common amongst brain (0.6% [1/172], OR: 0.03, P=0.001), breast (3.8% [7/185], OR: 0.18, P<0.0001), soft tissue sarcomas (5.4% [3/56], OR: 0.24, P=0.02), head and neck (5.8% [6/103], OR: 0.23, P=0.001) and hematologic malignancies (12.5% [26/208], OR: 0.53, P=0.01) (all p-values after multivariate analysis) (**Supplemental Table 2**).

Patients with *RAS* abnormalities had frequent co-alterations

Among patients with *RAS* alterations, 95.3% (386/405) had co-alterations (median: 3, range: 0-51). When compared to tumors bearing wild-type *RAS*, tumors harboring *RAS* alterations had significantly increased rates of co-alterations in the following genes: *STK11* (OR: 2.81, P=0.01), *SMAD4* (OR: 2.25, P=0.003) and *GNAS* (OR: 1.95, P=0.02). In contrast, alterations in *RET* (OR: 0.19, P=0.03), *KIT* (OR: 0.21, P=0.01), *EGFR* (OR: 0.27, P=0.001) and *BRAF* (OR: 0.29, P=0.0001) were less frequently associated with *RAS* alterations (all p-values after multivariate analysis) (**Supplemental Table 2**).

When co-alterations were grouped depending on their oncogenic pathways (e.g. *EGFR* and *FGFR* alterations grouped into tyrosine kinase families), *RAS* altered cases were most commonly co-altered with genes impacting PI3K signaling (31.4% [127/405]) followed by cell cycle-

associated genes (31.1% [126/405]), tyrosine kinase families (21.5% [87/405]), MAPK signaling (18.3% [74/405]), *BRCA*-associated genes (12.8% [52/405]) and mismatch repair and immune-associated genes (*MLH1*, *MSH2*, *MSH6, PMS2, CD274* [*PD-L1*] and *PDCD1LG2* [*PD-L2*], 4% [16/405]) **(Figure 1.B and Supplemental Table 3**).

RAS alterations were associated with shorter survival among patients with metastatic or recurrent solid tumors (N=1,526)

Among 1,937 patients with diverse cancers, patients with metastatic or recurrent solid tumors were included in the survival analysis (N=1,526) (excluded patients with lymphoma [N=40], hematological malignancies [N=208] and patients without recurrent or metastatic disease condition [N=163]) (**Supplemental Figure 1**). Among patients with metastatic or recurrent solid tumors, 23.5% (358/1,526) had *RAS* alterations. When compared to patients whose tumors harbored wild-type *RAS*, those who harbored cancers with *RAS* alterations had significantly shorter OS from date of metastatic/recurrent disease. (hazard ratio [HR]: 1.24, 95% confidence interval [CI]: 1.03-1.48, P=0.02 [multivariate]) (**Table 1 and Figure 2.A.**).

Specific _RAS_ alterations correlated with worse outcome: Among *K-*, *N-* and *H- RAS* alterations, *KRAS* alterations were the only alterations significantly associated with worse OS (HR: 1.30, 95% CI: 1.07-1.59, P=0.01). Moreover, among different *RAS* codon alterations, *KRAS* G12V (HR: 1.64, 95% CI: 1.18-2.30, P=0.004), *KRAS* G13D (HR: 2.07, 95% CI: 1.27-3.38, P=0.004) and *KRAS* amplification (HR: 1.88, 95% CI: 1.09-3.24, P=0.02) were independently associated with inferior OS (all p-values by multivariate analysis) (**Table 1 and Supplemental Table 4**).

RAS alterations accompanied by co-alterations in PI3K signaling and cell cycle-associated genes had worse survival

We also investigated survival based on the *RAS* alterations and the co-altered oncogenic pathways. Interrogating all 1,526 individuals (including *RAS* wild-type and *RAS* mutated), patients who had both *RAS* alterations and co-altered oncogenic pathways in other MAPK signaling genes, *BRCA*-associated genes and immune-related gene alterations showed no significant difference in OS when compared to patients with *RAS* wild-type without co-altered pathways (**Table 2**). However, harboring alterations in both *RAS* and tyrosine kinase family genes (HR: 1.37, 95% CI: 0.98-1.93, P=0.07 [trend]), PI3K signaling (HR: 1.52, 95% CI: 1.15-2.01, P=0.004) (**Figure 2.B.**) and cell cycle-associated genes (HR: 1.99, 95% CI: 1.49-2.67, P<0.0001) (**Figure 2.C.**) was associated with worse OS when compared to patients without those anomalies (all p-values by multivariate analysis) (**Table 2**).

When OS was evaluated just amongst 358 patients with *RAS* alterations (not including patients with wild-type *RAS*), co-alterations in cell cycle-associated genes showed a strong trend to worse OS (HR: 1.70, 95% CI: 1.20-2.41, P=0.001 [univariate], HR: 1.42, 95% CI: 0.99-2.02, P=0.056 [multivariate] [trend]) (**Table 2**).

Treatment outcomes among patients with metastatic or recurrent *RAS*-altered solid tumors (N=284)

Among patients with *RAS*-altered recurrent or metastatic solid tumors (N=358), 284 patients received systemic therapies and were evaluable for assessment of PFS (**Supplemental Figure 1**). When PFS was compared to patients who received unmatched therapy (therapies that were not based on genomic markers) (N=143), patients who received therapies targeting only the MAPK pathway (N=17) did not show a significant difference in PFS (HR: 1.14, 95% CI: 0.60-2.19, P=0.67 [univariate]). On the other hand, patients who received matched therapy targeting a non-MAPK pathway (N=124) had a trend for better PFS when compared to the unmatched therapy group (HR: 0.79, 95% CI: 0.61-1.03, P=0.07 [univariate]) (**Figure 3**). After multivariate analysis, targeting of non-MAPK pathway did not remain a significant factor predicting longer PFS

(HR: 0.89, 95% CI: 0.67-1.19, P=0.42) (**Supplemental Table 5**). Patients who received therapies targeting both MAPK and non-MAPK pathways were not included in the analysis due to the small sample size (N=9); however, 33.3% (3/9) achieved a partial response (PR) (**Figure 4**).

Illustrative Cases: Three patients with advanced lethal malignancies given trametinib and a therapy matched to a genomic co-alteration(s) who achieved partial responses lasting 9, 9.2 and 15 months are shown in **Figure 4.**

DISCUSSION

Herein we report the comprehensive genomic landscape of *RAS* alterations amongst 1,937 patients with diverse malignancies and clinical annotation. Overall, *RAS* alterations were found in 20.9% of patients (405/1,937). Among diverse cancer types, *RAS* alterations were significantly associated with pancreatic cancer (72.1% [44/61], odds ratio [OR]: 8.41), appendiceal malignancies (57.8% [37/64], OR: 3.37) and colorectal cancers (57.3% [106/185], OR: 4.45) (all P<0.0001 after multivariate analysis) (**Supplemental Table 2**), which is consistent with previous reports [4-6].

Since the presence of *RAS* alterations has been linked to significantly worse survival among lung, colorectal and pancreatic cancers when compared to patients with wild-type *RAS* [11-14], we also examined the impact of *RAS* alterations on survival among diverse solid tumors (N=1,526). Consistent with previous reports, we have observed that patients with *RAS*-altered tumors had significantly worse OS (HR: 1.24, 95% CI: 1.03-1.48, P=0.02 [multivariate]) (**Table 1**). Among different subtypes of *RAS* alterations (*K*-, *N*- and *H*- *RAS* alterations), *KRAS* alterations were associated with worse OS (HR: 1.30, 95% CI: 1.07-1.59, P=0.01 [multivariate]). Furthermore, among different codon or other alterations, *KRAS* G12V, *KRAS* G13D and *KRAS* amplification correlated with poor OS when compared to patients with *RAS* wild-type (HR: 1.64, 95% CI: 1.18-2.30, P= 0.004, HR: 2.07, 95% CI: 1.27-3.38, P= 0.004, and HR: 1.88, 95% CI: 1.09-3.24, P=0.02, respectively [multivariate]) (**Table 1**). Our findings are consistent with previous reports indicating that varied *RAS* alterations do not all have the same impact [13, 14, 27]. Distinct clinical outcomes may be attributable to dissimilar degrees of GTP-binding ability amongst *RAS* anomalies, thus leading to a differential impact on downstream signaling and effectors [28]. For example, Ihle *et al* [8], showed that PI3K signaling was preferentially activated in cell lines with *KRAS* G12D alterations; meanwhile, activation of the Ral A/B pathway associated with *KRAS* G12C alterations. In the study by Ihle *et al*, differences in downstream effectors, depending on

the specific codon alterations, also affected colony number and tumor growth, which may explain the heterogeneous clinical outcomes among different *RAS*-altered patients.

Importantly, we have demonstrated that co-altered oncogenic pathways associated with *RAS* abnormalities can also influence survival outcome. Notably, co-alterations in both *RAS* and PI3K signaling or cell cycle-associated genes were significantly correlated with worse OS when compared to patients without those anomalies (HR: 1.52, 95% CI: 1.15-2.01, P=0.004 and HR: 1.99, 95% CI: 1.49-2.67, P<0.0001, respectively [multivariate]) (**Table 2 and Figure 2.B and 2.C**). This observation is in line with a previous report that showed that a combination of alterations, especially *KRAS* and *CDKN2A* abnormalities, had significantly worse disease-free survival and OS among patients with pancreatic adenocarcinoma [13]. Further investigations that incorporate an understanding of RAS downstream signaling and effectors as well as the functional impact of genomic co-alterations are necessary.

Therapeutically, comprehensive understanding of *RAS* and its genomic co-alterations is likely required to better manage *RAS*-altered malignancies. As mentioned, multiple attempts have been made to target *RAS*-altered cancers, mainly by blocking the RAS membrane association [16-18] or by inhibiting the RAS downstream effectors (mostly with single-agent targeting with a MEK inhibitor or in combination with PI3K, AKT or mTOR inhibitors) [20, 21]. However, most of the approaches to date have failed to yield satisfactory anti-tumor activities (and co-targeting of MEK and PI3K pathways has demonstrated significant toxicity [29]). Therefore, there is no standardized therapy targeting *RAS*-altered cancers (**Supplemental Table 1**).

In our current study, we have also demonstrated that targeting the MAPK pathway with MEK inhibitors (N=17) was not associated with improvement in PFS when compared to patients receiving unmatched therapy (N=143) (HR: 1.14, 95% CI: 0.60-2.19, P=0.67) (**Figure 3 and Supplemental Table 5**). Giving patients therapy matched to their co-alterations, without targeting the MEK pathway, was associated with improved PFS, albeit without reaching statistical

significance (**Figure 3**). However, in contrast to previous reports and the current study, a dramatic response has been reported with single-agent cobimetinib (MEK inhibitor) in a patient with Rosai-Dorfman disease (rare non-Langerhans'-cell histiocytosis associated with massive lymphadenopathy) who harbored a *KRAS* G12R alteration, but without any co-alterations [23]. Therefore, one of the potential challenges in targeting *RAS* alterations with agents such as MEK inhibitors may be due to resistance mediated by the high number of genomic co-alterations. Indeed, we observed that most patients with *RAS* alterations harbored genomic co-alterations (95.3% [386/405], median: 3, rang: 0-51), often potentially affecting important oncogenic pathways **(Figure 1.B. and Supplemental Table 3**). Hence, effective Ras targeting may require a tailored combination approach that addresses both Ras activation and the specific co-altered pathway in each patient [30-33]. In this regard, we have demonstrated a response rate of 33% (3 of 9 individuals) among patients who received matched therapies targeting both MAPK and non-MAPK pathways. For instance, in a patient with pancreatic cancer and a *KRAS* and a *CDKN2A* alteration given both trametinib and palbociclib, a partial response lasting 9 months was achieved after having failed therapies including a palbociclib-containing regimen that did not include a MEK inhibitor (**Figure 4.B.**). Although the low sample size precludes definitive conclusions, our observations suggest that such a customized combination approach warrants further investigation (I-PREDICT trial currently ongoing [ClinicalTrial.gov, NCT02534675]) [34].

