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Original Article

KRT-232 and navitoclax enhance trametinib's anti-Cancer activity in non-small cell lung cancer patient-derived xenografts with KRAS mutations

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Abstract: Activating mutations of the KRAS gene are one of the major genomic alterations associated with tumorigenesis of non-small cell lung cancer (NSCLC). Thus far, treatment of KRAS-mutant NSCLC remains an unmet medical need. We determined the *in vivo* treatment responses of 13 KRAS mutant and 14 KRAS wild type NSCLC patient-derived xenografts (PDXs) to agents that target known NSCLC vulnerabilities: the MEK inhibitor trametinib, the MDM2 inhibitor KRT-232, and the BCL-X_L/BCL-2 inhibitor navitoclax. The results showed that the tumor regression rate after single agent therapy with KRT-232, trametinib and navitoclax was 11%, 10% and 0%, respectively. Combination therapies of trametinib plus KRT-232 and trametinib plus navitoclax led to improved partial response rates over single-agent activity in a subset of PDX models. Tumor regression was observed in 23% and 50% of PDXs after treatment with trametinib plus KRT-232 and trametinib plus navitoclax, respectively. The disease control rates in KRAS-mutant PDXs tested were 90%-100% after treatment with trametinib plus KRT-232 or plus navitoclax. A correlation analysis of treatment responses and genomic and proteomic biomarkers revealed that sensitivity to KRT-232 was significantly associated with TP53 wild-type or STK11 mutant genotypes ($P<0.05$). The levels of several proteins, including GSK3b, Nrf2, LKB1/pS334, and SMYD3, were significantly associated with sensitivity to trametinib plus navitoclax. Thus, the combination of trametinib plus KRT-232 or navitoclax resulted in improved efficacy compared with the agents alone in a subgroup of NSCLC PDX model with KRAS mutations. Expanded clinical trials of these targeted drug combinations in NSCLC are warranted.

Keywords: NSCLC, target therapy, combination therapy, MEK, MDM2, Bcl2

Introduction

The mitogen-activated protein kinase (MAPK) pathway is one of the most commonly activated oncogenic pathways in a vast array of cancers, including non-small cell lung cancer (NSCLC). Constitutive activation of the MAPK pathway resulting from upstream activating mutations of oncogenes such as KRAS, BRAF, and EGFR is one of the major drivers of uncontrolled cell proliferation, cell de-differentiation, and tumorigenesis. The MAPK kinases (MEK1 and MEK2) function as gatekeepers in the MAPK pathway because they are the only known mediators that convey activating signals from RAF to ERK. Consequently, MEK inhibitors have been intensively investigated, both preclinically and clinically, for the treatment of NSCLC [1]. The combi-

nation of the MEK inhibitor trametinib and the BRAF inhibitor dabrafenib was approved by the United States Food and Drug Administration for the treatment of BRAF V600E mutant NSCLC in 2017 [2, 3]. Trametinib combined with chemoradiation therapy, erlotinib, navitoclax, lapatinib, and the immune checkpoint inhibitor pembrolizumab are also undergoing clinical trials for the treatment of NSCLC, with or without KRAS mutations [1]. The combination of the MEK inhibitor selumetinib and chemotherapy has been investigated in clinical trials for the treatment of KRAS mutant or KRAS wild-type (wt)/unknown NSCLC [4-6]. A randomized phase III trial showed that selumetinib plus docetaxel resulted in a higher objective response rate than did placebo and docetaxel in KRAS mutant NSCLC but did not improve the median progression-free survival or median overall survival [4]. The MEK inhibitors cobimetinib and binimetinib, in combination with BRAF, EGFR, or immune checkpoint blockade inhibitors or chemotherapy, are also being studied in various clinical trials for the treatment of NSCLC, with and without KRAS mutations [1, 7].

We previously reported that treatment of NSCLC cells with selumetinib inhibited cell proliferation and induced apoptosis by increasing the expression of the pro-apoptotic BCL2 family proteins Bim, PUMA, and NOXA through enhanced nuclear translocation of FOXO3a [8]. Because the interaction between pro-apoptotic and anti-apoptotic BCL2 family proteins strictly controls the intrinsic apoptotic process, the overall ratio of pro-apoptotic and anti-apoptotic BCL2 family proteins in cancer cells is a key factor in the apoptotic response to targeted therapy. We hypothesized that pharmaceutical interventions that further change the balance of pro-apoptotic and anti-apoptotic BCL2 family proteins inside cancer cells will enhance the anti-cancer activity of MEK inhibitors. To test this hypothesis and to identify predictive biomarkers for personalized therapy, we tested whether combinations of MEK inhibitors with the MDM2 inhibitor KRT-232 (AMG 232) or BCL-X_L/BCL2 inhibitor, navitoclax, lead to enhanced therapeutic effects in NSCLC PDX models. KRT-232 is a potent MDM2 inhibitor [9] that enhances TP53 activity by blocking the interaction between MDM2 and TP53 and inducing TP53-mediated pro-apoptotic protein expression [10]. A phase I study of KRT-232 in patients with advanced TP53 wt solid tumors or multiple myeloma revealed that it has acceptable safety and dose-proportional pharmacokinetics and has induced stable disease in some patients [11]. The combination of KRT-232 and trametinib was evaluated in a phase Ib study in patients with relapsed or refractory acute myeloid leukemia [12], and the results suggested that the pharmacokinetics of KRT-232 and trametinib were not affected by co-administration [12]. Navitoclax is a potent inhibitor of BCL-X_L and BCL-BCL2 [13] and is currently under clinical investigation for the treatment of hematologic malignancies and solid tumors, including in combination therapy with trametinib for the treatment of solid tumors with KRAS or NRAS mutations [1].

