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Prognostic Effects Of Different Tp53 Mutations In Lung And Pancreatic Adenocarcinomas

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1. **Title page**

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2. Abstract

Cancer patients with similar clinical and pathologic characteristics may have different response to treatments and survival, which may be attributable to distinct molecular properties of their tumor. The most common forms of lung and pancreatic tumors, the adenocarcinomas, often have mutations in the *TP53* gene. It is not clear, however, how mutations in this gene may provide prognostic information and/or inform the clinical management of patients with lung adenocarcinoma (LUAD) and pancreatic ductal adenocarcinoma (PDAC). Despite previous studies suggesting that *all* mutations in *TP53* are associated with worse prognosis, it is now known that not all *TP53* mutations lead to the same biological function of the protein. This study assessed the potential effect of wild-type, missense and truncating *TP53* mutations on the survival of patients with LUAD and PDAC, in two independent prospective clinical cohorts (n = 4,751 LUAD, n = 517 PDAC), using multivariable Cox proportional hazards regression models that adjusted for patient demographic (age at diagnosis, sex, race /ethnicity) and tumor characteristics (presence of distant metastasis at diagnosis). Additionally, we conducted stratified analyses by the status of other mutations in driver oncogenes (e.g., *KRAS, EGFR*). We found that among LUAD patients, both missense (HR = 1.12, 95% CI: 1.00-1.24; p=0.044) and truncating mutations (hazard ratio [HR] = 1.33, 95% confidence interval [CI]: 1.17-1.51; p<0.001) in *TP53* were associated with worse overall survival when compared to wild-type *TP53*, while the magnitude of association appeared somewhat different. Contrarily, in patients with PDAC, only missense *TP53* mutations were associated with a worse survival compared to wild-type *TP53* (HR = 1.47, 95% CI: 1.00-2.17; p=0.051). Furthermore, our results indicated that in patients with LUAD, *TP53* was associated with a worse survival in the subgroup of patients with co-occurring *EGFR* mutations, but not among those with co-existing *KRAS* mutations. These findings suggest that molecular characterization of otherwise indistinguishable tumors may help to break down cancers into distinct entities,

each of which ought to be recognized as having separate behaviors and different molecular fingerprints. More importantly, our results indicate that there are innate biological differences among tumors that can impact patients' survival, and that can be exploited to identify molecular abnormalities for potential targeted therapy.

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5.1. Introduction

In the United States (US), heart diseases and malignant neoplasms remained the top 2 leading causes of death[1]. According to the Centers for Disease Control and Prevention, in 2020, lung cancer accounted for the majority of cancer deaths in this country (23%), followed by colorectal (9%), pancreas (8%), female breast (7%), prostate (5%), and liver and intrahepatic bile duct (5%) cancer[2]. Due to the demographic shifts in the US population, and the projected rise in the prevalence of many risk factors (including tobacco smoking, alcohol consumption, high body mass index, and diabetes), pancreatic cancer will surpass colorectal cancer to become the second leading cause of cancer-related deaths in the US[3]. Additionally, lung cancer is projected to remain the leading cause of cancer deaths during the next 20 years[3].

In the last decades, an important body of evidence has shown that cancer patients with similar clinical and pathologic characteristics may have different response to available treatments and survival, which may be attributable to distinct molecular properties of their tumors[4–6]. Molecular testing is now part of the diagnostic workup for lung and pancreatic cancers. The most common forms of lung (50%)[7,8] and pancreatic tumors(~90%)[9], the adenocarcinomas, often have mutations in the *TP53* gene (46% of lung adenocarcinomas, LUADs, and 70-75% of pancreatic ductal adenocarcinoma, PDACs)[10–14]. Additionally, approximately one-third of LUADs have co-occurring oncogenic gain-of-function (GOF) mutations involving components of growth factor receptor signaling pathways, including mutations in *EGFR* (10-15%) and mutations in *KRAS* (~30%)[13]. Similarly, around 70% of PDAC patients have co-occurring mutations in *KRAS* and *TP53*[15]. Despite this, it is not clear how mutations may provide prognostic information and/or inform patient

management. In LUAD, only *EGFR* mutation status guides therapy[16]. In PDAC, only DNA repair genes guide treatment in <10% of cases[17]. Therefore, there is an urgent need to further understand mutational profiling to inform clinical treatment and improve the survival of patients with these malignancies.