There were several limitations to the current report. First, clinical correlations were assessed retrospectively. Second, since the number of cancer types included in the study was based on the samples sent for NGS by treating physicians, sample size bias cannot be ignored. Third, multiple assessments could result in overcalling the implication of positive P-values. Fourth, molecular analysis was performed on archival tumor samples that were obtained at various time points in relationship to the clinical history. However, despite these limitations, the current study provides a large and comprehensive clinical analysis of *RAS* alterations in diverse, clinically annotated cancers.

In conclusion, we have shown that 20.9% of 1,937 patients with varied cancers had *RAS* alterations. *RAS*-altered versus wild-type cases (especially those involving *KRAS*) were associated with significantly worse survival. Moreover, among different subtypes of *RAS* alterations, *KRAS* G12V and G13D mutations and *KRAS* amplification correlated with the shortest survival. The majority of *RAS*-altered cases also had genomic co-alterations (95.3% [386/405], median: 3) affecting critical oncogenic signals that could serve to mediate resistance. Co-alterations in both *RAS* and PI3K signaling or cell cycle-associated genes associated with worse survival when compared to patients without those alterations. Among *RAS*-altered cases, patients who received matched targeted therapy against non-MAPK pathway alterations had a trend for better PFS when compared to patients who received unmatched therapy, but targeting the Ras pathway alone with MEK inhibitors showed no improvement in outcome. In a small subset of nine patients given combination therapies targeting both MAPK and the specific non-MAPK gene altered, a response rate of 33% was achieved, as reflected by the illustrative cases (**Figure 4**). Further clinical investigation of individualized combinations that include agents that impact both MAPK and the precise co-altered gene(s) harbored by each tumor in patients with *RAS*-altered cancers is warranted.

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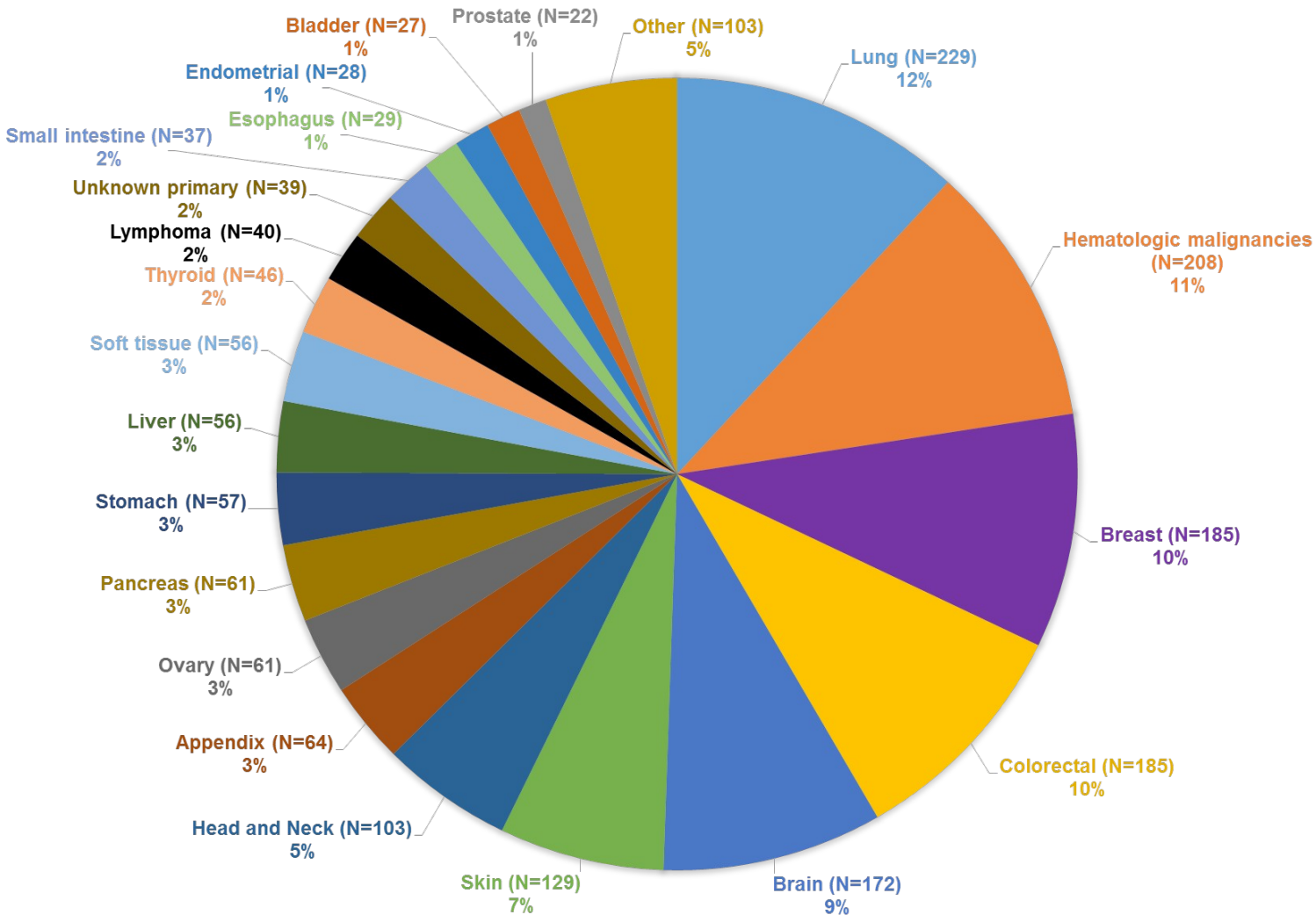
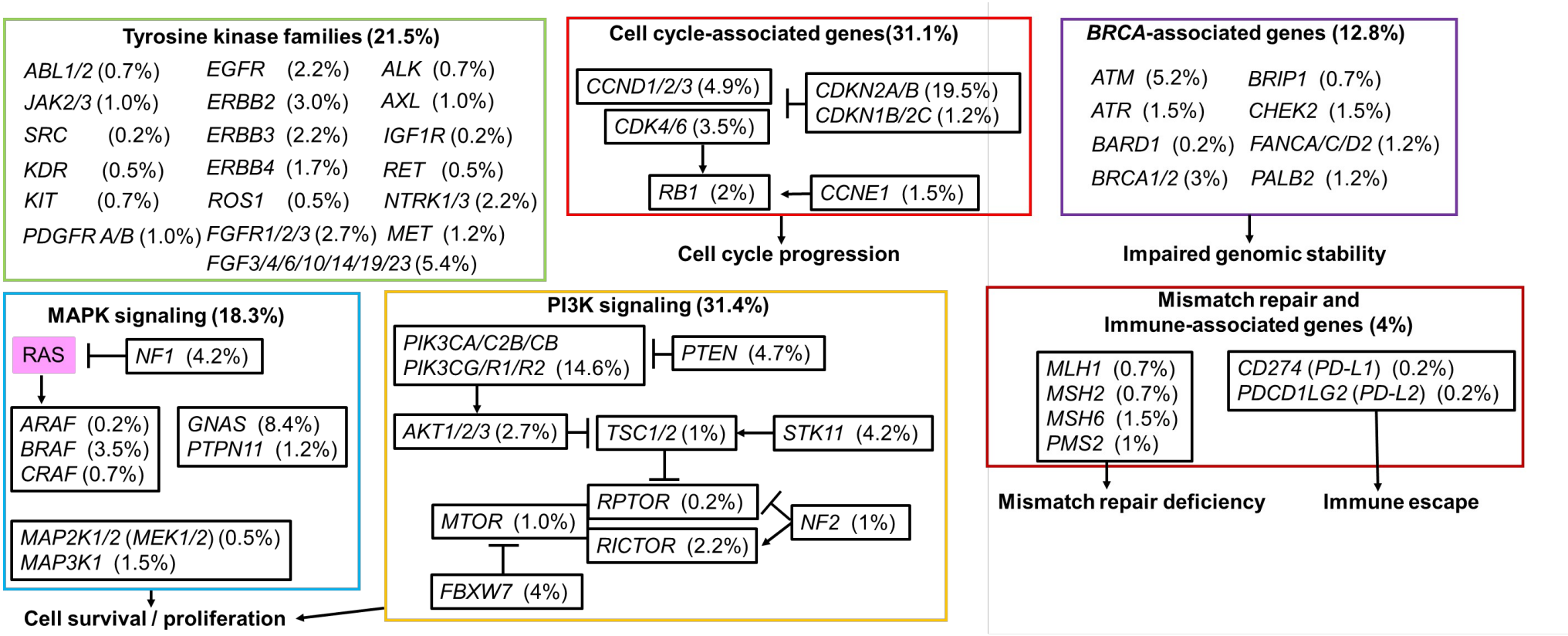
Figure 1.A. Frequency of analyzed cancer types included in this study (N=1937).



Included cancer diagnosis with N>20. Among diverse cancer types analyzed in this study, the most common cancer diagnosis was lung cancer (N=229, 12%) followed by hematologic malignancies (N=208, 11%), breast (N=185, 10%), colorectal (N=185, 10%) and brain cancer (N=172, 9%).

Figure 1.B. Co-altered oncogenic pathways associated with *RAS* alterations (N=405).

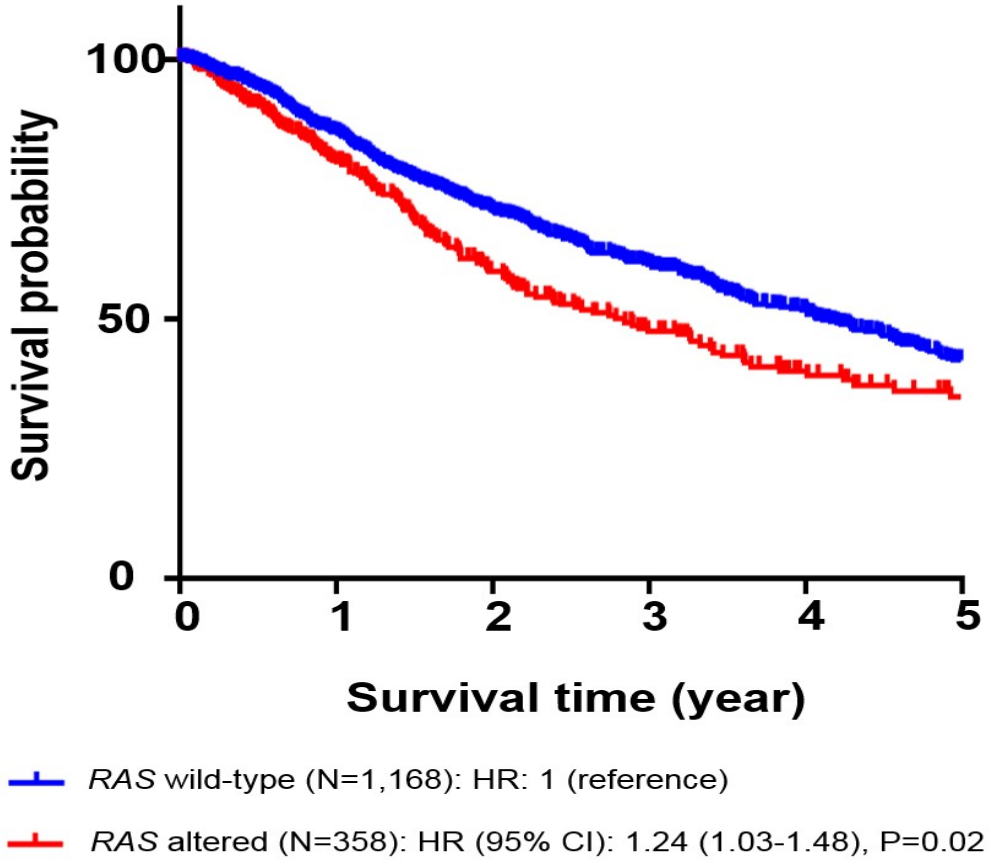


Among patients harboring *RAS* alterations (N=405), co-alterations in oncogenic pathways were observed in tyrosine kinase family genes (21.5%), cell cycle-associated genes (31.3%), *BRCA*-associated genes (12.8%), MAPK signaling pathway-associated genes (18.3%), PI3K signaling-associated genes (31.4%) and mismatch repair or immune associated genes (4%).
See **Supplemental Table 2** for detail.