Patient-derived xenografts (PDXs) are increasingly being used in preclinical anti-cancer drug development because of their ability to recapitulate the histological and molecular biological features of human primary tumors and predict clinical treatment responses [14, 15]. Over the past several years, we have generated about 200 NSCLC PDX models from surgically resected specimens, pleural fluid drainage samples, and biopsy samples. Histological and molecular characterizations on some of these models showed that they recapitulate the features of human primary tumors in their histology, genomic alterations, and tumor microenvironment [16-18]. Here we report the *in vivo* anti-cancer activity of trametinib, KRT-232, and navitoclax, in combination and as single agents, and biomarkers associated with treatment responses. The combination of trametinib and KRT-232 or navitoclax resulted in improved efficacy in a subgroup of NSCLC PDX models with KRAS mutations, supporting the feasibility of these combinations for the treatment of KRAS mutant NSCLC.

Materials and methods

Therapeutic agents

KRT-232 was provided by Amgen, Inc., and Kartos Therapeutics, Inc. Navitoclax was provided by Abbvie, Inc., both obtained through the Division of Cancer Treatment and Diagnosis of the National Cancer Institute. Trametinib was obtained from Selleck Chemicals.

NSCLC PDXs

The generation and passage of NSCLC PDXs, verification of PDX provenance by DNA fingerprint analysis, genomic characterization by next generation sequencing, and histological char-

acterization of PDXs were performed as we previously described [16, 17]. Next generation sequencing was performed at MD Anderson Moon Shot platform Cancer Genomics Laboratory. All clinical samples and data were collected with informed patient consent under a research protocol approved by the Institutional Review Board at The University of Texas MD Anderson Cancer Center (Houston, Texas). All animal studies were carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health Publication 85-23) and the institutional guidelines of MD Anderson.

Reverse-phase protein array (RPPA) assay

A small piece of tissue from each PDX model was lysed in RPPA lysis buffer and subjected to a RPPA assay at the Functional Proteomics RPPA Core facility at MD Anderson, as previously reported [19, 20]. In brief, serially diluted lysates were arrayed on nitrocellulose-coated slides. Each slide was probed with a validated primary antibody and then with a biotin-conjugated secondary antibody. After performing a colorimetric reaction of horseradish peroxidase and its substrate 3,3'-diaminobenzidine, we scanned the slides and quantified the signals on the basis of spot intensity. We used 196 antibodies specific for human proteins and protein phosphorylation sites to determine baseline protein levels in this study.

Animal experiments

In vivo drug testing studies was performed with randomizing animals in treatment groups and recording results in al blinded manner. Each PDX was subcutaneously inoculated into the dorsal flanks of female nude mice or non-obese diabetic/severe combined immunodeficiency (NOD-SCID) mice with null mutations of the gene encoding interleukin-2 receptor g (NSG). Subcutaneous tumors were measured with calipers, and tumor volume was calculated according to the formula $V=ab^2/2$, where *a* is the largest diameter and *b* is the smallest. Mice were added to treatment groups (n=3-5/group) randomly when tumors reached 200 mm^3 in size (about 7-9 mm in diameter). The following treatment regimens were used for each testing agent: (1) 45 mg/kg of KRT-232, oral gavage once a day for 5 days/week for 3 weeks; (2) 0.1 mg/kg of trametinib, oral gavage once a day for 4 weeks; and (3) 100 mg/kg of navitoclax, oral gavage once a day for 3 days/week for 3 weeks. Treatment with solvent was used as the control. For combination therapies, animals were treated with the same doses and regimens as for the single-agent therapy except that the 2 drugs were administered in combination. Tumor growth and body weight were monitored every 2 to 3 days. Tumor volume changes were calculated with an R software program, with tumor volume at the beginning of treatment set to a baseline of 0. The experiment was ended and the mice were euthanized when the tumors reached 15~18 mm in diameter. A total of 28 PDX models were tested for 6 treatment groups and one solvent control in this study. However, for each treatment group, the numbers of PDX models tested varied from 8 to 22 models. Some models were tested for certain treatment groups depending on numbers of animals available to be enrolled to the study.

Statistical analysis

For tumor volume data, the time points were transferred from the exact date to the relative days from the treatment start point (day 0 or t_0) for each mouse. For individual mice, the tumor volume change for each time point was calculated as a relative level of tumor growth change from the baseline: $\delta_t = \frac{V_t - V_0}{V_0} \times 100$, where V_t is the tumor volume at time t and V_0 is the tumor volume at baseline. For animals for which there was no tumor volume measurement at time t ($t_0<t<t_1$), we imputed the missing tumor volume data by linear interpolation: $\delta_t=\delta_0+\beta(t-t_0)$, where $\beta = \frac{\delta_{t_1} - \delta_{t_0}}{t_1 - t_0}$. The adjusted area under the curve (aAUC) was defined as the mean percentage change over time, which was computed as the area under the tumor growth curve from the baseline up to time *t*, divided by *t*. We determined the aAUC values at 21 days and the last observation time point for each individual mouse, and used the aAUC at 21 days for comparison among groups because the treatments with both KRT-232 and navitoclax ended by day 21.

