FULL LENGTH ARTICLE

The role of distinct co-mutation patterns with *TP53* mutation in immunotherapy for NSCLC

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Abstract *TP53* mutations was reported to be correlated to the efficacy of program death-1 (PD-1) and program death ligand-1 (PD-L1). The role of co-mutations of *TP53* with other recurrently mutated genes in outcome of anti-PD-(L)1 treatment for non-small cell lung cancer (NSCLC) is unknown. Here we mined a previously generated dataset to address the effect of co-mutations on the progression free survival (PFS) of NSCLC patients. Non-synonymous mutations and clinical data of 240 NSCLC patients with anti-PD-(L)1 based therapy was downloaded from cBioPortal. Totally 206 patients received monotherapy and 34 patients received combination therapy. In 240 NSCLC patients, TP53 mutation rate was 59.2%. For the monotherapy cohort, *TP53* mutated NSCLC patients have a significantly longer PFS (4.3 *vs.* 2.5 months, $P = 0.0019$) compared with *TP53* wild type NSCLC patients. The same tendency was also observed in the combination therapy cohort, but the difference in PFS (6.3 *vs.* 5.4 months, $P = 0.12$) was not significant. Ever-smoker had a longer PFS compared to never-smokers (4.0 *vs.* 2.7 months). For further co-mutation analysis with *TP53* including *KEAP1* mutation (53/240, 22.1%), *KMT3C* mutation (26/240, 10.8%), *STK11* mutation (56/240, 23.3%), *EGFR* mutation (28/240, 11.7%) and *KRAS* mutation (86/240, 35.8%). Patients with both *TP53* plus *KEAP1*

mutations in all 240 patients had a longer PFS compared with co-wild population (PFS 9.2 *vs.* 4.2 months, $P = 0.012$) when treated with PD-1/PD-L1 inhibitors. *TP53* might be the dominating mutation correlating with longer PFS in PD-1/PD-L1 monotherapy. Different genes displayed distinct effect when co-mutated with *TP53* in NSCLC patients.

Introduction

Checkpoint as PD-1 and PD-L1 inhibitors have emerged as the most promising therapeutics for non-small-cell lung cancer (NSCLC) which could prolong the 5-year survival in those responders.[1] However, the efficacy of checkpoint inhibitors in NSCLC was limited with an objective response rate of around 20%.[2] Identification of biomarkers with predictive power for the outcome of checkpoint inhibition could guide the clinical decision to employ checkpoint inhibitors. Currently, expression of PD-L1 and tumor mutation burden (TMB) are most wildly investigated as biomarkers to predict the effect of checkpoint inhibitors.[3,4] However, some PD-L1 negative and TMB low NSCLC populations could still respond to PD-1/PD-L1 antibodies. The complexity of checkpoint inhibition has yet to be investigated.

TP53 is one of the well-studied genes in human. With another tumor suppressor gene *CHEK2*, p53 checks whether DNA mutations in a damaged cell can be repaired or the cell has to be destroyed.[5] It was reported that *TP53* mutation is independently correlated with longer OS in advanced NSCLC patients.[6] This effect can be partially explained by the connection between *TP53* and TMB. If the functionality of p53 is intact, the magnitude of mutations in cancer will be kept at minimal level. TP53 mutation was reported to significantly increase the expression of immune checkpoints and activated T-effector and interferon-signature and *TP53/KRAS* co-mutation NSCLC showed remarkable clinical benefit to PD-1 inhibitors in a small sampled study with 34 patients,[7] which needs to be further confirmed.

The mutational landscape of cancer is rather complexed.[8] For NSCLC, there are many other recurrently mutated genes with mutation frequency above 10%.[9] Examples include *KRAS*, *KEAP1*, *STK11* and *EGFR*. *KRAS* mutations leads to hyperactivated downstream signaling controlling cell proliferation.[10] *KEAP1* is an important regulator of antioxidant response, determining the cellular outcome after exposure to oxidative stress.[11] *STK11* is a major modulator of lung cancer differentiation and metastasis.[12] *EGFR* is an important receptor regulating *RAS/MAPK*, *PI3K/AKT* signaling pathways, and the target of EGFR-TKI (Tyrosine Kinase Inhibitor). *EGFR* mutations critically impacts the clinical outcomes of NSCLC patients.[13]

It is not clear that how co-mutation of *TP53* with oncogenes or other tumor suppressor genes influence the response of NSCLC patients to checkpoint inhibitors. Our study took advantage of a recently published cohort of NSCLC patients with mutation data and survival data after receiving either monotherapy using anti-PD-1/PD-L1 therapy or combination therapy of anti-PD-1/PD-L1 and anti-CTLA-4.[14]

Specifically, this study aims to investigate the impact of co-mutation pattern on progression free survival of NSCLC patients.