Figure 2. Kaplan-Meier survival curves for overall survival. Tick marks represent censored time points for patients still alive at last follow up.

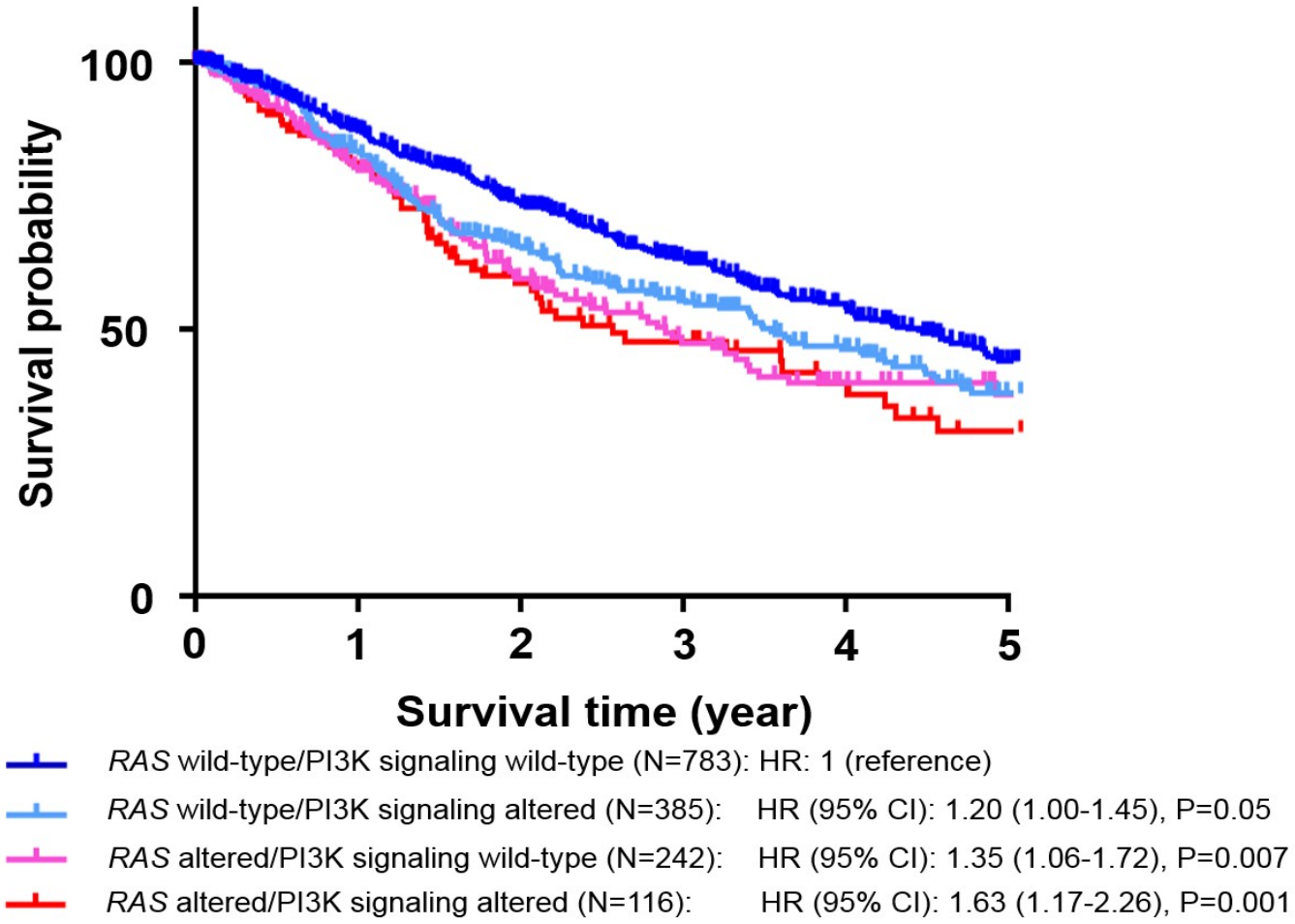
See **Table 1** for the Cox regression analysis that adjusted for age, sex and primary site of cancer diagnosis.

Figure 2.A. Overall survival from time of metastatic/advanced disease comparing patients with wild-type *RAS* and *RAS*-altered cancers.



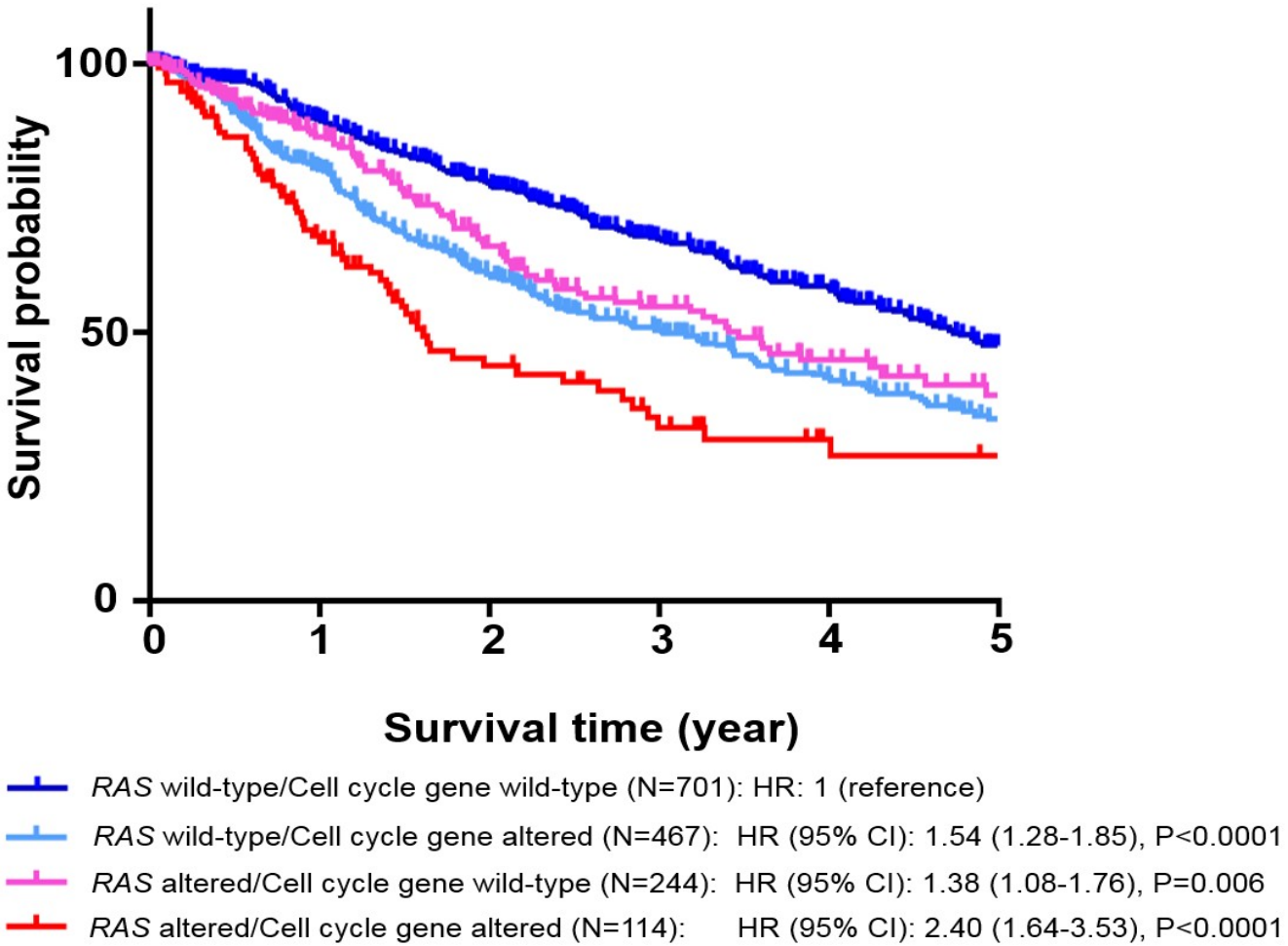
Overall survival analysis based on *RAS* alteration status. When compared to *RAS* wild-type cases (N=1,168), *RAS* altered cases (N=358) had worse overall survival with HR of 1.24 (95% CI: 1.03-1.48, P=0.02) by log-rank test.

Figure 2.B. Overall survival from time of metastatic/advanced disease comparing impact of *RAS* and PI3K signaling alterations.



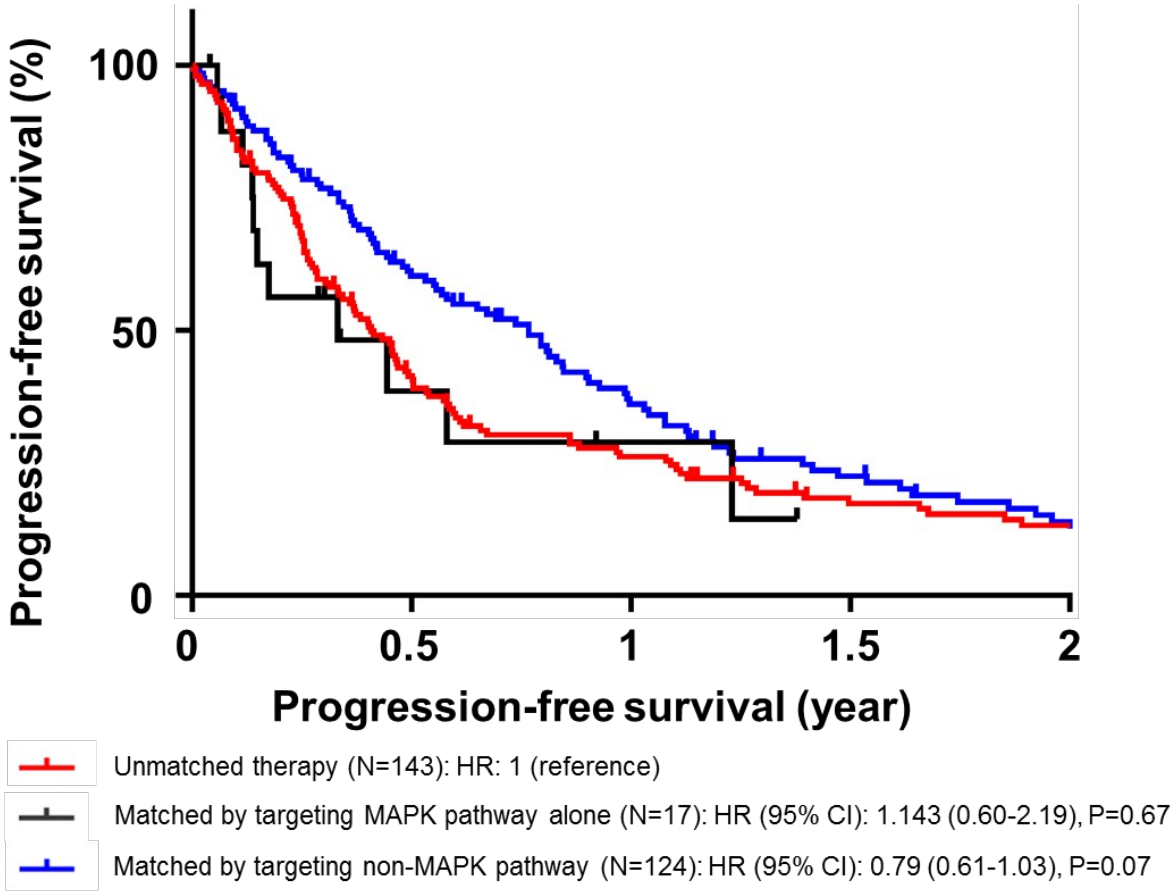
Overall survival analysis based on *RAS* and PI3K signaling-associated gene alteration status**.** When compared to *RAS* wild-type/PI3K signaling wild-type cases (N=783), *RAS* wild-type/PI3K signaling altered cases (N=385) had HR of 1.20 (95% CI: 1.00-1.45, P=0.05), *RAS* altered/PI3K signaling wild-type cases (N=242) had HR of 1.35 (95% CI: 1.06-1.72, P=0.007) and *RAS* altered/PI3K signaling altered cases (N=116) had HR of 1.63 (95% CI: 1.17-2.26, P=0.001) by log-rank test.