Studies assessing the prognostic effects of *TP53* mutations in these malignancies have suggested that *all* mutations in this gene are associated with worse prognosis[18–24], however, it is now well known that not all *TP53* mutations lead to the same biological function of the protein[25]. The majority of *TP53* mutations are caused by single nucleotide changes, resulting in mutant proteins that differ in only one amino acid from the wildtype molecule[26]. These mutations either result in: 1) the loss of p53 protein expression and therefore a loss in the tumor-suppressive function (loss of function [LOF] p53), as seen with truncating mutations, or 2) a neomorphic p53 protein, stably expressed and accumulated in tumoral cells with oncogenic properties that contrast the effects of wild-type p53 (gain of function [GOF] p53), as seen with missense mutations[27,28]. Over the last 25 years, experimental data on the functional impact of different amino-acid substitutions in p53 suggest that LOF and GOF mutants exhibit very distinct phenotypes, translating into different tumoral effects[25,26]. For example, GOF p53 proteins acquire novel oncogenic functions capable of regulating oncogenic signaling that supports sustained cell growth, migration, invasion, genomic instability and resistance to treatment[29–31]. The importance of functional differences in p53 proteins is perhaps better supported by the finding that patients with Li-Fraumeni Syndrome harboring missense mutations in the germline (leading to expression of a mutant p53 protein with GOF phenotype) have a significantly earlier cancer onset compared to patients with truncating mutations in *TP53*, in which tumors tend to develop later in life[32,33].

Given these genetic and functional differences in *TP53* mutations and to address the knowledge gap in the prognostic value of different types of mutations, this study assesses the prognostic effect of wild-type, missense and truncating *TP53* mutations in the survival of patients with LUAD and PDAC, in two independent prospective clinical cohorts (n= 4,751 LUAD, n=517 PDAC). Additionally, we will assess if the effect of *TP53* mutations is modified by the co-occurrence of mutations in driver oncogenes (e.g., *KRAS, EGFR*) in patients with LUAD. We hypothesized that different mutations in *TP53* might be associated with varied prognostic effects compared to the wild-type *TP53* in patients with LUAD and PDAC. Moreover, we hypothesized that co-occurrence of mutations in *TP53* and mutations in driver oncogenes are associated with a worse survival in patients with LUAD.

5.2. Review of Studies Relevant to the Problem

In recent years, advances in next-generation sequencing technologies have allowed researchers to understand better the unique features of each subtype of cancer. Approximately 50% of spontaneous cancers in humans have mutations in *TP53* and the majority, if not all, cancer cells with wild-type (WT) *TP53* gene have compromised p53-related activities[14]. Previous studies have demonstrated that in patients with LUAD, mutations in *TP53* are associated with shorter survival than wild-type *TP53*[18–22]. Increases in the risk of death in some of these studies range from 40% (hazard ratio (HR) = 1.40, 95% confidence interval (CI): 1.10–1.78; p=0.006)[18] to 91% (HR=1.91; 95% CI: 1.01–3.60, p =0.040)[20]. In a cohort of 6,297 patients with non–small cell lung cancer (NSCLC), followed for more than two decades, Saleh *et al.* found that in patients with localized tumors (Union for International Cancer Control classification: stages I, II, IIIA), those harboring *TP53* mutations have significantly shorter overall survival (OS) (HR=1.61, 95% CI: 1.22–2.12; p=0.001) compared with those with wild-type *TP53*. In the same study, the presence of *TP53* mutations was a less strong prognostic biomarker among NSCLC patients with more advanced disease (stages IIIB–IV)[34]. In a separate study carried out by Zhao and colleagues that assessed the association between *TP53* somatic mutations and immunotherapeutic outcomes in patients with NSCLC, the presence of *TP53* mutations was an independent predictor of both immunotherapeutic outcomes (HR=1.40, 95% CI: 1.13-1.73; p = 0.002) and OS (HR=1.36, 95% CI: 1.05-1.76; p = 0.019)[35]. The association between *TP53* mutations and immunotherapy outcomes was more profound in patients with lower tumor mutation burden, and*TP53* mutation status improved the prognostic prediction in NSCLC patients who underwent immunotherapy[35].