Materials and methods

Data collection

Non-synonymous mutations and clinical data of 240 NSCLC patients with anti-PD-(L)1 based therapy was downloaded from cBioPortal.[14,15] Patient samples were analyzed by MSK-IMPACT assay as previously described. Sequencing libraries were generated for a custom panel of 341 (56 patients, version 1), 410 (164 patients, version 2) and 468 (20 patients, version 3) genes. In total, 206 patients received monotherapy with PD-1/PD-L1 inhibitors and 34 patients received combination therapy with PD-1/PD-L1 inhibitor and anti-CTLA-4 therapy. All patients were enrolled in Memorial Sloan Kettering Cancer Center between April 2011 and January 2017.

Survival analysis

Survival analysis was performed with Kaplan–Meier method. Survminer was used to implement survival analysis. All plots were generated with R statistical programming environment. For each patient stratification method, survival curves were plotted for the monotherapy cohort, the combination therapy cohort and the complete patient cohort.

To determine single-cell mutation and double-gene mutation, only non-synonymous mutations were considered. Kaplan–Meier curves analysis of progression-free survival (PFS) were compared using the log-rank test.

Statistics

No statistical method was carried out to estimate the sample number. All reported P values are two-tailed, and for all analyses, P less than 0.05 is considered statistically significant, unless otherwise specified. Hazard ratios (HRs) were calculated by the Mantel–Haenszel test. Given that smoking acts as a possible treatment selection bias, we performed multivariable extended cox regression when accessing the effect of co-mutation.

Table 1 Patients characteristics.

| Item		number	mPFS (all)	logrank_P	cox_P	HR	0.95LCI	0.95UCI	mPFS (mono)	logrank_P	cox_P	HR	0.95LCI	0.95UCI	mPFS (combination)	logrank_P	cox_P	HR	0.95LCI	0.95UCI
Diagnosis Age	18–60	76	3.13	0.39					3.05	0.91					5.73	0.26				
	≥60	164	3.50		0.393	1.1	0.84	1.5	3.07		0.915	1	0.73	1.4	7.9		0.267	1.6	0.7	3.6
Sex	Female	122	3.07	0.54					2.77	0.74					6.33	0.43				
	Male	118	3.50		0.542	1.1	0.83	1.4	3.23		0.738	1.1	0.78	1.4	7.90		0.427	1.4	0.63	3
smoking	Ever	193	4.00	0.031					3.3	0.0025					6.33	0.93				
	never	47	2.67		0.032	1.4	1	2	2.1		0.003	1.8	1.2	2.6	11.83		0.933	1	0.43	2.5
Pathology	squamous cell carcinioma	34	2.92	LUAD-LUSC: 0.9782	0.981	1.01	0.67	1.5	3.23	LUAD-LUSC: 0.8644	0.426	0.84	0.54	1.3	1.83	LUAD-LUSC: 0.0709	0.043	3.96	1.04	15
	adenocarcinoma	186	3.50	LUAD-Others: 0.9639					3.07	LUAD-Others: 0.8644					8.63	LUAD-Others: 0.6416				
	Others	20	3.68	LUSC-Others: 0.9639	0.535	0.84	0.47	1.5	2.52	LUSC-Others: 0.8644	0.753	0.91	0.49	1.7	6.33	LUSC-Others: 0.2571	0.668	0.73	0.17	3.1
Lines.of. treatment of PD-1/ PD-L1	1 st	51	7.50	0.00046					5.47	0.2					10.46	0.087				
	≥2	189	2.73		0.005	1.7	1.2	2.4	2.67		0.201	1.3	0.86	2	4.33		0.093	2	0.89	4.5
Detection panel	IMPACT341	56	2.92	IMPACT341-IMPACT410: 0.4389					2.1	IMPACT341-IMPACT410: 0.4389					7.9	IMPACT341-IMPACT410: 0.4057				
	IMPACT410	164	3.50	IMPACT341-IMPACT468: 0.4389	0.401	0.87	0.63	1.2	3.17	IMPACT341-IMPACT468: 0.4389	0.035	0.69	0.48	0.97	6.33	IMPACT341-IMPACT468:0.4057	0.405	1.4	0.61	3.3
	IMPACT468	20	4.17	IMPACT410-IMPACT468: 0.4389	0.238	0.68	0.36	1.3	6.03	IMPACT410-IMPACT468: 0.4389	0.038	0.5	0.26	0.96	3.43	IMPACT410-IMPACT468:0.4057	0.209	4	0.46	34.2
KEAP1	yes	53	2.80	0.53	0.538	0.9	0.64	1.3	2.5	0.77					22.63	0.1	0.12	0.31	0.072	1.4
	no	187	3.50						3.07		0.777	0.95	0.67	1.4	5.43					
KMT2C	yes	26	7.33	0.049	0.052	0.62	0.39	1	4.17	0.11					22.43	0.23	0.247	0.42	0.099	1.8
	no	214	3.17						2.9		0.118	0.67	0.41	1.1	5.43					
STK11	yes	56	2.54	0.23	0.229	1.2	0.88	1.7	2.47	0.21					9.10	0.65	0.651	0.78	0.27	2.3
	no	184	3.80						3.23		0.207	1.2	0.89	1.8	6.33					
EGFR	yes	28	3.07	0.038	0.04	1.6	1	2.4	3.07	0.12					2.92	0.044	0.052	2.8	0.99	8
	no	212	3.50						3.03		0.123	1.4	0.9	2.3	7.9					
KRAS	yes	86	3.43	0.83	0.824	0.97	0.72	1.3	3.07	0.56					4.88	0.79	0.783	1.1	0.5	2.5
	no	154	3.30						2.8		0.559	0.91	0.67	1.2	8.63					
TP53	yes	142	4.27	0.0019	0.002	0.64	0.49	0.85	4.00	0.0078					6.33	0.12	0.127	0.55	0.25	1.2
	no	98	2.47						2.47		0.008	0.67	0.49	0.9	5.43					