Figure 2.C. Overall survival from time of metastatic/advanced disease comparing impact of *RAS* and cell cycle-associated alterations.



Overall survival analysis based on *RAS* and cell cycle-associated gene alteration status. When compared to *RAS* wild-type/Cell cycle gene wild-type cases (N=701), *RAS* wild-type/Cell cycle gene altered cases (N=467) had HR of 1.54 (95% CI: 1.28-1.85, P<0.0001), *RAS* altered/Cell cycle gene wild-type cases (N=244) had HR of 1.38 (95% CI: 1.08-1.76, P=0.006) and *RAS* altered/Cell cycle gene altered cases (N=114) had HR of 2.40 (95% CI: 1.64-3.53, P<0.0001) by log-rank test.
Abbreviations: CI; confidence interval, HR; hazard ratio

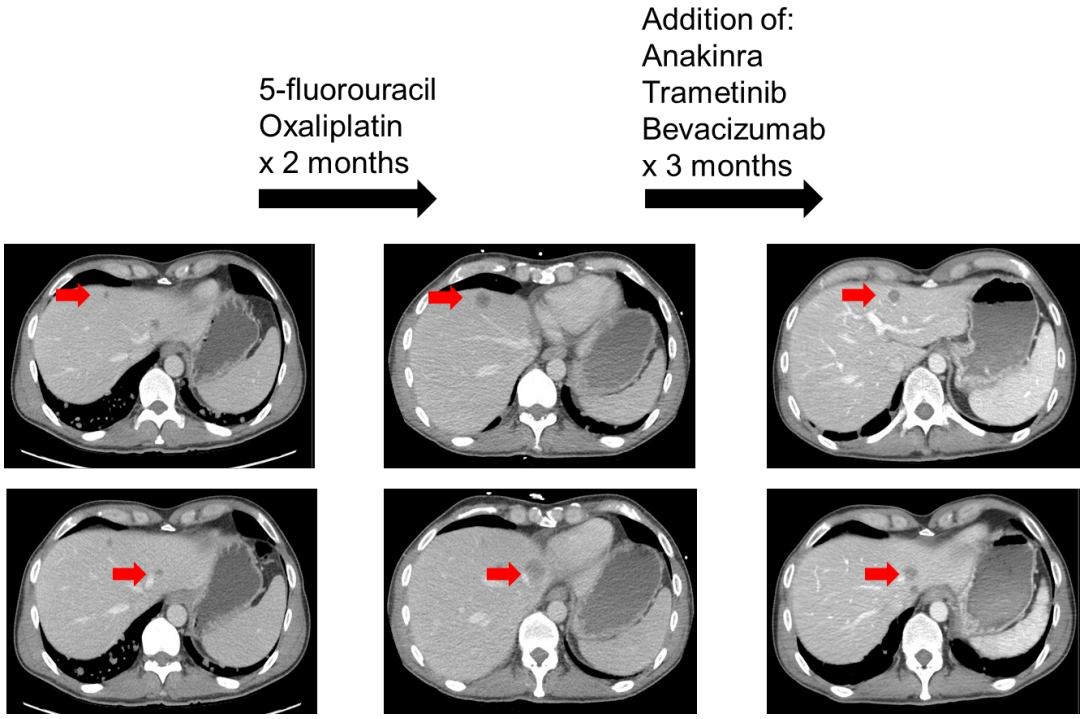
Figure 3. Progression-free survival among patients with *RAS*-altered solid tumors who received systemic therapies (N=284). Tick marks represent censored time points for patients still progression free at last follow up.



Progression-free survival among patients with *RAS* mutations who received systemic therapies (N=284) were evaluated (Patients who received therapies targeting both MAPK and non-MAPK pathways were not included in the analysis due to the small sample size [N=9]). When compared to patients who received unmatched therapy (N=143), patients who received matched therapy only against MAPK pathway had HR of 1.14 (95% CI: 0.60-2.19, P=0.67) (N=17) and patients who received matched targeted therapy targeting non-MAPK pathway had HR of 0.79 (95% CI: 0.61-1.03, P=0.07) (N=124).

Figure 4. Illustrative cases of patients with *RAS* alterations treated with MEK inhibitor based therapies

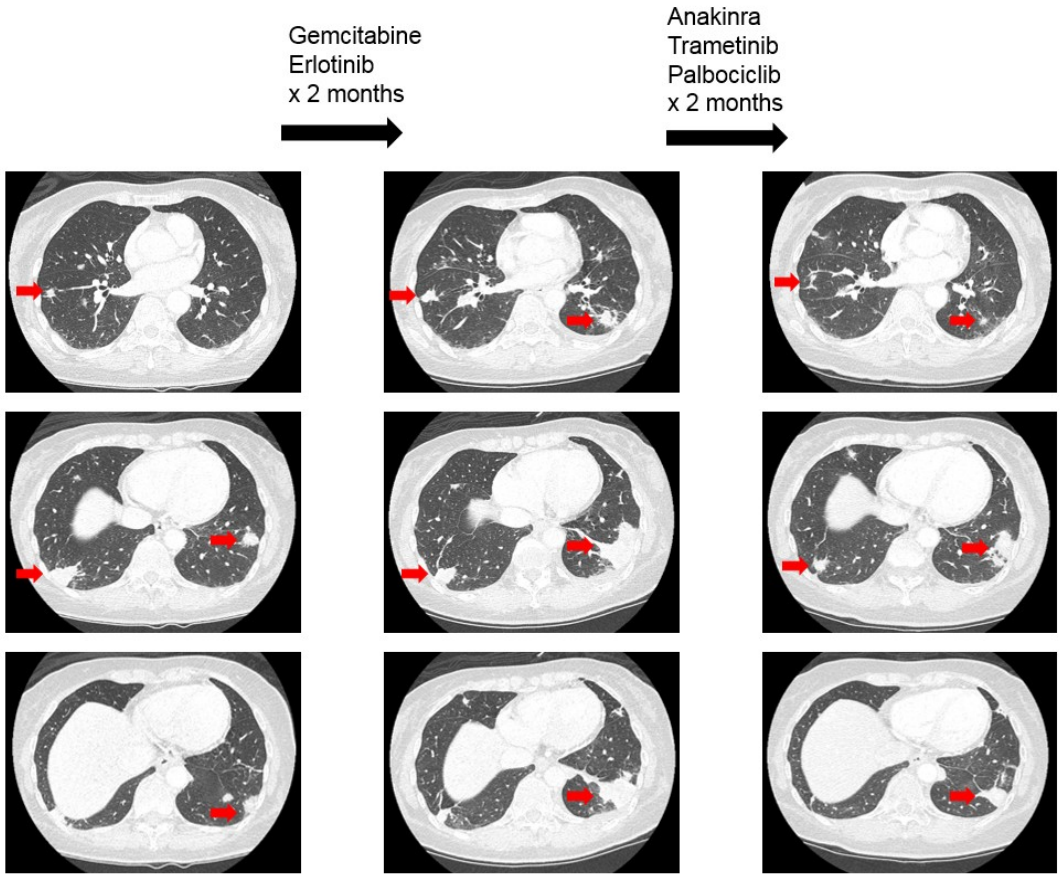
Figure 4.A. Colon cancer patient with *KRAS* G13D and *TP53* alterations managed with trametinib and bevacizumab-based targeted therapy approach.



A 50-year-old man presented with rectal pain. Further evaluation with colonoscopy revealed a rectal mass. Biopsy was consistent with moderately differentiated invasive colorectal adenocarcinoma. Further imaging with computed tomography (CT) showed lung and liver metastases (**Figure 4.A**. left panel). Patient was initially started on 5-fluorouracil and oxaliplatin. However, two months after the initiation of therapy, CT showed progression in the liver metastases (**Figure 4.A.** middle panel). The case was discussed at the Molecular Tumor Board and suggestions included continuing on 5-fluorouracil/ oxaliplatin, adding trametinib (MEK inhibitor) and anakinra (interleukin-1 [IL-1] receptor antagonist) for *KRAS* G13D (MEK inhibitor to attenuate signals downstream of KRAS. IL-1 having been shown to be a downstream mediator of cell growth in *KRAS*-mutated cells [35-38]) and bevacizumab (anti-VEGF antibody) for *TP53* C242fs*5 (*TP53* alterations are associated with increased VEGF expression [39]; clinical

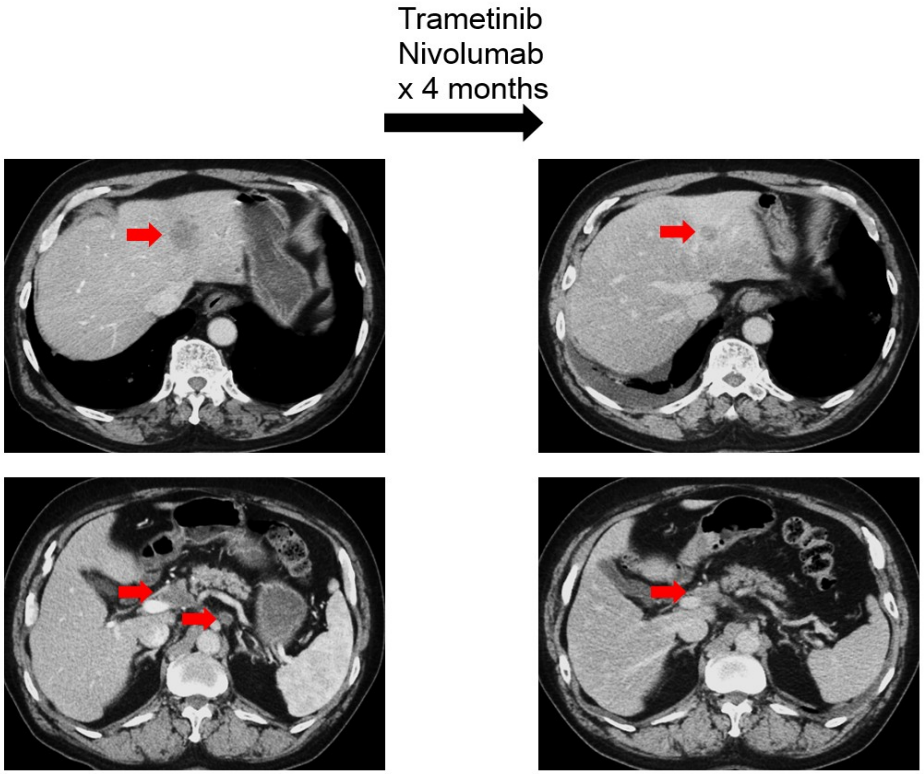
data suggested that patients with *TP53* alterations have longer PFS with bevacizumab [anti-VEGF antibody] or pazopanib [small molecule VEGFR inhibitor] containing regimens when compared to treatment without anti-angiogenesis agents [40, 41]). After adding the targeted agents, the patient achieved a partial response (30% decrease by RECIST 1.1) (**Figure 4.A.** right panel). No significant toxicities occurred. After nine months of therapy, patient elected to switch the therapy to non-conventional approach that consisted of herbal medicine.

Figure 4.B. Pancreatic cancer patient with *KRAS* G12D and *CDKN2A* R80* alteration managed with trametinib and palbociclib based combination therapy.