For drug response data, at a given time (t=21 days), each animal was classified into 1 of 3 groups by comparing tumor volume changes, as calculated by $aAUC_{0\text{-}21\ day}$ or at day 21 after treatment initiation compared with the baseline: partial response ([PR], tumor regression ≥ 30% or $\delta_t \leq$-30%), stable disease ([SD], tumor growth was significantly suppressed when compared with animals treated with solvent control ($P<0.05$) but $\delta_t >$-30%), or progressive disease [PD], tumor volume was not significantly differ-

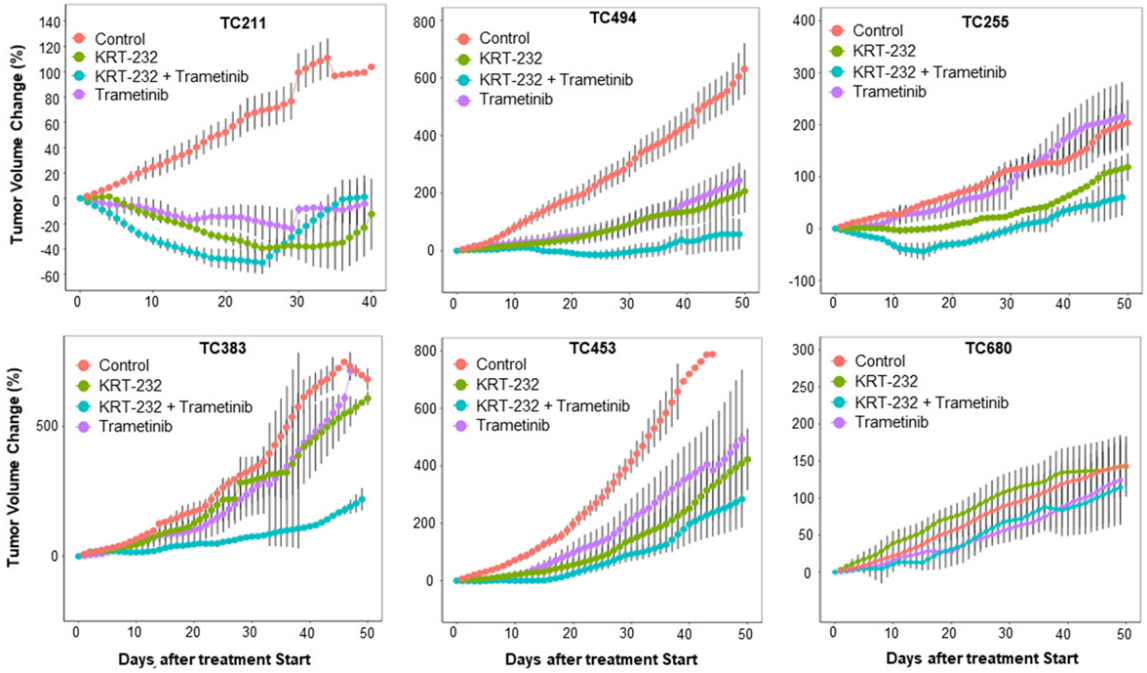

Figure 1. Effect of trametinib and KRT-232 combination therapy in NSCLC PDXs. NSCLC PDXs were treated with trametinib, KRT-232, or both, as described in the Materials and Methods. Mice treated with vehicles were used as controls. Tumor volume changes after treatment were determined by an R software program. The Y axis shows the mean ± standard error (n=3-5/group) of tumor volume changes for each PDX, with the starting tumor volume (about 200 mm^3) and treatment starting day set as 0. The tumor volume changes for 6 PDXs are shown as examples. The combination therapy had more activity than did single-agent therapy in PDX TC211, TC494, TC255, and TC383, but not in TC453 (TP53 wt/KRAS mutated) and TC680 (TP53 wt/KRAS wt).

ent from control group (P>0.05). For the PDX model, 1-way ANOVA or 2-sample t-tests were performed to test for the difference in tumor volume change (δ_t) and aAUC at a given time point between the treatment and control groups, if applicable. For association studies of mutation and RPPA data, we combined PR and SD into a sensitive group and compared it with the resistant group PD using Fisher's exact tests for mutation data and 2-sample t-tests for RPPA data. A multiplicity adjustment was performed by controlling the false discovery rate, as described previously [21]. Fisher exact test was used to compare the difference in treatment responses among different models. All statistical analyses were performed using R software version 3.5.2. A *P* value <0.05 was regarded as significant.

Results

Combination of KRT-232 and trametinib resulted in greater anti-cancer activity than did single agents in NSCLC PDXs

We determined the *in vivo* activity of the combination of KRT-232 and trametinib in 22 NSCLC PDX models, including 11 KRAS mutant and 11 KRAS wild type NSCLC PDXs. **Figure 1** shows examples of tumor volume changes in mice treated with solvent (control), single agents, and combination therapy over time. We observed 2 patterns of tumor growth: 1) combination therapy resulted in significantly more anti-cancer activity than did single-agent therapy, as shown in PDXs TC211, TC494, TC255, and TC383; and 2) combination therapy resulted in either similar tumor volume changes as did single-agent therapy in PDX TC453 or having no activity as did solvent or single-agent treatment in PDX TC680.

To categorize treatment responses, we determined tumor volume changes at the end of treatment (day 21) and the aAUC from the start of treatment (day 0) to the end of treatment ($AUC_{0\text{-}21\ day}$) to measure duration of response (**Figure 2**). We used the criteria to categorize treatment responses into three groups PR, SD, and PD, as described in Materials and Methods. On the basis of these criteria, 5 (23%), 10 (46%), and 7 (32%) of 22 PDXs treated with KRT-232 plus trametinib had a PR, SD, and PD,