A

B
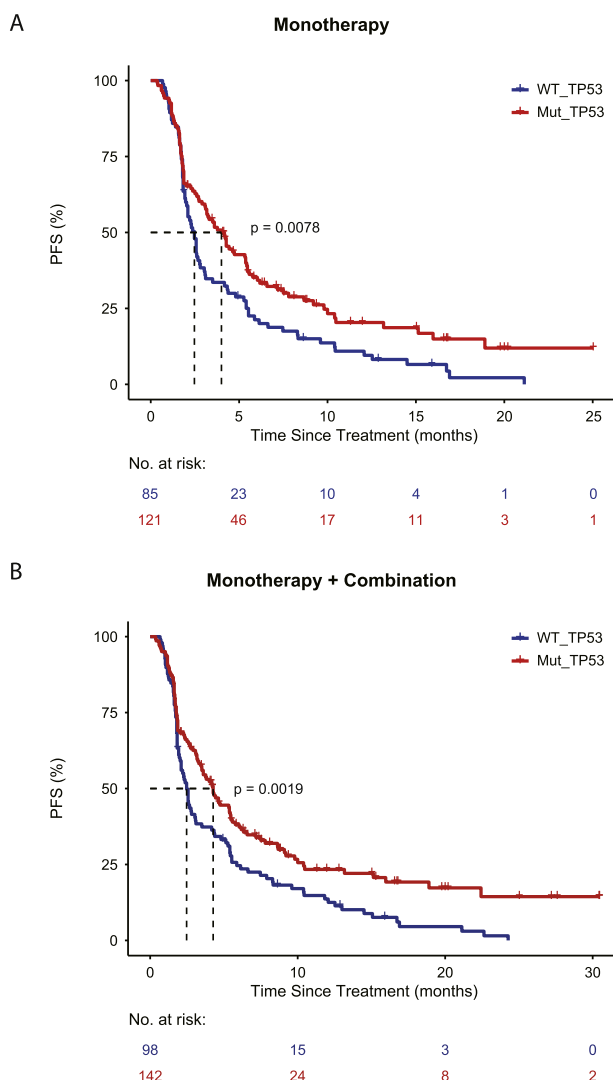
Figure 1   (A) Patients treated with monotherapy were stratified with *TP53* mutation status. The survival curve was plotted with PFS for the distinct group. Wild type *TP53* is shown with blue and mutated *TP53* is displayed with red. (B) Patients treated either with monotherapy or combination therapy were stratified with *TP53* mutation status. The survival curve was plotted with PFS for the distinct group. Wild type *TP53* is shown with blue and mutated *TP53* is displayed with red.