A 63 year-old woman was initially diagnosed with locally advanced adenocarcinoma of pancreas. Patient was started on neoadjuvant therapy with 5-fluorouracil, oxaliplatin and irinotecan followed by pancreaticoduodenectomy. Surgery was followed by adjuvant gemcitabine. Ten months after the surgery for local recurrence, patient received chemoradiation therapy with capecitabine. Patient was subsequently found to have lung metastases and received albumin-bound paclitaxel in combination with palbociclib until progression (PFS = 3.6 months; best response = stable disease) (on a clinical trial) followed by gemcitabine plus erlotinib with progression (**Figure 4.B.** Left and middle panels). Case was discussed at the Molecular Tumor Board with suggestion of trametinib (MEK inhibitor) and anakinra (IL-1 receptor antagonist) for *KRAS* G12D (IL-1 having been shown to be a downstream mediator of cell growth in *KRAS*-mutated cells [35-38]) and re-administration of palbociclib (CDK4/6 inhibitor) for *CDKN2A* R80*. After two months of combination therapy, the patient achieved a partial response (31.7% decrease by RECIST 1.1). PFS lasted 9.2 months. There was no significant toxicity.

Figure 4.C. Patient with adenocarcinoma of unknown primary with *KRAS* G12D and *MLH1* alteration managed with trametinib plus nivolumab.



An 82 year-old-man presented with abdominal pain. Further work up revealed patient to have adenocarcinoma of unknown primary with liver and abdominal lymph node metastases. Genomic analysis revealed *KRAS* G12D and *MLH1* splice site 1989+1G>T. The case was discussed at the Molecular Tumor Board and it was suggested to enroll the patient into I-PREDICT protocol (NCT02534675) and to start therapy with trametinib for *KRAS* G12D and nivolumab (anti-PD-1 inhibitor) for *MLH1* alteration (checkpoint inhibitor associated with anti-tumor activity in patients with mismatch repair gene alteration including *MLH1*) (microsatellite instability status: ambiguous; tumor mutational burden: intermediate [15 mutations/megabase]). Patient achieved a partial response (36.4% decrease per RECIST 1.1) (**Figure 4.C.** left to right) along with normalization of carbohydrate antigen 19-9 (CA-19-9) tumor marker (>10,000 U/ml down to 20 U/ml). PFS lasted 15 months [42]. There was no significant toxicity.

Table 1. Overall survival by *RAS* (*K/N/H-RAS)* subtype *alteration* and different *RAS* codon alteration status (N=1,526)***.**

Patient characteristics (N=1,526)	HR for overall survival (95% CI) (univariate) [1]	P-value (univariate) [1]	HR for overall survival (95% CI) (multivariate) [2]	P-value (multivariate) [2]
Overall survival comparing *RAS* wild-type and *RAS* altered cases				
RAS wild-type (N=1,168)	1 (reference)			
RAS alteration (N=358)	1.34 (1.11-1.63)	0.001	**1.24 (1.03-1.48)**	**0.02**
Overall survival among *K/N/H- RAS* alterations [3]				
RAS wild-type (N=1,168)	1 (reference)			
KRAS (N=295)	1.46 (1.18-1.80)	<0.0001	**1.30 (1.07-1.59)**	**0.01**
NRAS (N=48)	0.86 (0.54-1.37)	0.55		
HRAS (N=16)	1.05 (0.51-2.15)	0.89		
Overall survival among different codon alterations				
RAS wild-type (N=1,168)	1 (reference)			
KRAS G12D (N=91)	1.1 (0.76-1.6)	0.59		
KRAS G12V (N=68)	1.76 (1.14-2.7)	0.001	**1.64 (1.18-2.30)**	**0.004**
KRAS G13D (N=27)	2.01 (1.02-3.94)	0.004	**2.07 (1.27-3.38)**	**0.004**
KRAS amplification [4] (N=24)	1.84 (0.9-3.75)	0.02	**1.88 (1.09-3.24)**	**0.02**
KRAS G12C (N=22)	0.84 (0.4-1.75)	0.66		
KRAS G12R (N=17)	2.15 (0.87-5.34)	0.01	1.58 (0.83-2.99)	0.16
KRAS Q61H (N=11)	2.00 (0.64-6.20)	0.09	1.39 (0.61-3.19)	0.43
NRAS Q61K (N=12)	0.38 (0.16-0.91)	0.16		
NRAS Q61R (N=18)	1.24 (0.54-2.85)	0.57		

*****Included characteristics with N≥10.

[1] HR and P-values with univariate analysis by log-rank test.

[2] HR and P-values with multivariate analysis by Cox regression analysis. Adjusted for age, sex, primary site of cancer diagnosis and variables among sub-categories with P-value <0.1 by univariate analysis (all variables in **Table 1** and **Supplemental Table 4** with univariate p values <0.1 were included in the multivariate analysis shown in this Table)

[3] N=1 had both *KRAS* and *NRAS* alterations.

[4] *KRAS* amplification indicates amplification in wild-type *KRAS*.
Abbreviations: CI; confidence interval, HR; hazard ratio

Table 2. Overall survival by co-altered oncogenic pathways associated with *RAS* alterations (N=1,526)*.

Patient characteristics	HR for overall survival (95% CI) (univariate) [1]	P-value (univariate) [1]	HR for overall survival (95% CI) (multivariate) [2]	P-value (multivariate) [2]
Overall survival depending on *RAS* and tyrosine kinase family gene alterations (N=1,526) [3]				
RAS wild-type/Tyrosine Kinases wild-type (N=769)	1 (reference)			
RAS wild-type/Tyrosine Kinases altered (N=399)	1.11 (0.93-1.34)	0.25		
RAS altered/Tyrosine Kinases wild-type (N=278)	1.34 (1.07-1.67)	0.006	1.20 (0.98-1.47)	0.07
RAS altered/Tyrosine Kinases altered (N=80)	1.55 (1.04-2.32)	0.01	1.37 (0.98-1.93)	0.07
Overall survival depending on tyrosine kinase family gene alterations among *RAS* altered cases (N=358)				
RAS altered/Tyrosine Kinases wild-type (N=278)	1 (reference)			
RAS altered/Tyrosine Kinases altered (N=80)	1.11 (0.76-1.61)	0.58		
Overall survival depending on *RAS* and MAPK signaling alterations (N=1,526) [3]				
RAS wild-type/other MAPK wild-type (N=919)	1 (reference)			
RAS wild-type/other MAPK altered (N=249)	0.98 (0.79-1.22)	0.88		
RAS altered/other MAPK wild-type (N=294)	1.34 (1.08-1.65)	0.003	1.20 (0.99-1.46)	0.07
RAS altered/other MAPK altered (N=64)	1.35 (0.86-2.10)	0.13		
Overall survival depending on MAPK signaling alterations among *RAS* altered cases (N=358)				
RAS altered/other MAPK wild-type (N=294)	1 (reference)			
RAS altered/MAPK altered	1.04 (0.69 to 1.56)	0.87		

(N=64)				
Overall survival depending on *RAS* and PI3K signaling alterations (N=1,526) [3]				
RAS wild-type/PI3K signaling wild-type (N=783)	1 (reference)			
RAS wild-type/PI3K signaling altered (N=385)	1.20 (1.00-1.45)	0.05	**1.29 (1.07-1.56)**	**0.01**
RAS altered/PI3K signaling wild-type (N=242)	1.35 (1.06-1.72)	0.007	**1.26 (1.00-1.58)**	**0.05**
RAS altered/PI3K signaling altered (N=116)	1.63 (1.17-2.26)	0.001	**1.52 (1.15-2.01)**	**0.004**
Overall survival depending on PI3K signaling alterations among *RAS* altered cases (N=358)				
RAS altered/PI3K signaling wild-type (N=242)	1 (reference)			
RAS altered/PI3K signaling altered (N=116)	1.16 (0.84-1.61)	0.36		
Overall survival depending on *RAS* and cell cycle associated gene alterations (N=1,526) [3]				
RAS wild-type/Cell cycle gene wild-type (N=701)	1 (reference)			
RAS wild-type/Cell cycle gene altered (N=467)	1.54 (1.28-1.85)	<0.0001	**1.52 (1.27-1.81)**	**<0.0001**
RAS altered/Cell cycle gene wild-type (N=244)	1.38 (1.08-1.76)	0.006	**1.28 (1.01-1.61)**	**0.04**
RAS altered/Cell cycle gene altered (N=114)	2.40 (1.64-3.53)	<0.0001	**1.99 (1.49-2.67)**	**<0.0001**
Overall survival depending on cell cycle associated gene alterations among *RAS* altered cases (N=358)				
RAS altered/Cell cycle gene wild-type (N=244)	1 (reference)			
RAS altered/Cell cycle gene altered (N=114)	1.70 (1.20-2.41)	0.001	1.42 (0.99-2.02)	0.056
Overall survival depending on *RAS* and *BRCA* associated gene alterations (N=1,526) [3]				
RAS wild-type/*BRCA* associated gene wild-type (N=994)	1 (reference)			

RAS wild-type/*BRCA* associated gene altered (N=174)	0.91 (0.71-1.16)	0.47		
RAS altered/*BRCA* associated gene wild-type (N=309)	1.31 (1.07-1.61)	0.005	1.20 (0.99-1.46)	0.06
RAS altered/*BRCA* associated gene altered (N=49)	1.34 (0.84-2.14)	0.16		
Overall survival depending on *BRCA* associated gene alterations among *RAS* altered cases (N=358)				
RAS altered/*BRCA* associated gene wild-type (N=309)	1 (reference)			
RAS altered/*BRCA* associated gene altered (N=49)	1.00 (0.65-1.55)	0.99		
Overall survival depending on *RAS* and immune related gene alterations (N=1,526) [3]				
RAS wild-type/immune related genes wild-type (N=1,139)	1 (reference)			
RAS wild-type/immune related genes altered (N=29)	1.01 (0.57-1.80)	0.97		
RAS altered/immune related genes wild-type (N=342)	1.35 (1.11-1.64)	0.001	**1.23 (1.03-1.48)**	**0.03**
RAS altered/immune related genes altered (N=16)	1.04 (0.42-2.57)	0.92		
Overall survival depending on immune related gene alterations among *RAS* altered cases (N=358)				
RAS altered/immune related genes wild-type (N=342)	1 (reference)			
RAS altered/immune related genes altered (N=16)	0.74 (0.34-1.60)	0.50		

*Included characteristics with N≥10.

[1] HR and P-values with univariate analysis by log-rank test.

[2] HR and P-values with multivariate analysis by Cox regression analysis. Adjusted for age, sex, primary site of cancer diagnosis and variables among sub-categories with P-value <0.1 by univariate analysis (all

variables in **Table 1** or **Supplemental Table 3** with univariate p values <0.1 were included in the multivariate analysis shown in this Table)

3 See **Figure 1** And **Supplemental Table 2** for the description of co-altered oncogenic pathways; for example, immune related genes included *MLH1*, *MSH2*, *MSH6, PMS2, CD274 (PD-L1)* and *PDCD1LG2 (PD-L2)* alterations.

Abbreviations: CI; confidence interval, HR; hazard ratio

Supplemental information

Supplemental Table 1. Selected clinical trials targeting *RAS*-altered cancers.