In addition, recent literature has suggested that the prognostic effect of *TP53* mutations in patients with lung cancer remains after adjusting for the effect of other driver mutations. When LUAD patients are stratified to study the effect of co-occurrence of mutations in driver oncogenes (e.g., *EGFR*) and mutations in *TP53,* those who had both *EGFR* and *TP53* mutations had a worse prognosis[19,20,36]. In a study that included 136 patients with NSCLC harboring mutations in *EGFR,* compared to patients with wild-type *TP53,* patients with *TP53* mutations had a significantly shorter median OS (16.2 vs. 32.3 months; p= 0.114) and an increased risk of death (HR=4.75, 95% CI: 1.38–16.29; p=0.013). Additionally, in a cohort of 569 patients with LUAD from The Cancer Genome Atlas project, Zheng *et al.* found that the co-occurrence of $EGFR^{L858R}$ and *TP53* mutations was a significant predictor of survival (HR=2.77, 95% CI: 1.26–6.10; p= 0.012) when compared to $EGFR^{WT}/TP53^{WT}$ tumors[19]. This study suggested that patients harboring $EGFR^{L858R}$/*TP53* co-mutations had increased expression of cartilage oligomeric matrix protein and integrin subunit beta 8, involving pathways related to extracellular matrix organization and cell surface receptor signaling and therefore, contributing to poor prognosis in LUAD[19].

As for PDAC, patients harboring mutations in *TP53* had a worse clinical prognosis than those with wild-type *TP53*[24]. In a study by Quian *et al.*, PDAC patients with *TP53* mutant tumors had worse disease-free survival (DFS) (median [interquartile range]: 10.8 [6.2-24.5]) months) and OS (20.3 [11.1-37.8] months) compared with patients with wild-type *TP53* tumors (DFS: 14.8 [8.1-30.5] months; OS: 24.6 [13.5-44.6] months). In another study carried out by McIntyre *et al.,* the median OS for patients with *TP53* alterations was almost half the time (37.4 months, 95% CI: 32.1-42.8 months) of those with wild-type TP53 (65.0 months, 95% CI: 33.0 months to not reached) (p=0.035)[23].

While multiple studies have examined the prognostic effect of *TP53* mutations in LUAD and PDAC, they all considered *TP53* mutations a single entity (Table 1). To the best of our

knowledge, this study is the first to distinguish the specific types of *TP53* mutations (truncating versus missense mutations) and assess the prognostic effect of different types of *TP53* mutations.

5.3. Research Design

5.3.1. Patient Cohorts

This study is a secondary analysis of data from two large-scale, prospective clinical-genomic profiling initiatives: The Cancer Genome Atlas (TGCA) cohort[12] and the Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) cohort[23]. Demographic, clinical, pathologic, mutation profiles, and time-to-event data can be accessed from a public data repository known as cBioPortal (cBio Cancer Genomics Portal)[37], a web-based resource for researchers to explore and share cancer genomics datasets from thousands of patients worldwide[38]. We selected these two cohorts because they are comparable in terms of the included population, patients´ tumor characteristics, and the types of treatments that patients received.

A total of 11,315 and 25,775 patients were initially identified from the TCGA[39] and MSK-IMPACT[40] cohorts, respectively. Those who fulfilled the following eligibility criteria were included in our study: 1) diagnosis of LUAD or PDAC based on the cancer type variable available in cBioPortal; 2) have information available on *TP53* mutation status. Our final study population consists of 4,751 patients with LUAD and 517 patients with PDAC.

5.3.2. Exposure assessment and outcome ascertainment

For all patients included in this study, mutations in the *TP53* gene were classified into three distinct categories: missense, truncating, or wild-type (reference), according to genomic profiling data available in cBioPortal. The study outcome, OS, was defined in the original cohort as the time from surgery to the date of death or last follow-up, whichever

was earlier. If death had not occurred, patients were censored at the date of the last follow-up. Additionally, we limited our analysis to those patients whose survival time was shorter than 60 months (5-years), based on the fact that the overall five-year survival for LUAD and PDAC were 22% and 11%, respectively[41].

5.3.3. Statistical Analysis

Continuous data were reported as medians and interquartile ranges (e.g., age of diagnosis), and categorical variables (e.g., sex, race /ethnicity) were reported as frequencies and percentages. Bivariate associations between covariates and the exposure of interest (*TP53* mutation status) were assessed using Fisher's exact test and Wilcoxon rank-sum test.

For patients with different types of *TP53* mutations, Kaplan-Meier curves were generated, from which median survival was calculated. Additionally, Kaplan-Meier curves of patients with different types of *TP53* mutations were compared with the log-rank test.