Results

In total, there were 206 patients who received mono-therapy with PD-1/PD-L1 inhibitor and 34 patients who received combination therapy with PD-1/PD-L1 inhibitor and anti-CTLA-4 therapy. First, we evaluated the effect of all the patients' characteristics on PFS (Table 1), and results showed that smoking status (4.0 *vs.* 2.7 months, $P = 0.031$), lines of treatment (7.5 *vs.* 2.7 months, $P = 0.00046$), *TP53* mutation (4.3 *vs.* 2.5 months, $P = 0.00019$), *EGFR* mutation (3.1 *vs.* 3.3 months, $P = 0.0038$) and *KMT2C* mutation (7.3 *vs.* 3.2 months, $P = 0.049$) were significantly correlated with PFS in the entire cohort of patients. For monotherapy of PD-1 and PD-

L1 inhibitors, only smoking status (3.3 *vs.* 2.1 months, $P = 0.0025$) and *TP53* mutation (4.0 *vs.* 2.5 months, $P = 0.0078$) had significant effect on PFS.

Then we plotted the survival curve for both the mono-therapy cohort and the combination cohort (Fig. 1). Patients were stratified based on the status of *TP53* mutation. In 240 NSCLC patients, *TP53* mutation rate was 59.2%. Consistently, it was found that NSCLC patients with *TP53* mutations had significantly longer PFS either using the monotherapy cohort or the entire cohort. For the mono-therapy (PD-1, PD-L1) cohort, *TP53* mutated NSCLC patients have a significantly longer PFS (4.3 months *vs.* 2.5 months, $P = 0.0019$) compared with *TP53* wild type NSCLC patients. The same tendency was also observed in the combination therapy cohort, but the difference in PFS (6.3 months *vs.* 5.4 months, $P = 0.12$) was not significant due to limited number of patients. We focused on three recurrently mutated tumor suppressor genes: *KMT2C*, *STK11* and *KEAP1* (Table 2). Patients with co-mutation in *TP53* and *KMT2C* have longer PFS (9.2 months *vs.* 2.5 months, $P = 0.005$) compared with patients without *TP53* and *KMT2C* mutations (Fig. 2). Co-mutations seemed to confer a favorable survival compared with the patients with only mutation in one gene. For co-mutation analysis, patients with *TP53* and *STK11* co-mutations have better PFS (3.3 months *vs.* 2.6 months), but this was not statistically significant. Patients with mutant *TP53* and wild type *STK11* had significantly longer PFS (4.3 months *vs.* 2.6 months) as compared with patients with wild type *TP53* and *STK11*. Similarly, in the case of *TP53* and *KEAP1* co-mutation, it seemed that *TP53* mutation was dominating the outcome of checkpoint inhibition. *KEAP1* mutation diminished the effect of *TP53* mutation.

We next checked the effect of *KRAS* co-mutation on the outcome of patients in response to checkpoint inhibitors (Fig. 3). We found that patients with co-mutation of *TP53* and *KRAS* had significantly longer PFS (5.8 months *vs.* 2.6 months, $P = 0.005$), as compared to patients harboring wild type *TP53* and *KRAS*. Patient with *TP53* mutation and wild type *KRAS* had a median PFS of 3.6 months. When smoking factor is included in multivariate analysis, only the *TP53*/*KRAS* co-mutation stood out as a significant factor ($P = 0.024$). Finally, we evaluated the effect of co-occurring *TP53* and *EGFR* mutations. Patients with mutated *TP53* and wild type *EGFR* had significantly longer PFS (4.3 months *vs.* 2.5 months, $P = 0.001$) as compared with patients with wild type *TP53* and *EGFR*, while patients with co-occurring *TP53* and *EGFR* mutations exerted no significant improvement of PFS (3.4 months *vs.* 2.5 months, $P = 0.707$).

To sum up, we proposed a model to explain the effect of mutations in key driver genes on the sensitivity of ICI treatment (Fig. 4). The effect of individual gene could be additive or subtractive to *TP53* mutations.