Drug	**Target**	**Status (Phase of trial)**	**Type of cancer**	**Results**	**References**
Selected clinical trials with inhibitors that blocks RAS membrane association					
Tipifarnib (R115777)	**Farnesyltransferase**				
Tipifarnib	" "	Phase II	Pancreatic adenocarcinoma	No responses were observed (N=53).	S1
Tipifarnib	" "	Phase II	Pancreatic adenocarcinoma	No responses were observed (N= 20).	S2
Tipifarnib	" "	Phase II	*HRAS* mutant head and neck squamous cell carcinoma	PR of 67% (4/6).	S3
Tipifarnib	" "	Phase III	*KRAS* mutant colorectal cancer	No responses were observed (N=46).	S4
Tipifarnib plus Gemcitabine	" "	Phase III	Pancreatic adenocarcinoma	RR of 6% (20/341) with tipifarnib plus gemcitabine. RR was 8% (28/347) with gemcitabine alone.	S5
BMS-214662	**Farnesyltransferase**				
BMS-214662	" "	Phase I	Advanced solid tumors.	No response was observed (N=44) including patients with pancreatic cancer (N=14).	S6
L-778,123	**Farnesyltransferase and geranylgeranyltransferase type 1**				

L-778,123 plus concomitant radiotherapy	“ “	Phase I	Locally advanced pancreatic cancer	PR of 12.5% (1/8).	S7
Salirasib	**Farnesylcysteine mimetic**				
Salirasib plus gemcitabine	“ “	Phase I	Pancreatic adenocarcinoma	No response was observed (N=19).	S8
Salirasib	“ “	Phase II	*KRAS* mutant lung adenocarcinoma	No response was observed (N=33).	S9
Simvastatin	**HMG-CoA reductase inhibitors**				
Simvastatin plus cetuximab and irinotecan	“ “	Phase II	*KRAS* mutant colorectal cancer	RR of 1.9% (1/52).	S10
Simvastatin plus cetuximab	“ “	Phase II	*KRAS* mutant colorectal cancer	No response was observed (N=18).	S11
Simvastatin plus gemcitabine	“ “	Phase II	Pancreatic adenocarcinoma	PR of 6.9% (4/58) with simvastatin plus gemcitabine. Placebo plus gemcitabine had PR of 14.3% (8/56).	S12
Direct RAS inhibitor					
AMG 510	*KRAS* G12C	Phase I	*KRAS* G12C mutated solid tumors.	PR of 50% (5/10) among NSCLC patients. Stable disease of 72.2% (13/18) among colorectal cancer patients.	S13
Combination of MEK and PI3K pathway inhibitors					
Sorafenib	**RAF**				
Sorafenib	“ “	Phase I	*NRAS* mutated melanoma	No response observed (0/5).	S14
Sorafenib	“ “	Phase	*KRAS* mutated	PR was seen in 9% (5/57) of patients.	S15

		II	NSCLC		
Sorefenib plus gemcitabine	“ “	Phase II	Pancreatic adenocarcinoma	No response observed in sorafenib alone arm (N=15). Gemcitabin plus sorafenib arm had 1/37 (3%) PR.	S16
Sorafenib plus irinotecan	“ “	Phase II	*KRAS* mutated colorectal cancer	PR was seen in 1.8% (1/54) of patients.	S17
Sorafenib plus gemcitabine	“ “	Phase III	Pancreatic adenocarcinoma	Similar response rates were seen between gemcitabine plus sorafenib (23% [12/52]) and gemcitabine alone (19% [10/52]). Also, there were no statistical difference for PFS and OS.	S18
Sorafenib plus carboplatin and paclitaxel	“ “	Phase III	*NRAS* mutated melanoma	Response were seen in 22.7% (5/22) of patients. However *NRAS* WT patients also had 20% (5/25) RR.	S19
Selumetinib (AZD6244)	**MEK1/2**				
Selumetinib	“ “	Phase I	Advance solid tumors	No response observed (N=57). Among 57 patients enrolled, there were patients with *KRAS* (N=5), *NRAS* (N=4) and *BRAF* (N=1) mutations.	S20
Selumetinib	“ “	Phase II Basket trial	*KRAS* mutated NSCLC	No response observed (0/10).	S21
Selumetinib	“ “	Phase II	Pancreatic adenocarcinoma	PR was seen in 5.2% (2/38) of patients.	S22
Selumetinib plus irinotecan	“ “	Phase II	*KRAS* mutated colorectal cancer	PR was see in 9.7% (3/31) of patients.	S23
Selumetinib plus docetaxel	“ “	Phase III	*KRAS* mutated NSCLC	Addition of selumetinib to docetaxel did not improve PFS or OS when compared to placebo plus docetaxel. Selumetinib plus docetaxel vs. placebo	S24

				plus docetaxel: PFS: 3.9 months vs. 2.8 months (HR: 0.93, 95% CI: 0.77-1.12, P = 0.44), OS: 8.7 months vs. 7.9 months (HR: 1.05, 95% CI: 0.85-1.30, P = 0.64).	
Pimasertib (AS-703026)	**MEK1/2**				
Pimasertib plus FOLFIRI	" "	Phase I	*KRAS* mutated colorectal cancer	PR was seen in 13% (2/15) of patients.	S25
Trametinib (GSK1120212)	**MEK1/2**				
Trametinib plus gemcitatinbe	" "	Phase II	Pancreatic adenocarcinoma	Addition of trametinib to gemcitabine did not improve OS, PFS or RR. RR 22% with gemcitabine plus trametinib, 18% with gemcitabine alone.	S26
Trametinib	" "	Phase II	*KRAS* mutated NSCLC	PR seen in 10/86 (12%) in trametinib arm, while docetaxel arm also had PR of 5/43 (12%).	S27
Trametinib	" "	Phase I	*NRAS* mutated melanoma	No response observed (0/7).	S28
Trametinib	" "	Phase I	Advance solid tumors	Pancreatic cancer with PR of 8% (2/26). *KRAS* mutated colorectal cancer had no response (0/13). *KRAS* mutated NSCLC with PR of 11% (2/18).	S29
Trametinib plus paclitaxel	" "	Phase I	*NRAS* mutated melanoma	PR was seen in 50% of patients with *NRAS* mutated melanoma (4/8). Meanwhile PR was also seen in 33% of *NRAS* wild type melanoma (2/6).	S30
Binimetinib (MEK162, ARRY-162)	**MEK 1/2**				
Binimetinib	" "	Phase II	*NRAS* mutated melanoma	PR was seen in 20% (6/30) of patients.	S31
CI-1040 (PD184352)	**MEK1/2**				
CI-1040	" "	Phase	Advanced	Pancreatic cancer with PR of 17% (1/6).	S32

(PD184352)		I	solid tumors		
CI-1040 (PD184352)	“ “	Phase II	Advanced solid tumors	No response observed among patients with pancreatic cancer (0/15).	S33
RO4987655 (CH4987655)	**MEK1/2**				
RO4987655 (CH4987655)	“ “	Phase I	Advanced solid tumors	*KRAS* mutated colorectal cancer had no response (0/30). *KRAS* mutated NSCLC with PR of 11% (2/18).	S34
Combination of MEK and PI3K pathway inhibitors					
Selumetinib plus MK-2206 (AKT 1/2/3 inhibitor)	MEK and AKT inhibitors	Phase II	*KRAS* mutated colorectal cancer	No response observed (N = 11).	S35
Selumetinib plus MK-2206 (AKT 1/2/3 inhibitor)	MEK and AKT inhibitors	Phase I	Advance solid tumors	PR seen in 14% (4/29) patients with *KRAS*-mutant advanced solid tumors. No response was seen in *KRAS* wild-type cancers (N=33).	S36
Trametinib plus BKM120 (pan-PI3K inhibitor)	MEK and pan-PI3K inhibitors	Phase I/II	Advance solid tumors	Among *KRAS*-mutated cancers, RR was 8% (7/84). Ovarian cancer with *KRAS* mutation had RR of 29% (6/21; 1 CR, 5 PR). NSCLC with *KRAS* mutation had PR of 6% (1/17).	S37
Trametinib plus everolimus	MEK and mTOR inhibitors	Phase I	Advance solid tumors	PR seen in 5% (1/21) of patients with pancreatic cancer.	S38
Selumetinib plus MK-2206 (AKT 1/2/3 inhibitor)	MEK and AKT inhibitors	Phase II	Pancreatic adenocarcinoma	Combination of selumetinib plus MK-2206 had shorter PFS and OS when compared to 5-fluorouracil plus oxaliplatin (PFS: 1.9 vs 2.0 months, HR: 1.61, 95% CI: 1.07-2.43, P = 0.02, OS: 3.9 vs 6.7 months, HR: 1.37, 95% CI: 0.90-2.08, P = 0.15).	S39

Abbreviation: CR, complete response; CI, confidence interval; HR, hazard ratio; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; PR, partial response; RR, response rate; WT, wild type

Supplemental Table 2. Basic characteristics and types co-genomic alterations associated with *RAS* alterations (N=1,937).

Patient characteristics (N=1,937)	***RAS*** **wild-type (N=1532) (%)**	***RAS*** **altered (N=405) (%)**	***KRAS*** **altered (N=324) (%)**	***HRAS*** **altered (N=20) (%)**	***NRAS*** **altered (N=65) (%)**	**OR (95% CI) (Univariate) [1]**	**P-value (Univariate) [1]**	**OR (95% CI) (Multivariate) [2]**	**P-value (Multivariate) [2]**
Age at diagnosis									
Age <50 (N=607)	500 (82.4%)	107 (17.6%)	82 (13.5%)	1 (0.2%)	26 (4.3%)	0.74 (0.58-0.94)	0.02	0.77 (0.57-1.05)	0.10
Age ≥50 (N=1330)	1032 (77.6%)	298 (22.4%)	242 (18.2%)	19 (1.4%)	39 (2.9%)				
Gender									
Women (N=995)	790 (79.4%)	205 (20.6%)	166 (16.7%)	8 (0.8%)	32 (3.2%)	0.96 (0.77-1.2)	0.74		
Men (N=942)	742 (78.8%)	200 (21.2%)	158 (16.8%)	12 (1.3%)	33 (3.5%)				
Primary site of cancer diagnosis									
Lung (N=229)	174 (76%)	55 (24%)	52 (22.7%)	0 (0%)	3 (1.3%)	1.23 (0.89-1.7)	0.23		
Hematologic malignancies (N=208)	182 (87.5%)	26 (12.5%)	13 (6.3%)	0 (0%)	16 (7.7%)	0.51 (0.33-0.77)	0.001	**0.53 (0.33-0.85)**	**0.01**
Breast (N=185)	178 (96.2%)	7 (3.8%)	1 (0.5%)	4 (2.2%)	2 (1.1%)	0.13 (0.06-0.28)	<0.0001	**0.18 (0.08-0.4)**	**<0.0001**
Colorectal (N=185)	79 (42.7%)	106 (57.3%)	97 (52.4%)	0 (0%)	10 (5.4%)	6.52 (4.72-8.98)	<0.0001	**4.45 (2.72-7.28)**	**<0.0001**
Brain (N=172)	171 (99.4%)	1 (0.6%)	1 (0.6%)	0 (0%)	0 (0%)	0.02 (0-0.11)	<0.0001	**0.03 (0.004-0.24)**	**0.001**
Skin (N=129)	99 (76.7%)	30 (23.3%)	5 (3.9%)	7 (5.4%)	18 (14%)	1.16 (0.76-1.77)	0.5		
Head and Neck (N=103)	97 (94.2%)	6 (5.8%)	2 (1.9%)	2 (1.9%)	2 (1.9%)	0.22 (0.1-0.5)	<0.0001	**0.23 (0.1-0.54)**	**0.001**
Appendix (N=64)	27 (42.2%)	37 (57.8%)	36 (56.3%)	0 (0%)	1 (1.6%)	5.6 (3.41-9.23)	<0.0001	**3.37 (1.87-6.07)**	**<0.0001**
Ovary (N=61)	44 (72.1%)	17 (27.9%)	16 (26.2%)	0 (0%)	1 (1.6%)	1.48 (0.82-2.63)	0.2		
Pancreas (N=61)	17 (27.9%)	44 (72.1%)	44 (72.1%)	0 (0%)	0 (0%)	10.86 (6.1-19.7)	<0.0001	**8.41 (4.51-15.69)**	**<0.0001**
Stomach (N=57)	50 (87.7%)	7 (12.3%)	5 (8.8%)	0 (0%)	2 (3.5%)	0.52 (0.23-1.12)	0.14		
Liver (N=56)	46 (82.1%)	10 (17.9%)	9 (16.1%)	0 (0%)	1 (1.8%)	0.82 (0.39-1.6)	0.74		
Soft tissue sarcomas (N=56)	53 (94.6%)	3 (5.4%)	3 (5.4%)	0 (0%)	0 (0%)	0.21 (0.07-0.62)	0.002	**0.24 (0.07-0.79)**	**0.02**
Thyroid (N=46)	38 (82.6%)	8 (17.4%)	0 (0%)	3 (6.5%)	5 (10.9%)	0.79 (0.38-1.67)	0.71		
Lymphoma (N=40)	37 (92.5%)	3 (7.5%)	1 (2.5%)	1 (2.5%)	1 (2.5%)	0.3 (0.1-0.93)	0.03	0.3 (0.09-1.02)	0.054
Unknown primary (N=39)	29 (74.4%)	10 (25.6%)	7 (17.9%)	2 (5.1%)	1 (2.6%)	1.31 (0.64-2.69)	0.43		
Small intestine (N=37)	30 (81.1%)	7 (18.9%)	6 (16.2%)	0 (0%)	1 (2.7%)	0.88 (0.37-1.95)	>0.9999		
Types of co-genomic alterations									
TP53 (N=857)	644 (75.1%)	213 (24.9%)	186	10 (1.2%)	18 (2.1%)	1.53 (1.23-1.91)	0.0002	1.06 (0.8-1.41)	0.67