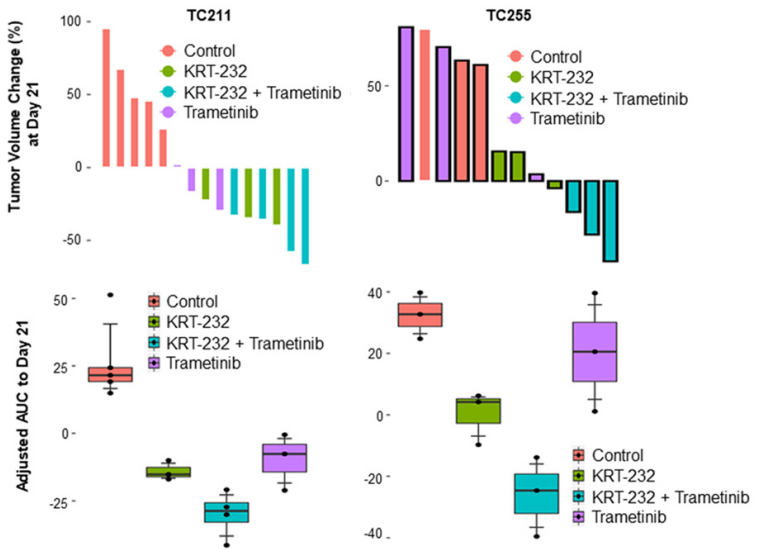

Figure 2. Treatment responses are presented as waterfall graphs and aAUC graphs. Tumor volume changes for each individual mouse at day 21 after treatment started are presented as a waterfall graph in the top panel for PDX TC211 and TC255. The box plots (bottom panel) shows the aAUC, with overall tumor volume changes from day 0 to day 21. The values represent the median (line inside box) and the third and first quartile (box) ± 1.5 x the interquartile range from the top and bottom of the box (error bar). The aAUC in the combination group is significantly different from the control and single-agent groups (P<0.05).

Table 1. Summary of treatment responses in lung PDX models

Treatment	PR (%)	SD (%)	Resistance (%)	Total
KRT-232 + Trametinib	5 (23)	10 (46)	7 (32)	22
KRT-232 + Navitoclax	1 (9)	9 (82)	1 (9)	11
Trametinib + Navitoclax	5 (50)	3 (30)	2 (20)	10
KRT-232	2 (11)	5 (28)	11 (61)	18
Navitoclax	0 (0)	4 (50)	4 (50)	8
Trametinib	2 (10)	10 (48)	9 (43)	21

respectively (**Table 1**). In the 11 KRAS mutant PDXs, the PR, SD, and PD rates were 36% (4), 55% (6), and 9% (1), respectively (**Table 2**).

Combination of navitoclax and trametinib resulted in greater anti-cancer activity than did single agents in KRAS mutant NSCLC PDXs

We determined the efficacy of combined treatment with navitoclax and trametinib in 10 NSCLC PDXs, including 7 KRAS mutant PDXs. **Figure 3** shows the responses to combination therapy versus single-agent therapy in 3 selected KRAS mutant PDXs. Combination therapy resulted in significantly greater *in vivo* activity, as determined by the $aAUC_{0\text{-}21\ day}$, in all the 3 PDX models shown in **Figure 3** (P<0.05). Of 10 PDX models treated with navitoclax plus trametinib 5 (50%), 3 (30%), and 2 (20%) NSCLC PDXs experienced a PR, SD, and PD, respectively (**Table 1**). All 7 KRAS mutant PDXs also responded to this combination treatment (PR, 5 of 7 [71%], or SD, 2 of 7 [29%]) (**Table 2**).

KRT-232 and navitoclax combined did not result in greater anti-cancer activity than did single agents in NSCLC PDXs

We tested 11 NSCLC PDX models to determine the *in vivo* anti-cancer activity of KRT-232 plus navitoclax combination therapy, including 4 TP53 mutant and 5 KRAS mutant PDXs. All KRAS mutant models tested were TP53 wild type. The combination led to 1 PR, 9 SD, and 1 PD. We did not observe any significant improvement in anti-cancer activity with combination therapy compared with single-agent activity (**Figure 4**). In three KRAS mutant PDXs that were tested for all six treatment groups, combination therapies of trametinib plus KRT-232 and/or trametinib plus navitoclax had enhanced anticancer activity when compared with all other groups (**Figure 5**).

The responses in all treatment groups are summarized in **Table 1** and Supplementary Table 1. A PR was induced in 11% (2 of 18), 10% (2 of 21), and 0% (0 of 8) of mice after treatment with KRT-232, trametinib, and navitoclax alone, respectively. The PR rate in animals treated with trametinib plus navitoclax was significantly higher than those treated with trametinib alone (P=0.022) or those treated with navitoclax alone (P=0.036). In the KRAS mutant PDXs tested, the PR in the trametinib plus navitoclax group was significantly higher than in the trametinib alone group (P=0.035) but was not sig-

Table 2. Summary of treatment responses in KRAS mutant lung PDX models

Treatment	PR (%)	SD (%)	Resistance (%)	Total
KRT-232 + Trametinib	4 (36)	6 (55)	1 (9)	11
KRT-232 + Navitoclax	1 (20)	4 (80)	0 (0)	5
Trametinib + Navitoclax	5 (71)	2 (29)	0 (0)	7
KRT-232	1 (13)	4 (50)	3 (38)	8
Navitoclax	0 (0)	2 (50)	2 (50)	4
Trametinib	1 (10)	7 (70)	2 (20)	10

PR: Tumor Regression ≥ 30%. SD: Tumor growth inhibition (tumor regression <30%; Tumor growth significantly suppressed vs controls).

nificant when compared with the navitoclax alone group (P=0.06). We did not observe significant differences in body weight in any treatment groups, suggesting that the treatments, including combination therapy, were not toxic at the doses tested in this study.

Molecular biomarkers associated with treatment response

We determined whether treatment responses in NSCLC PDX models were associated with genotype in TP53, KRAS, STK11, and EGFR or with protein expression levels by performing a reverse-phase protein microarray (RPPA) analysis. For this purpose, we combined PR and SD as a sensitive group and compared with PD as a resistant group. Fisher exact test revealed that the TP53 and STK11 genotypes were significantly associated with sensitivity to KRT-232 single-agent therapy. Seven of 11 TP53 wt PDXs were sensitive to KRT-232 (PR or SD [64%]), while none of the 7 TP53 mutant PDXs were sensitive (Supplementary Table S2) (P=0.013). Among 11 TP53 WT PDXs, 4/4 with mutated STK11 and 3/7 with STK11 WT were sensitive to KRT-232 treatment. Interestingly, all 4 STK11 mutant PDXs were sensitive to KRT-232 therapy compared with only 3 of 14 STK11 wt PDXs (21%) (P=0.011). Among 10 PDXs treated with trametinib plus navitoclax, all 7 KRAS mutant PDXs were sensitive, compared with 1 of 3 KRAS wt PDXs was sensitive, indicating that trametinib plus navitoclax is more effective in KRAS mutant tumors; however, the difference was not statistically significant (P= 0.067).