Furthermore, a multivariable Cox proportional hazards regression model was used to evaluate the impact of *TP53* mutations on OS, with an estimation of HRs and 95% CIs. To build this model, we first defined baseline covariates to be included due to their *a priori* potential for confounding. To identify additional potential confounders, we selected covariates associated with: (1) the exposure of interest using Fisher's exact test with a threshold of $P \leq 0.05$, and (2) the outcome (OS) using stepwise forward selection Cox proportional hazards regression with a threshold of $P \leq 0.1$. The proportionality of hazards assumption was verified by evaluating time-dependent variables of the cross-product of each *TP53* mutation status and time. We tested heterogeneity across cohorts using the Q statistic (P heterogeneity <0.05).

Preplanned subgroup analyses were performed that assessed the prognostic value of different types of *TP53* mutations while accounting for the presence of other driver mutations in patients with LUAD*,* including mutations in *EGFR* and *KRAS.*

All hypothesis tests were 2-sided, and a 2-sided $P < 0.05$ indicated statistical significance. SAS (Version 9.4; SAS Institute, Inc, Cary, North Carolina) and RStudio (version 3.5.1; R Foundation for Statistical Computing, Vienna, Austria) were used to analyze the data, and Prism 9 was utilized to plot the Kaplan-Meier curves.

5.4. Presentation of Findings

5.4.1. LUAD harboring truncating mutations in *TP53* have a worse overall survival compared with patients with wild-type *TP53* and missense mutations in *TP53*.

The demographic and tumor characteristics for LUAD patients from the TCGA (n=575), MSK-IMPACT (n=4,176), and the combined cohorts are presented in Table 2. Briefly, of the 4,755 patients included in the combined cohort, the majority of them were women (60.2%), White/Caucasians (77.4%), with a median (interquartile range, IQR) age of 66.3 (58.6-73.0) years. In addition, most patients had distant metastasis at the time of diagnosis (73.0%). Regarding the *TP53* status, 51.0% of patients were wild-type, 32.6% harbored missense mutations, and 16.4% harbored truncating mutations (Table 2, Fig. 1). Overall, patients harboring truncating *TP53* mutations, missense *TP53* mutations, and wild-type *TP53* had significantly different patterns of survival ($p<0.0001$) (Fig. 2A). Corresponding median survivals for patients with truncating and missense mutations, as well as wild-type *TP53* were 28.10 (IQR: 24.22-33.84) months, 36.65 (IQR: 32.66-39.35) months and 50.10 (IQR: 47.41,54.37) months, respectively (Fig. 2).

Based on the multivariable Cox proportional hazards regression analysis, compared to LUAD patients with wild-type *TP53*, patients with truncating mutations had a worse OS (HR=1.33, 95% CI: 1.17-1.51; $p<0.001$), so did patients with missense mutations (HR=1.12, 95% CI: 1.00-1.24; $p=0.044$) (Table 3, Fig. 3A). Of note, males had a significantly shorter OS compared to women (HR, 1.34, 95% CI: 1.22-1.47; $p<0.001$) (Table 3).

5.4.2. *TP53* mutations are associated with worse survival among LUADs harboring co-occurring *EGFR* mutations

To determine if the co-occurrence of oncogenic driver mutations in *EGFR or KRAS* and *TP53* would impact the OS of LUAD patients, we stratified patients in three different categories as follows: wild-type *EGFR/KRAS,* mutant *EGFR*, *and* mutant *KRAS*. Among patients with wild-type *EGFR* and *KRAS,* both truncating (HR=1.41, 95% CI: 1.16-2.72; p<0.001) and missense *TP53* mutations (HR=1.24, 95% CI: 1.05-1.46; p=0.013) were associated with a significant worse survival compared to wild-type *TP53* (Table 3, Fig. 2B). Similarly, we found that among patients with *EGFR* mutations, those who had truncating (HR=1.86, 95% CI: 1.43-2.42; p<0.001) or missense (HR=1.56, 95% CI: 1.24-1.96; p<0.001) *TP53* mutations experienced a significant worse survival than patients with wild-type *TP53* (Table 3, Fig. 2C). Contrarily, we found that in LUAD patients with *KRAS* mutations*,* neither truncating nor missense commutations in *TP53* were prognostic predictors of survival (Table 3, Fig. 2D).