			(21.7%)						
CDKN2A (N=367)	288 (78.5%)	79 (21.5%)	55 (15%)	7 (1.9%)	18 (4.9%)	1.05 (0.8-1.38)	0.78		
CDKN2B (N=211)	182 (86.3%)	29 (13.7%)	22 (10.4%)	1 (0.5%)	6 (2.8%)	0.57 (0.38-0.86)	0.0069	0.9 (0.55-1.46)	0.66
PIK3CA (N=187)	143 (76.5%)	44 (23.5%)	37 (19.8%)	4 (2.1%)	3 (1.6%)	1.18 (0.82-1.69)	0.35		
TERT (N=182)	154 (84.6%)	28 (15.4%)	6 (3.3%)	8 (4.4%)	14 (7.7%)	0.66 (0.44-1.01)	0.06	1.3 (0.8-2.11)	0.29
MYC (N=165)	126 (76.4%)	39 (23.6%)	32 (19.4%)	1 (0.6%)	6 (3.6%)	1.19 (0.81-1.73)	0.37		
APC (N=162)	84 (51.9%)	78 (48.1%)	73 (45.1%)	0 (0%)	5 (3.1%)	4.11 (2.95-5.71)	<0.0001	0.98 (0.59-1.63)	0.93
PTEN (N=152)	133 (87.5%)	19 (12.5%)	13 (8.6%)	3 (2%)	3 (2%)	0.52 (0.31-0.84)	0.01	0.85 (0.48-1.53)	0.60
EGFR (N=139)	130 (93.5%)	9 (6.5%)	7 (5%)	2 (1.4%)	0 (0%)	0.25 (0.13-0.47)	<0.0001	**0.27 (0.13-0.57)**	**0.001**
BRAF (N=130)	116 (89.2%)	14 (10.8%)	7 (5.4%)	3 (2.3%)	4 (3.1%)	0.44 (0.24-0.75)	0.002	**0.29 (0.16-0.55)**	**0.0001**
NF1 (N=112)	95 (84.8%)	17 (15.2%)	11 (9.8%)	4 (3.6%)	2 (1.8%)	0.66 (0.39-1.12)	0.15		
ARID1A (N=110)	77 (70%)	33 (30%)	26 (23.6%)	2 (1.8%)	5 (4.5%)	1.68 (1.08-2.54)	0.02	**1.73 (1.03-2.9)**	**0.04**
RB1 (N=101)	93 (92.1%)	8 (7.9%)	5 (5%)	2 (2%)	1 (1%)	0.31 (0.15-0.64)	0.0006	**0.41 (0.19-0.91)**	**0.03**
SMAD4 (N=91)	45 (49.5%)	46 (50.5%)	45 (49.5%)	0 (0%)	2 (2.2%)	4.23 (2.75-6.53)	<0.0001	**2.25 (1.31-3.89)**	**0.003**
MLL2 (N=90)	78 (86.7%)	12 (13.3%)	8 (8.9%)	0 (0%)	4 (4.4%)	0.57 (0.3-1.04)	0.08	0.6 (0.29-1.23)	0.16
ERBB2 (N=85)	73 (85.9%)	12 (14.1%)	11 (12.9%)	0 (0%)	1 (1.2%)	0.61 (0.32-1.13)	0.13		
CCND1 (N=83)	74 (89.2%)	9 (10.8%)	6 (7.2%)	0 (0%)	3 (3.6%)	0.45 (0.23-0.89)	0.02	1.46 (0.27-7.82)	0.66
GNAS (N=82)	48 (58.5%)	34 (41.5%)	32 (39%)	0 (0%)	2 (2.4%)	2.83 (1.8-4.43)	<0.0001	**1.95 (1.09-3.49)**	**0.02**
LRP1B (N=81)	63 (77.8%)	18 (22.2%)	13 (16%)	3 (3.7%)	2 (2.5%)	1.09 (0.64-1.84)	0.78		
MDM2 (N=79)	66 (83.5%)	13 (16.5%)	12 (15.2%)	0 (0%)	1 (1.3%)	0.74 (0.41-1.34)	0.4		
FGF19 (N=72)	65 (90.3%)	7 (9.7%)	5 (6.9%)	0 (0%)	2 (2.8%)	0.4 (0.18-0.86)	0.02	0.75 (0.04-16.08)	0.85
FGF3 (N=72)	65 (90.3%)	7 (9.7%)	5 (6.9%)	0 (0%)	2 (2.8%)	0.4 (0.18-0.86)	0.02	4.58 (0.12-173.08)	0.41
FGF4 (N=72)	65 (90.3%)	7 (9.7%)	5 (6.9%)	0 (0%)	2 (2.8%)	0.4 (0.18-0.86)	0.02	0.18 (0.002-16.47)	0.46
NOTCH1 (N=71)	64 (90.1%)	7 (9.9%)	4 (5.6%)	2 (2.8%)	1 (1.4%)	0.4 (0.18-0.88)	0.02	0.48 (0.2-1.13)	0.09
IDH1 (N=69)	64 (92.8%)	5 (7.2%)	2 (2.9%)	0 (0%)	3 (4.3%)	0.29 (0.12-0.7)	0.003	0.75 (0.26-2.15)	0.59
CDK4 (N=66)	54 (81.8%)	12 (18.2%)	11 (16.7%)	0 (0%)	1 (1.5%)	0.84 (0.43-1.55)	0.65		
DNMT3A (N=65)	47 (72.3%)	18 (27.7%)	13 (20%)	0 (0%)	5 (7.7%)	1.47 (0.84-2.53)	0.17		
ASXL1 (N=64)	50 (78.1%)	14 (21.9%)	9 (14.1%)	1 (1.6%)	5 (7.8%)	1.06 (0.58-1.89)	0.88		
BRCA2 (N=62)	51 (82.3%)	11 (17.7%)	9 (14.5%)	1 (1.6%)	1 (1.6%)	0.81 (0.43-1.56)	0.63		
ATRX (N=58)	55 (94.8%)	3 (5.2%)	2 (3.4%)	0 (0%)	1 (1.7%)	0.2 (0.07-0.59)	0.002	0.38 (0.1-1.53)	0.17
TET2 (N=56)	45 (80.4%)	11 (19.6%)	8 (14.3%)	1 (1.8%)	2 (3.6%)	0.92 (0.48-1.75)	>0.9999		
FBXW7 (N=54)	38 (70.4%)	16 (29.6%)	14 (25.9%)	0 (0%)	3 (5.6%)	1.62 (0.9-2.91)	0.13		
ATM (N=54)	33 (61.1%)	21 (38.9%)	19 (35.2%)	1 (1.9%)	1 (1.9%)	2.48 (1.39-4.3)	0.002	1.67 (0.84-3.32)	0.15

KIT (N=51)	48 (94.1%)	3 (5.9%)	2 (3.9%)	0 (0%)	1 (2%)	0.23 (0.07-0.69)	0.005	**0.21 (0.06-0.71)**	**0.01**
ZNF217 (N=51)	45 (88.2%)	6 (11.8%)	5 (9.8%)	1 (2%)	0 (0%)	0.5 (0.23-1.16)	0.12		
CTNNB1 (N=50)	38 (76%)	12 (24%)	7 (14%)	1 (2%)	4 (8%)	1.2 (0.62-2.27)	0.6		
MCL1 (N=49)	40 (81.6%)	9 (18.4%)	7 (14.3%)	1 (2%)	1 (2%)	0.85 (0.41-1.77)	0.86		
CREBBP (N=48)	40 (83.3%)	8 (16.7%)	4 (8.3%)	1 (2.1%)	3 (6.3%)	0.75 (0.36-1.57)	0.59		
STK11 (N=44)	27 (61.4%)	17 (38.6%)	16 (36.4%)	1 (2.3%)	1 (2.3%)	2.44 (1.34-4.54)	0.01	**2.81 (1.36-5.81)**	**0.01**
FGFR1 (N=44)	39 (88.6%)	5 (11.4%)	5 (11.4%)	0 (0%)	0 (0%)	0.48 (0.2-1.18)	0.13		
ARID2 (N=43)	31 (72.1%)	12 (27.9%)	5 (11.6%)	3 (7%)	4 (9.3%)	1.48 (0.77-2.94)	0.26		
BCOR (N=39)	27 (69.2%)	12 (30.8%)	8 (20.5%)	2 (5.1%)	2 (5.1%)	1.7 (0.88-3.34)	0.16		
PTCH1 (N=38)	31 (81.6%)	7 (18.4%)	7 (18.4%)	0 (0%)	0 (0%)	0.85 (0.36-1.87)	0.84		
CCNE1 (N=37)	31 (83.8%)	6 (16.2%)	6 (16.2%)	0 (0%)	0 (0%)	0.73 (0.32-1.71)	0.68		
NOTCH2 (N=36)	31 (86.1%)	5 (13.9%)	3 (8.3%)	2 (5.6%)	0 (0%)	0.61 (0.25-1.46)	0.41		
CDH1 (N=36)	34 (94.4%)	2 (5.6%)	1 (2.8%)	1 (2.8%)	0 (0%)	0.22 (0.05-0.82)	0.02	0.38 (0.08-1.87)	0.23
PIK3R1 (N=35)	26 (74.3%)	9 (25.7%)	8 (22.9%)	1 (2.9%)	0 (0%)	1.32 (0.61-2.73)	0.53		
SF3B1 (N=35)	32 (91.4%)	3 (8.6%)	3 (8.6%)	0 (0%)	1 (2.9%)	0.35 (0.11-1.1)	0.09	0.44 (0.12-1.67)	0.23
JAK2 (N=34)	30 (88.2%)	4 (11.8%)	3 (8.8%)	0 (0%)	1 (2.9%)	0.5 (0.19-1.36)	0.28		
FAT1 (N=34)	30 (88.2%)	4 (11.8%)	3 (8.8%)	1 (2.9%)	0 (0%)	0.5 (0.19-1.36)	0.28		
AURKA (N=33)	28 (84.8%)	5 (15.2%)	5 (15.2%)	0 (0%)	0 (0%)	0.67 (0.28-1.65)	0.52		
NF2 (N=32)	28 (87.5%)	4 (12.5%)	2 (6.3%)	1 (3.1%)	1 (3.1%)	0.54 (0.2-1.48)	0.28		
NFKBIA (N=32)	25 (78.1%)	7 (21.9%)	6 (18.8%)	1 (3.1%)	0 (0%)	1.06 (0.44-2.46)	0.83		
SMARCA4 (N=32)	23 (71.9%)	9 (28.1%)	9 (28.1%)	0 (0%)	0 (0%)	1.49 (0.68-3.21)	0.38		
SPTA1 (N=32)	23 (71.9%)	9 (28.1%)	6 (18.8%)	1 (3.1%)	2 (6.3%)	1.49 (0.68-3.21)	0.38		
GATA3 (N=31)	31 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0-0.39)	0.001	Not applicable	1.00
BRCA1 (N=31)	29 (93.5%)	2 (6.5%)	2 (6.5%)	0 (0%)	0 (0%)	0.26 (0.06-0.98)	0.05	0.25 (0.05-1.14)	0.07
MET (N=31)	26 (83.9%)	5 (16.1%)	5 (16.1%)	0 (0%)	0 (0%)	0.72 (0.3-1.81)	0.66		
PBRM1 (N=31)	24 (77.4%)	7 (22.6%)	5 (16.1%)	1 (3.2%)	1 (3.2%)	1.11 (0.45-2.59)	0.82		
RET (N=30)	28 (93.3%)	2 (6.7%)	1 (3.3%)	0 (0%)	1 (3.3%)	0.27 (0.06-1.03)	0.07	**0.19 (0.04-0.85)**	**0.03**
SOX9 (N=30)	12 (40%)	18 (60%)	18 (60%)	0 (0%)	0 (0%)	5.89 (2.86-12.05)	<0.0001	2.14 (0.88-5.2)	0.09

Included characteristics with N≥30.
N=4 had both *KRAS* and *NRAS* alterations.
[1] OR and P-values with univariate analysis by Fisher's exact test. Evaluated between *RAS* wild-type and *RAS* altered cases for each characteristics.
[2] OR and P-values with multivariate analysis by logistics regression analysis. Characteristics with P-value <0.1 from univariate analysis were selected for the analysis. P < 0.05 was considered to be statistically significant.