A comparison of protein expression levels between treatment-sensitive (PR + SD) and -resistant (PD) groups revealed that levels of Bcl-X_L, DUPS4, Rb, and BAP1 were significantly associated with sensitivity to KRT-232 as a single agent (P<0.05), when analyzed with either all PDX models tested or with TP53 wt PDXs only. However, in either case, the false discovery rate was greater than 0.3 (Supplementary Table 3). No significant association between KRT-232 and MDM2pS166 expression was observed when analyzed with all tested models or with TP53 wt only.

In the trametinib plus navitoclax treated group, the protein levels of GSK3b, Nrf2, LKB1/pS334, catalase, CTLA4, SMYD3, PCNA, and RAD50 were significantly different between the sensitive and resistant groups (P<0.005 at false discovery rate of 0.07). PDXs that were sensitive to trametinib plus navitoclax expressed higher levels of Nrf2, LKB1/pS334, catalase, and SMYD3 but lower levels of GSK3b, CTLA4, PCNA, and RAD50 than did those that were resistant (**Figure 6**). For the other treatment groups tested in this study, we did not identify any proteomic biomarkers that were significantly associated with sensitivity at the genomic and proteomic levels.

Discussion

We determined treatment responses to single-agent and combination therapies with the MEK inhibitor trametinib, the MDM2 inhibitor KRT-232, and the BCL-X_L/BCL-2 inhibitor navitoclax in molecularly annotated NSCLC PDX models. Our results showed that trametinib plus KRT-232 or navitoclax led to greater *in vivo* anti-cancer activity than did single-agent therapy, whereas KRT-232 plus navitoclax did not result in improved activity. We did not observe obvious weight loss in any of the treatment groups and tested models, suggesting that all treatment regimens were not toxic. Moreover, we identified genomic and proteomic biomarkers that were significantly associated with treatment responses to single-agent KRT-232 and trametinib plus navitoclax.

KRT-232 is a potent MDM2 inhibitor that activates TP53 signaling and inhibits tumor cell proliferation in TP53 wt tumor cells but has no significant effect on TP53 mutant tumor cells [10]. Similar to the published results from cell lines, we found that among 18 NSCLC PDX models tested, none of the TP53 mutated tumors was sensitive to KRT-232 treatment. KRT-

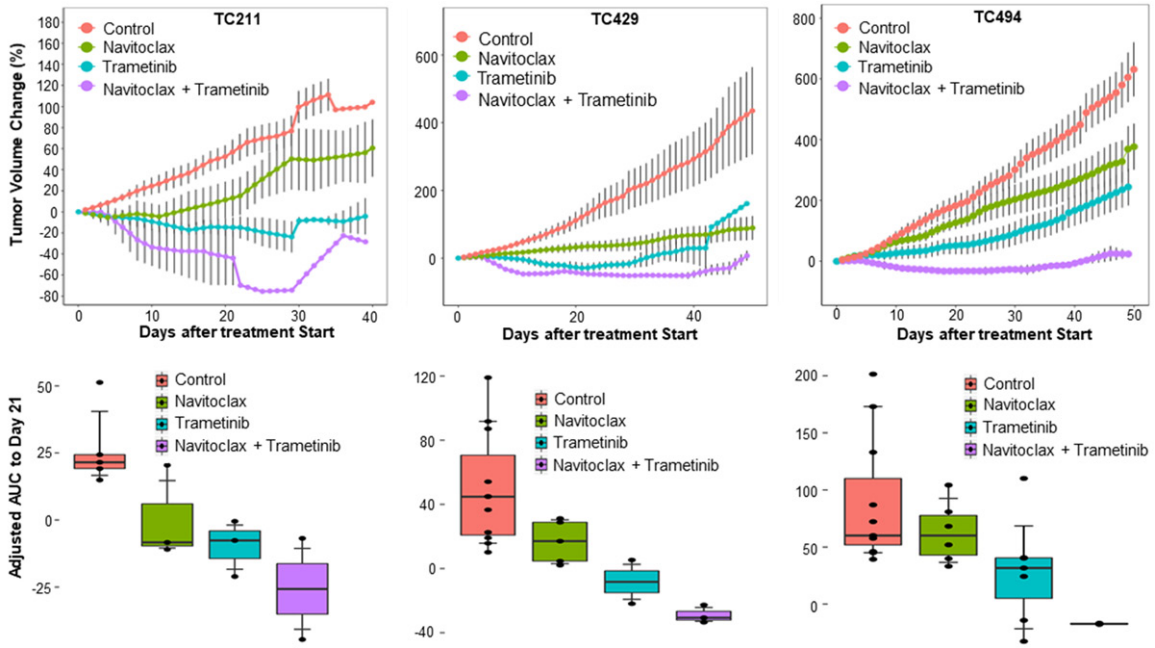

Figure 3. Combined effect of trametinib and navitoclax in NSCLC PDXs. The tumor volume changes and aAUC for 3 KRAS mutant PDXs are presented after treatment with trametinib, navitoclax, or both. The tumor volume changes and aAUC were determined as described in **Figures 1** and **2**. All 3 PDXs had an enhanced response to the combination therapy compared with single-agent therapies.