5.4.3. PDAC harboring missense mutations in *TP53* have a worse overall survival compared with patients with wild-type *TP53*

The demographic and tumor characteristics for PDAC patients from the TCGA (n=141), MSK-IMPACT (n=376), and combined cohorts are presented in Table 4. Briefly, of the 517 patients included in the combined cohort, the majority of them were men (53.2%), White/Caucasians (81.0%), with a median age of 65.0 (IQR: 56.0-72.1) years. In addition, half of patients had distant metastasis at the time of diagnosis (50.0%). Regarding the *TP53* status, 30.6% of patients were wild-type, 46.4% harbored missense mutations, and 23.0% harbored truncating mutations, respectively (Table 4, Fig. 1). Overall, patients harboring missense *TP53* mutations, truncating *TP53* mutations, and

wild-type *TP53* had significantly different patterns of survival (p<0.031) (Fig. 3). Corresponding median survivals for patients with missense and truncating mutations, as well as wild-type *TP53* were 16.17 (IQR: 13.84-20.84) months, 19.96 (IQR: 19.07-71.74) months and 20.19 (IQR: 15.81-24.26) months, respectively (Fig. 3).

With the multivariable Cox proportional hazards regression analysis, patients with missense mutations in *TP53* appeared to have a worse OS than patients with wild-type *TP53* (HR=1.47; 95% IC, 1.00-2.17; p=0.051), while patients with truncating mutations in *TP53* had a similar OS to those with wild-type *TP53* (HR=1.11; 95% CI, 0.70-1.76; p=0.654) compared to patients with wild-type *TP5*3, however, these associations were not significant (Table 5, Fig. 3). Additionally, we found that patients with distant metastasis at the time of diagnosis had almost 2.60 times the hazard of dying compared to those without metastatic disease (HR=2.58, 95% CI=1.74-3.81; p<0.001) (Table 5).

5.5. Discussion of findings

This analysis of cancer genomics data from two large-scale, multi-institutional clinical cohorts is the first to examine the prognostic effect of specific types of TP53 mutations (missense versus truncating) in LUAD and PDAC. Among LUAD patients, both missense and truncating mutations in *TP53* were associated with worse overall survival when compared to wild-type *TP53*, while the magnitude of association appeared somewhat different. Additionally, we found that in patients with LUAD, *TP53* was associated with a worse survival in the subgroup of patients with co-occurring *EGFR* mutations, but not among those with co-existing *KRAS* mutations. In patients with PDAC, only missense *TP53* mutations were associated with a worse clinical prognosis compared to wild-type *TP53*. Collectively, these findings suggested that molecular characterization of otherwise indistinguishable tumors can help to break down cancers into distinct entities, each of which ought to be recognized as having separate behaviors and different molecular fingerprints. More importantly, our results indicated that there are innate biological differences among tumors that can impact patients' survival, and that man be exploited to identify molecular abnormalities for potential targeted therapy.

Our results are novel in that for the first time we evaluated the two different types of *TP53* mutations separately. Previous studies have consistently shown that in patients with LUAD and PDAC, mutations in *TP53* are associated with shorter survival than wild-type *TP53*[18–22], however, these studies are shortsighted in that they considered all mutations in *TP53* as part of the same entity[18,24,42,43], without recognizing the important role of new oncogenic properties conferred by missense and truncating mutations, which are independent and different from the wild-type p53[44,45]. Extensive epidemiological studies involving many patients and experimental data have suggested that different types of

mutations in *TP53* are in fact distinct[25–27], which may have different manifestation in the prognosis of patients with LUAD and PDAC.

Interestingly, our study found that unlike co-occurring mutations in *KRAS* and *TP53*, which have not been shown to have consistent prognostic effects across studies[18,42,46], co-occurring mutations in *EGFR* and *TP53* are associated with a worse overall survival. Specifically, we uncovered a rather high HR for LUAD patients with co-occurring truncating mutations in *TP53* and *EGFR*, representing a refinement in the molecular categorization of LUAD that could be potentially explored in further studies to predict the survival of this subset of patients. Current guidelines for treatment of LUADs harboring mutant *EGFR* includes the use of EGFR tyrosine kinase inhibitors (TKIs)[47]. However, our findings indicated that even in patients that are candidates to receive immunotherapy with TKIs, identifying patients with co-occurring truncating *TP53* mutations and *EGFR* mutations early in the disease processes can be pivotal, as they might benefit from additional treatments or adjustments to the current standard of care.