Discussion

Our study approached the problem of patient stratification in immune checkpoint inhibition by extensive data mining and re-analysis of a publicly available dataset, uncovering a complex interplay between recurrently occurring

Table 2 Co-mutation status with PFS of PD-1/PDL1 treatment.

		Number of case	mPFS (all)	log-rank_P	COX P	rmsmoker_cox_P	mPFS (mono)	log-rank_P	COX P	rmsmoker_cox_P	mPFS (combine)	log-rank_P	COX P
TP53 mut	KEAP1 mut	34	3.38	0.1348	0.037 (0.62, 0.39–0.97)	0.093	3.17	0.3252	0.109 (0.68, 0.42–1.09)	0.314	NA	0.3489	0.083 (0.16, 0.02–1.3)
TP53 mut	KEAP1 wild	108	4.27	0.0086	0.002 (0.61, 0.44–0.84)	0.009	4.27	0.0231	0.005 (0.61, 0.43–0.86)	0.032	6.33	0.5283	0.191 (0.59, 0.26–1.3)
TP53 wild	KEAP1 mut	19	2.27	0.4491	0.363 (0.78, 0.46–1.33)	0.566	2.18	0.4712	0.351 (0.76, 0.43–1.34)	0.684	22.63	0.5283	0.379 (0.4, 0.05–3.1)
TP53 wild	KEAP1 wild	79	2.57				2.57				5.28		
TP53 mut	KMT2C mut	19	9.2	0.0204	0.005 (0.44, 0.24–0.78)	0.012	5.47	0.061	0.02 (0.48, 0.26–0.89)	0.056	22.43	0.3828	0.141 (0.21, 0.028–1.7)
TP53 mut	KMT2C wild	123	4.2	0.0204	0.007 (0.67, 0.5–0.9)	0.019	3.6	0.061	0.021 (0.69, 0.5–0.95)	0.071	4.63	0.3828	0.205 (0.59, 0.266–1.3)
TP53 wild	KMT2C mut	7	2.57	0.5119	0.42 (0.71, 0.31–1.63)	0.523	2.47	0.671	0.571 (0.77, 0.31–1.91)	0.769	13	0.8451	0.64 (0.61, 0.079–4.8)
TP53 wild	KMT2C wild	91	2.47				2.47				5.28		
TP53 mut	STK11 mut	21	3.3	0.5177	0.285 (0.75, 0.44–1.28)	0.58	2.83	0.7648	0.527 (0.83, 0.47–1.48)	0.932	9.1	0.6947	0.267 (0.42, 0.094–1.9)
TP53 mut	STK11 wild	121	4.27	0.0349	0.007 (0.64, 0.46–0.88)	0.043	4.2	0.0732	0.013 (0.64, 0.45–0.91)	0.119	6.33	0.6947	0.165 (0.55, 0.237–1.3)
TP53 wild	STK11 mut	35	2.27	0.9195	0.884 (1.03, 0.67–1.59)	0.507	2.27	0.9021	0.941 (0.98, 0.62–1.55)	0.481	12.13	0.6947	0.77 (0.8, 0.173–3.7)
TP53 wild	STK11 wild	63	2.6				2.47				5.43		
TP53 mut	EGFR mut	20	3.4	0.633	0.707 (1.1, 0.66–1.84)	0.747	3.3	0.7633	0.782 (1.08, 0.61–1.92)	0.902	3.5	0.3627	0.449 (1.58, 0.48–5.2)
TP53 mut	EGFR wild	122	4.33	0.0069	0.001 (0.61, 0.45–0.82)	0.003	4.2	0.0315	0.005 (0.63, 0.46–0.87)	0.018	9.1	0.0963	0.082 (0.46, 0.19–1.1)
TP53 wild	EGFR mut	8	2.47	0.4153	0.336 (1.46, 0.67–3.18)	0.428	3.07	0.7633	0.662 (1.2, 0.52–2.78)	0.974	1.27	0.0574	0.022 (26.78, 1.61–446.3)
TP53 wild	EGFR wild	90	2.52				2.33				6.66		
TP53 mut	KRAS mut	32	5.77	0.0113	0.005 (0.47, 0.27–0.8)	0.024	5.47	0.0142	0.005 (0.42, 0.23–0.77)	0.042	6.33	0.5253	0.354 (0.57, 0.17–1.9)
TP53 mut	KRAS wild	110	3.6	0.1495	0.122 (0.75, 0.52–1.1)	0.282	3.3	0.1031	0.048 (0.66, 0.44–1)	0.247	8.63	0.6536	0.58 (0.76, 0.29–2)
TP53 wild	KRAS mut	54	2.33	0.5746	0.718 (1.08, 0.72–1.6)	0.478	2.37	0.4977	0.468 (0.85, 0.54–1.32)	0.988	1.63	0.3114	0.179 (2.18, 0.7–6.8)
TP53 wild	KRAS wild	44	2.57				2.47				9.87		