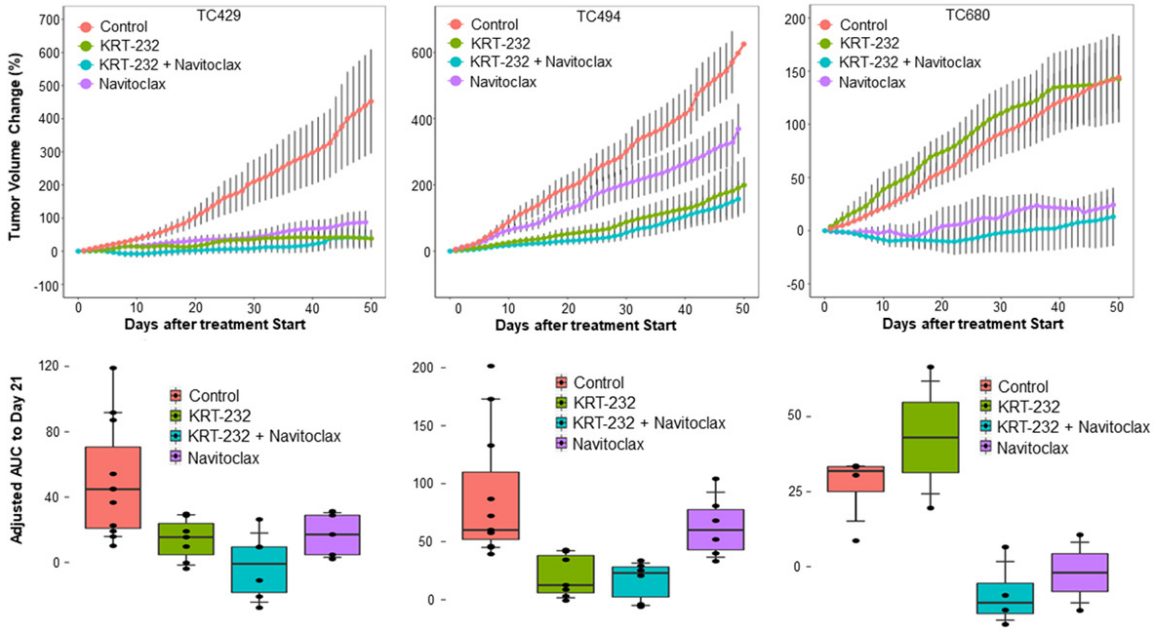

Figure 4. Combination effect of KRT-232 and navitoclax in NSCLC PDXs. The tumor volume changes and aAUC for 3 KRAS mutant PDXs are presented after treatment with KRT-232, navitoclax, or both. The tumor volume changes and aAUC were determined as described in **Figures 1** and **2**. The treatment responses to combination therapy were not significantly different to those to single-agent therapies in all 3 PDXs.

232 anti-cancer activity was observed in 7 of 11 TP53 wt PDXs but none of the 7 TP53 mutant PDXs. Interestingly, we also found an association between response to KRT-232 and

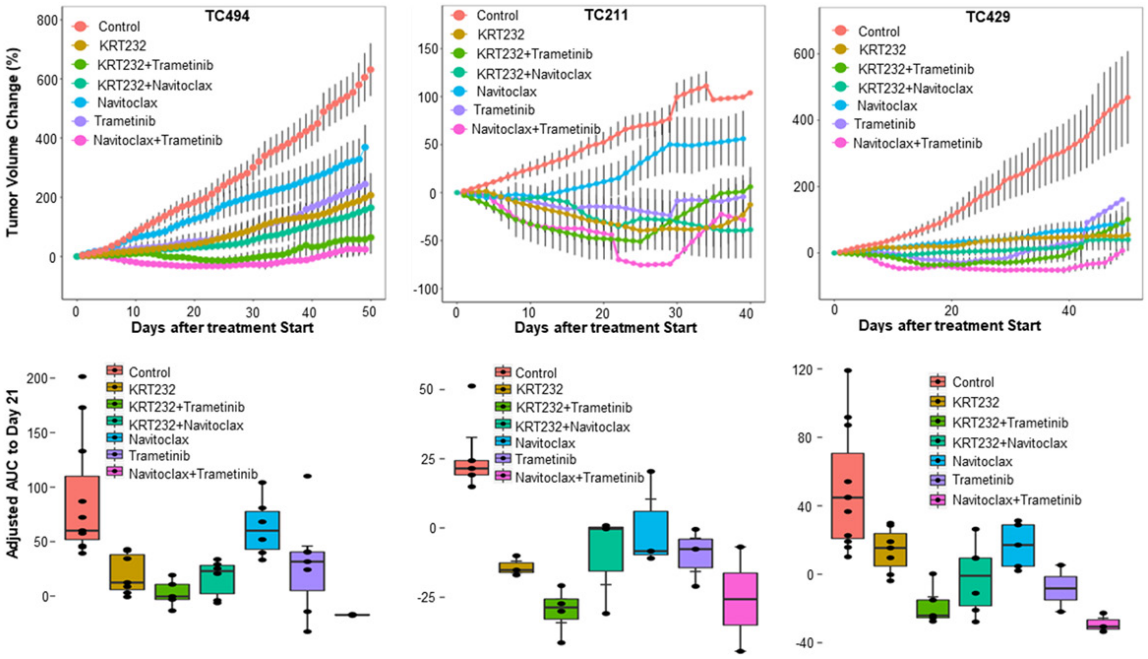

Figure 5. The tumor volume changes and aAUC for 3 KRAS mutant PDXs that were tested for all six treatments. The tumor volume changes and aAUC were determined as described in **Figures 1** and **2**. The combination therapies of trametinib plus KRT-232 and/or trametinib plus navitoclax had enhanced anticancer activity in these 3 PDXs.