Abbreviations: CI; confidence interval, OR; odds ratio

Supplemental Table 3. Co-altered oncogenic pathways associated with *RAS* alterations (N=405)

Types of co-genomic alterations with *RAS* mutations	**Frequencies**
Tyrosine Kinase families (N=87)	**21.5%**
ABL1/2 (N=3)	0.7%
ALK (N=3)	0.7%
AXL (N=4)	1%
EGFR (N=9)	2.2%
ERBB2 (N=12)	3%
ERBB3 (N=9)	2.2%
ERBB4 (N=7)	1.7%
FGF3/4/6/10/14/19/23 (N=22)	5.4%
FGFR1/2/3 (N=11)	2.7%
IGF1R (N=1)	0.2%
JAK2/3 (N=4)	1%
KDR (N=2)	0.5%
KIT (N=3)	0.7%
MET (N=5)	1.2%
NTRK1/3 (N=9)	2.2%
PDGFR A/B (N=4)	1%
RET (N=2)	0.5%
ROS1 (N=2)	0.5%
SRC (N=1)	0.2%
MAPK signaling (N=74)	**18.3%**
ARAF (N=1)	0.2%
BRAF (N=14)	3.5%
GNAS (N=34)	8.4%
MAP2K1/2 (N=2)	0.5%
MAP3K1 (N=6)	1.5%
NF1 (N=17)	4.2%
PTPN11 (N=5)	1.2%
RAF1 (N=3)	0.7%
PI3K signaling (N=127)	**31.4%**
AKT1/2/3 (N=11)	2.7%

FBXW7 (N=16)	4%
MTOR (N=4)	1%
NF2 (N=4)	1%
PIK3CA/C2B/CB/CG/R1/R2 (N=59)	14.6%
PTEN (N=19)	4.7%
RICTOR (N=9)	2.2%
RPTOR (N=1)	0.2%
STK11 (N=17)	4.2%
TSC1/2 (N=4)	1%
Cell cycle associated genes (N=126)	**31.1%**
CCND1/2/3 (N=20)	4.9%
CDK4/6 (N=14)	3.5%
CDKN2A/B (N=79)	19.5%
CDKN1B/2C (N=5)	1.2%
CDK8 (N=7)	1.7%
CDK12 (N=4)	1%
CCNE1 (N=6)	1.5%
RB1 (N=8)	2%
***BRCA* associated genes (N=52)**	**12.8%**
BRCA1/2 (N=12)	3%
ATM (N=21)	5.2%
ATR (N=6)	1.5%
BARD1 (N=1)	0.2%
BRIP1 (N=3)	0.7%
CHEK2 (N=6)	1.5%
FANCA/C/D2 (N=5)	1.2%
PALB2 (N=5)	1.2%
Mismatch repair and immune associated genes (N=16)	**4%**
MLH1 (N=3)	0.7%
MSH2 (N=3)	0.7%
MSH6 (N=6)	1.5%
PMS2 (N=4)	1%

CD274 (PD-L1) (N=1)	0.2%
PDCD1LG2 (PD-L2) (N=1)	0.2%

Supplemental Table 4. Association between basic characteristics and overall survival (N=1,526)

Patient characteristics (N=1,526)	**HR for overall survival (95% CI) (univariate)**	**P-value (univariate)**
Age at diagnosis		
Age <50 year (N=482) vs. ≥ 50 year	0.63 (0.54-0.74)	<0.0001
Gender		
Woman (N=812) vs. man	0.79 (0.68-0.92)	0.003
Primary site of cancer diagnosis		
Lung (N=208) vs. other	1.33 (1.04-1.70)	0.01
Colorectal (N=184) vs. other	0.94 (0.74-1.20)	0.62
Breast (N=181) vs. other	0.89 (0.73-1.09)	0.26
Skin (N=116) vs. other	0.71 (0.53-0.95)	0.05
Brain (N=114) vs. other	0.86 (0.65-1.13)	0.32
Head and Neck (N=100) vs. other	0.88 (0.66-1.18)	0.42
Appendix (N=61) vs. other	0.86 (0.57-1.29)	0.49
Ovary (N=59) vs. other	0.52 (0.37-0.73)	0.003
Pancreas (N=56) vs. other	2.82 (1.61-4.94)	<0.0001
Liver (N=53) vs. other	3.72 (1.98-7.00)	<0.0001
Soft tissue sarcomas (N=47) vs. other	1.18 (0.75-1.88)	0.44
Thyroid (N=44) vs. other	0.4 (0.28-0.58)	0.001
Stomach (N=40) vs. other	1.26 (0.75-2.12)	0.33
Unknown primary (N=37) vs. other	1.49 (0.87- 2.55)	0.08
Endometrial (N=28) vs. other	0.47 (0.27-0.83)	0.06
Esophagus (N=27) vs. other	1.44 (0.75-2.78)	0.19
Small intestine (N=27) vs. other	0.65 (0.34-1.24)	0.28
Bladder (N=25) vs. other	1.19 (0.66-2.17)	0.53
Prostate (N=22) vs. other	0.45 (0.26-0.77)	0.04
Gallbladder/ bile duct (N=19) vs. other	2.24 (0.79-6.31)	0.02
Kidney (N=16) vs. other	2.19 (0.91-5.24)	0.01

Peritoneum (N=14) vs. other	1.59 (0.58-4.34)	0.26
Anus (N=11) vs. other	1.41 (0.54-3.66)	0.40

Included characteristics with N≥10.
HR and P-values with univariate analysis by log-rank test
All variables in **Table 1** and **Supplemental Table 4** with univariate p values <0.1 were included in the multivariate analysis for **Table 1**.

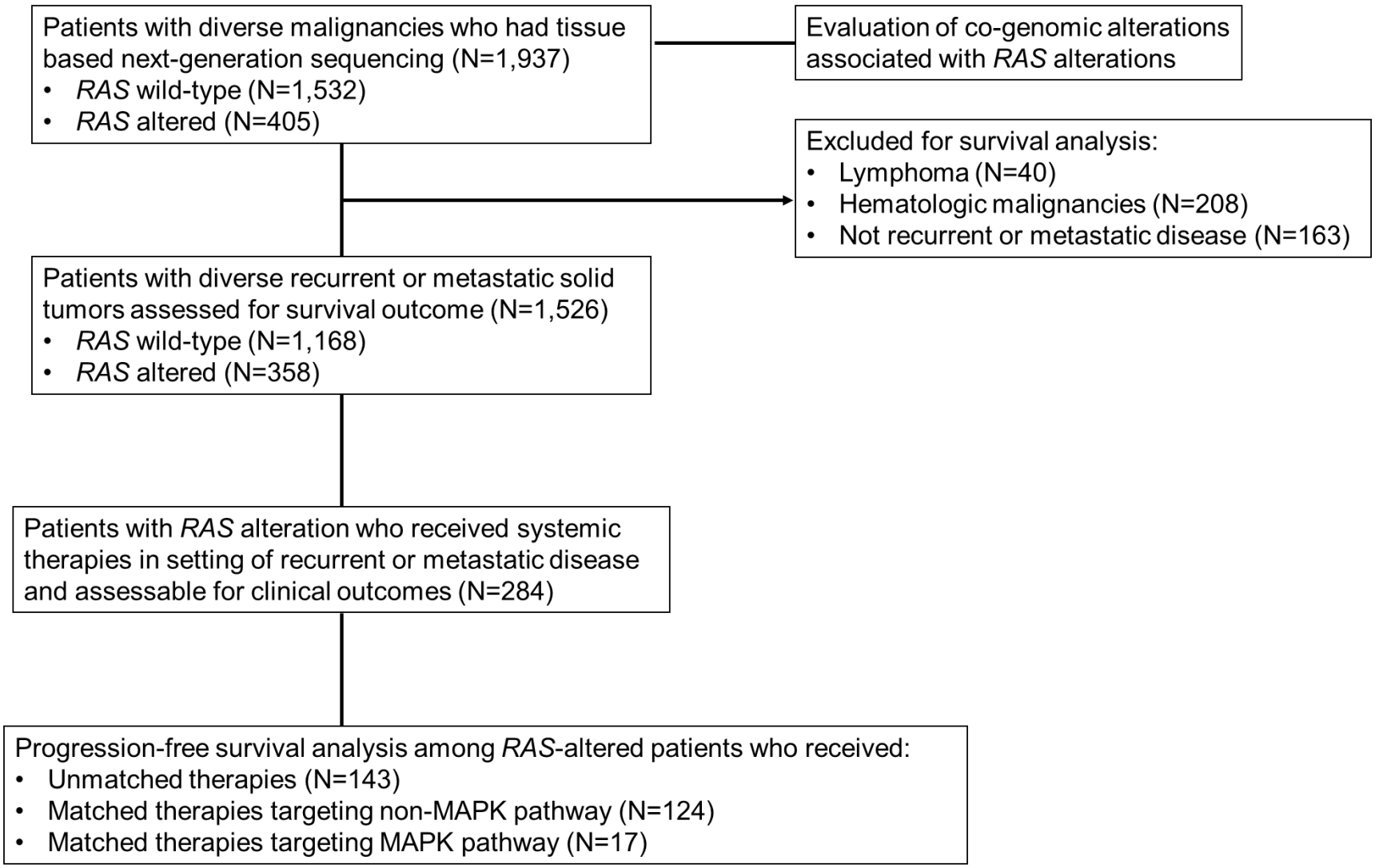
Abbreviations: CI; confidence interval, HR; hazard ratio

Supplemental Table 5. Progression-free survival analysis among patients with *RAS* alterations who received systemic therapies (N=284).

Patient characteristics (N=284)	HR for PFS (95% CI) (univariate) [1]	P-value (univariate) [1]	HR for PFS (95% CI) (multivariate) [2]	P-value (multivariate) [2]
Age at diagnosis				
Age <50 (N=73) vs. ≥ 50 year	0.82 (0.61-1.08)	0.17		
Gender				
Women (N=141) vs. man	0.85 (0.66-1.10)	0.21		
Primary site of cancer diagnosis				
Colorectal (N=88) vs. other	0.74 (0.57-0.97)	0.04	0.83 (0.62-1.13)	0.24
Lung (N=42) vs. other	0.92 (0.65-1.30)	0.63		
Pancreas (N=36) vs. other	1.86 (1.14-3.04)	0.001	**1.70 (1.11-2.59)**	**0.01**
Appendix (N=26) vs. other	0.84 (0.54-1.29)	0.45		
Skin (N=22) vs. other	1.18 (0.71-1.99)	0.49		
Ovary (N=11) vs. other	0.99 (0.54-1.80)	0.97		
Lines of therapy				
First line therapy (N=222) vs. ≥2 lines of therapy	1.00 (0.72-1.40)	1.00		
Types of therapy				
Unmatched therapy (N=143)	1 (Reference)			
Matched by targeting MAPK pathway alone with MEK inhibitor based therapies (N=17)	1.14 (0.60-2.19)	0.67		
Matched by targeting non-MAPK pathway (N=124)	0.79 (0.61-1.03)	0.07	0.89 (0.67-1.19)	0.42

Included characteristics with N≥10.
[1] HR and P-values with univariate analysis by log-rank test.
[2] HR and P-values with multivariate analysis by Cox regression analysis. Included factors with P-value <0.1 by univariate analysis.
Abbreviations: CI; confidence interval, HR; hazard ratio, PFS; progression-free survival

Supplemental Figure 1. Consort diagram of study among patients who had tissue based next-generation sequencing (N=1,937)



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