mutations. We confirmed the beneficial effect of *TP53* mutations in immune checkpoint inhibition treated patients. This is in agreement with previous reports.[6,9,14,16] However, the outcome of immune checkpoint inhibition depends on multiple factors, rendering a multivariate analysis necessary. Here we comprehensively surveyed the effect of *TP53* with co-occurring mutations in common oncogenes and other tumor suppressor genes on the response to immune checkpoint inhibition.

KMT2C is a gene frequently mutated in non-small cell lung cancer. Our analysis suggested that co-mutation of *TP53* with *KMT2C* seemed to confer a favorable response of NSCLC patients to immune checkpoint inhibition. Co-occurring *KMT2C* mutations significantly enhanced the response of NSCLC patients to ICIs, serving as proof of principle that finer patient stratification is more informative to guide clinical decision. The other two tumor suppressor genes *STK11* and *KEAP1* analyzed in this study did not significantly alter the response profile of NSCLC patients to immune checkpoint inhibitors. There is still limited evidence to completely rule out roles played by those tumor suppressor genes, as functionality is always context dependent.

Recently, there was a case report documenting a durable response to combination therapy with PD-1 antibody and chemotherapy in a NSCLC patient with co-occurring *TP53* and *KRAS* mutations.[17] One potential explanation for this is that *TP53* and *KRAS* double mutated patients had significantly higher expression of PD-L1 in their cancer samples.[7] PD-L1 is a well-accepted biomarker to predict the sensitivity to immune checkpoint inhibition.[18]

EGFR mutations were shown to correlate with a worse response of patients to immune checkpoint inhibition.[19,20]
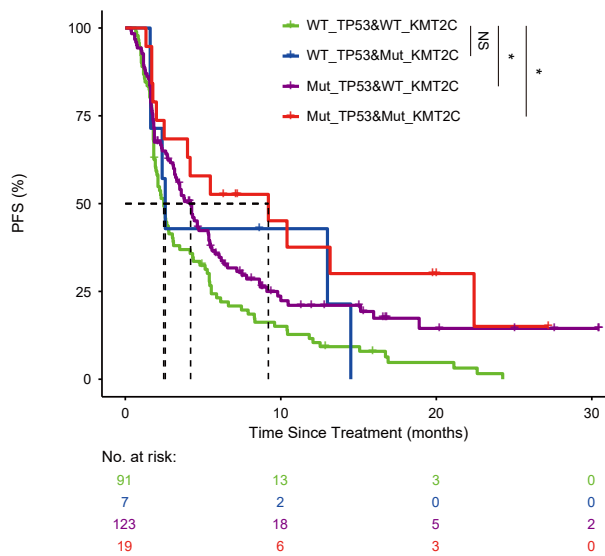