STK11 mutations: all 4 SKT11 mutant PDXs responded to KRT-232. As these 4 STK11 mutant NSCLC were TP53 wt and KRAS mutant, it is possible that the response to KRT-232 was primarily due to activation of TP53. Nevertheless, this result indicated that STK11 mutations would not have a negative effect on the anti-cancer activity of KRT-232. Because mutations of SKT11 and TP53 are mutually exclusive in lung adenocarcinoma with KRAS mutations [22] and because lung adenocarcinoma with concomitant mutations of KRAS and STK11 is highly resistant to immune checkpoint blockade therapy [23], it will be important to determine whether KRT-232 can sensitize these tumors to immune checkpoint blockade therapy.

Trametinib plus KRT-232 or navitoclax is currently being studied in clinical trials for cancer treatment [1, 12]. Both combination therapies have been found to be more effective than single-agent therapy in several models, particularly in KRAS mutant models. All 7 KRAS mutant PDX models were sensitive to trametinib plus navitoclax in our study (5 PR and 2 SD). Ten of the 11 KRAS mutant PDX models were sensitive to trametinib plus KRT-232 (4 PR and 6 SD).

Our study of proteomic biomarkers revealed that the expression levels of several proteins or phosphorylation sites were associated with treatment responses to trametinib plus navitoclax. It is not yet clear whether the levels of these protein markers were associated with KRAS mutations. Increased expression of NRF2 and catalase may indicate the presence of oxidative stress, whereas increased expression of LKB1/pS334 may indicate increased AKT activity in tumors, as phosphorylation of LKB1 at Ser334 by AKT was reported to block the tumor suppressor activity of LKB1 [24]. KRAS mutations are known to increase oxidative stress and activate the PI3K/AKT pathway [25, 26]. In addition, SMYD3, a histone lysine methyltransferase, is reported to promote RAS-driven tumorigenesis by methylating MAP3K2 and potentiating activation of the RAS/RAF/MEK/ERK signaling pathway [27]. Thus, several protein biomarkers that are associated with response to trametinib and navitoclax are involved in RAS-induced alterations in cellular metabolism, signal transduction, and cooperation with oncogenic RAS in tumorigenesis.

Nevertheless, this study has some limitations. Because all PDX models tested in this study were established in immune-compromised

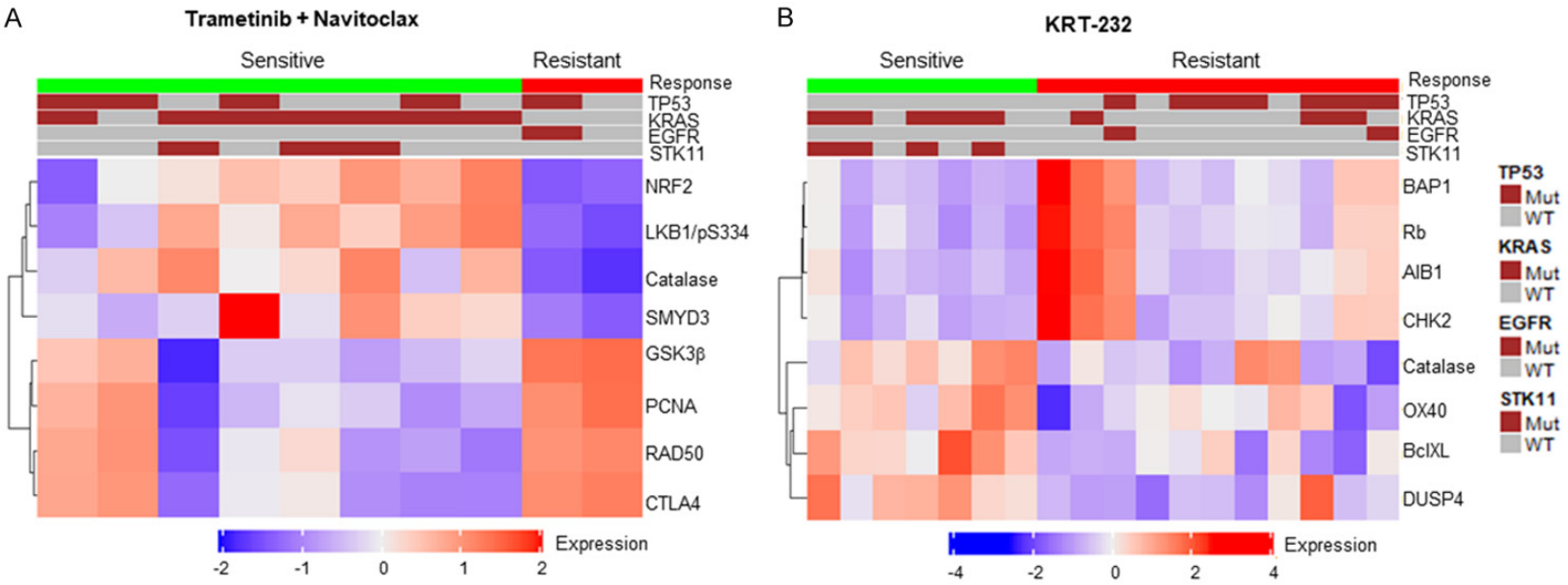

Figure 6. Heatmaps of baseline protein levels in sensitive and resistant PDXs. The heatmaps for the top 8 proteins whose levels were significantly different between sensitive and resistant PDXs are presented for trametinib plus navitoclax (A) and KRT-232 monotherapy (B). Each vertical column represents each PDX model tested. Side bars for treatment responses and genotypes of TP53, KRAS, STK11, and EGFR are shown on the top of each graph. WT: wild type; Mut: mutant.

mice, the effects of host immune responses and the tumor immune microenvironment could not be determined. Although we tested treatment responses in multiple PDX models, the number of models tested was relatively small and may not cover all subtypes of KRAS mutant lung adenocarcinoma. It may be important to note that not all of the models were tested with all of the treatments, so the statistical comparisons between the treatments and between markers of sensitivity/resistance may be underpowered. It is not yet clear whether the molecular biomarkers identified in this study could be used for patient stratification in future clinical trials, in addition to TP53 wild type as the current patient selection marker for treatment with KRT-232. However, because effective therapy for KRAS-mutant NSCLC, one of the most common molecular subtypes of NSCLC, is not yet available clinically, our results support the initiation of expanded clinical trials of combination therapies of trametinib plus KRT-232 or navitoclax for KRAS mutant NSCLC.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. PDX Models and Response

PDX ID	Histology	Grade	TP53	KRAS	STK11	EGFR	KRT-232	KRT-232		Trametinib		KRT-232
								Trametinib	Trametinib	Navitoclax	Navitoclax	Navitoclax
TC286	ACA	Poor	Mut	G12D	Mut	WT	NA	Resistant	NA	NA	NA	NA
TC211	ACA	Poor	WT	Q61H	Mut	WT	PR	PR	SD	PR	SD	PR
TC247	ACA	Moderate	WT	G12C	Mut	WT	SD	NA	NA	NA	Resistant	SD
TC255	ACA	Poor	WT	Q61H	Mut	WT	SD	PR	SD	NA	NA	NA
TC453	ACA	Poor	WT	G12C	Mut	WT	SD	SD	Resistant	SD	NA	SD
TC494	ACA	Moderate	WT	G12V	Mut	WT	SD	SD	SD	PR	Resistant	SD
TC219	Pleo	Poor	Mut	G13D	WT	WT	NA	NA	Resistant	NA	NA	NA
TC241	ACA	Moderate	Mut	G12S	WT	WT	Resistant	PR	SD	PR	NA	NA
TC303	ACA	Poor	Mut	G12C	WT	WT	NA	SD	SD	NA	NA	NA
TC314	ACA	Moderate	Mut	G12C	WT	WT	Resistant	SD	SD	PR	NA	NA
TC333	Pleo	Poor	Mut	G12D	WT	WT	NA	SD	NA	SD	NA	NA
TC429	ACA	Moderate	WT	G12V	WT	WT	SD	PR	PR	PR	SD	SD
TC551	ACA	Poor	WT	G12C	WT	WT	Resistant	SD	SD	NA	NA	NA
TC479	ACA	Poor	WT	WT	WT	Mut	NA	Resistant	Resistant	NA	NA	NA
TC618	SquCA	Poor	Mut	WT	WT	WT	NA	Resistant	NA	NA	NA	NA
TC386	ACA	Poor	Mut	WT	WT	Mut	Resistant	SD	SD	Resistant	NA	SD
TC393	SquCA	Moderate	Mut	WT	WT	WT	Resistant	Resistant	Resistant	NA	Resistant	SD
TC397	SquCA	Moderate	Mut	WT	WT	WT	NA	SD	SD	SD	NA	NA
TC562	ACA	Poor	Mut	WT	WT	Mut	Resistant	Resistant	NA	NA	SD	SD
TC616	SquCA	Moderate	Mut	WT	WT	WT	Resistant	NA	Resistant	NA	NA	NA
TC664	SquCA	Moderate	Mut	WT	WT	WT	Resistant	SD	SD	NA	NA	SD
TC371	ACA	Moderate	WT	WT	WT	WT	PR	PR	PR	NA	NA	NA
TC383	SquCA	Moderate	WT	WT	WT	WT	Resistant	SD	Resistant	NA	NA	NA
TC464	ACA	Poor	WT	WT	WT	WT	NA	Resistant	NA	Resistant	NA	NA
TC680	ACA	Poor	WT	WT	WT	WT	Resistant	Resistant	Resistant	NA	SD	SD
TC257	SquCA	Moderate	WT	WT	WT	WT	Resistant	NA	NA	NA	Resistant	Resistant
TC423	ACA	Moderate	WT	WT	WT	WT	NA	NA	Resistant	NA	NA	NA

Supplementary Table 2. Gene Mutation Association

	KRT-232		
STK11	Sensitive	Resistant	*P* value
Mut	5	0	
WT	2	11	0.0025
	KRT-232		
TP53	Sensitive	Resistant	*P* value
Mut	0	7	0.013
WT	7	4	
	Trametinib+Navitoclax		
KRAS	Sensitive	Resistant	*P* value
Mut	7	0	0.067
WT	1	2	

Supplementary Table 3. RPPA in TP53wt PDXs

Proteins	KRT-232 (Mean)		*P* value	Adjust P
	Resistant	Sensitive		
DUSP4	-3.024	-2.067	0.006	0.497
STAT3/pY705	-0.514	0.059	0.020	0.497
Rb	1.461	-0.754	0.020	0.497
AIB1	0.833	-0.702	0.027	0.497
BclXL	0.924	1.201	0.027	0.497
BAP1	0.158	-1.465	0.029	0.497
Cdc2	0.947	-1.267	0.031	0.497
AMPKa/pT172	1.761	2.321	0.038	0.497
CHK2	0.973	-1.210	0.041	0.497
Snail	0.419	-1.196	0.045	0.497
	KRT-232+Trametinib		*P* value	Adjust P
	Resistant	Sensitive		
cJUN/pS73	0.428	-0.012	0.007	0.975
CD38	-0.225	0.547	0.028	0.975
AKT	1.620	2.020	0.038	0.975
TSC2/pT1462	-0.508	-0.907	0.038	0.975
AMPKa/pT172	1.408	2.170	0.039	0.975
Met/pY1234/1235	-0.797	-1.767	0.044	0.975
IRS1	0.189	-0.260	0.049	0.975