Figure 2 Patients treated either with monotherapy or combination therapy were stratified with *TP53* mutation status and *KMT2C* mutation status. The survival curves were plotted for four distinct groups, including wild type *TP53* and wild type *KMT2C* (green), wild type *TP53* and mutated *KMT2C* (blue), mutated *TP53* and wild type *KMT2C* (purple), mutated *TP53* and mutated *KMT2C* (red).
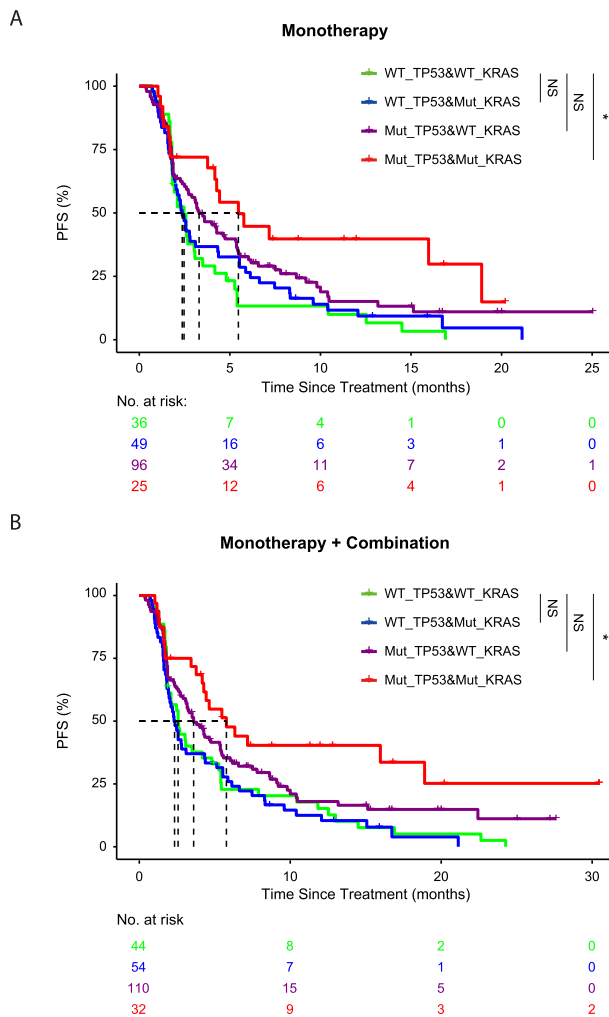
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Figure 3 (A) Patients treated with monotherapy were stratified with *TP53* mutation status and *KRAS* mutation status. The survival curves were plotted for four distinct groups, including wild type *TP53* and wild type *KRAS* (green), wild type *TP53* and mutated *KRAS* (blue), mutated *TP53* and wild type *KRAS* (purple), mutated *TP53* and mutated *KRAS* (red). (B) Patients treated either with monotherapy or combination therapy were stratified with *TP53* mutation status and *KRAS* mutation status. The survival curves were plotted for four distinct groups, including wild type *TP53* and wild type *KRAS* (green), wild type *TP53* and mutated *KRAS* (blue), mutated *TP53* and wild type *KRAS* (purple), mutated *TP53* and mutated *KRAS* (red).
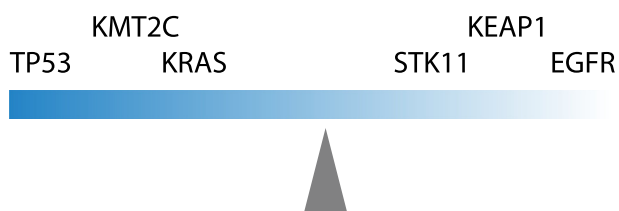

Figure 4 Proposed model for the influence of distinct *TP53* co-mutation patterns on the outcome of checkpoint inhibition.

Despite this, the effect of *EGFR* mutations might be context dependent.[21] The negative effect of *EGFR* mutations and the positive effect of *TP53* mutations seemed to neutralize each other, as double mutants were similar to double wild type. This suggested that the first line therapy for *TP53*/ *EGFR* double mutated NSCLC patients should be TKIs. To sum up previously discussed points, it is clear that the mechanisms for a cancer gene mutation to alter the ICIs response are decoupled from its roles played in tumorigenesis as an oncogene or tumor suppressor gene.

Conclusions

Immune checkpoint inhibition has emerged as a promising cancer therapeutic that can induce durable clinical benefit in a subset of patients. However, many patients are insensitive to checkpoint inhibitors, while the mechanistic insights remain lacking. It's urgent to develop a finer patient stratification method to guide clinical decision. As next generation sequencing had become routine in clinic to inform clinical decision regarding the use of targeted drugs,[22] the mutation status of recurrently mutated genes analyzed in this study is generally available for cancer patients. Thus, future studies using a larger population of patients are merited to further confirm the effect of distinct co-mutation patterns on the response of NSCLC patients to immune checkpoint inhibition.

Author contributions

Ning Li and Xiaoyun Huang contributed to concept and design. Shuhang Wang and Miaomiao Jiang contributed to the literature search, data acquisition, data analysis, statistical analysis, manuscript preparation. Zuozhen Yang contributed to manuscript editing and manuscript review.

Conflict of interests

All authors declare no conflict of interest.

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