As for patients with PDAC, our study is the first one to decipher the different effects of missense versus truncating *TP53* mutations. Compared to patients with wild-type *TP53*, those with truncating mutations had a similar OS, while patients with missense mutations had a worse OS. We were able to identify such a contrast because our study included the largest number of PDAC patients to date. Unlike other tumor suppressor genes, majority of mutations in *TP53* in patients with PDAC are missense mutations. Numerous studies have revealed that in addition to abrogate the tumor-suppressive role of p53, missense mutations also confer upon the mutant proteins new oncogenic GOF activities. It is believed that such GOF of activities of the mutant is due to its ability to interact and partner with a variety of proteins and thereby modulate the transcriptional landscape of the cell

and impact directly/indirectly a variety of cellular processes[27,30,31,45]. A recent translational study found that in the context of GOF mutant p53, tumoral cells increase the expression of the splicing regulator hnRNPK to promote the inclusion of cytosine-rich exons within GTPase-activating proteins, negative regulators of RAS family members. Mutant p53-enforced GTPase-activating protein isoforms lose cell membrane association, leading to heightened *KRAS* activity[48]. Furthermore, they found that preventing cytosine-rich exon inclusion in mutant *KRAS/p53* PDACs decreases tumor growth, indicating a putative therapeutic strategy for this hard-to-treat cancer. Our results are encouraging as they indicated potential therapeutic drivers that can be potentially targeted to treat this lethal malignancy. Further studies are needed to better define these genomic alterations as biomarkers in patients with PDAC.

5.6. Conclusions

In summary, our study provides the first comprehensive mutational catalogue of *TP53* mutations in LUAD and PDAC, quantifying the relative effect on overall survival of different types of TP53 mutations. Our findings support the importance of refining the molecular categorization of LUAD and PDAC, with the goal of identifying markers that can potentially be used to predict patient's prognosis or response to specific treatments. Such knowledge is paramount to personalize treatment of cancer.

This study has multiple strengths, including its large sample size, which improved our statistical power. In addition, by including different validated clinical-genomic profiling datasets that enrolled patients treated in both private and public settings, we have addressed a major limitation of previous studies and increased the representativeness of our sample as well as the generalizability of our findings. On the other hand, there are some limitations due to the nature of the data source. First, because of limited clinical information (e.g., clinical stage, systemic treatment protocols, clinical responses), especially for the MSK-IMPACT cohort, it is possible that some potential confounders were not included in the multivariable analysis, which could have biased the estimates presented in this study. Nevertheless, we included the majority of reported prognostic factors in the literature (e.g., age, sex, race/ethnicity, driver mutations, metastasis at time of diagnosis), and our results for TP53 mutations overall (i.e., without the distinction of missense versus truncating mutations) are consistent with those from previous studies. Future studies would benefit from incorporating more clinical data. Second, recent studies have revealed that in addition to driver mutations, there are likely other mechanisms through which tumors are initiated, maintained, and able to evade therapy such as epigenetic changes, or alternative RNA splicing [49]. Future studies could include p53

isoforms and further delineate prognostic subgroups in patients with wild-type *TP53*. Third, most patients included in this study identified themselves as White/Caucasians, so racial/ethnic minorities are underrepresented in our analysis. This limits the generatability of our findings and highlights the importance of increasing diversity in genomic studies and oncology trials [50].

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6. Appendix or Appendices

Table 1. Review of Studies Relevant to the Problem Regarding the Prognostic Value of *TP53* Mutations in Patients with Lung Adenocarcinoma and Pancreatic Ductal Adenocarcinoma

Type of cancer	Effect they assessed for	Authors	Year	Paper title	Aim	Main findings
LUAD*	*TP53*	Mogi et al	2010	*TP53* Mutations in Nonsmall Cell Lung Cancer	Review: This paper will focus on the role of *TP53* in the molecular pathogenesis, epidemiology, and therapeutic strategies of *TP53* mutation in NSCLC.	NSCLC with *TP53* alterations carries a worse prognosis and may be relatively more resistant to chemotherapy and radiation.
LUAD*	*TP53*	Villa et al	2014	Nondisruptive p53 Mutations Are Associated with Shorter Survival in Patients with Advanced Non-Small Cell Lung Cancer	To analyze *TP53* mutations in a training cohort of 318 patients with advanced NSCLC [125 *ECFR*-wt and 193 ECFR-mutated (mut)] and correlate *TP53* mutations with clinical parameters.	Nondisruptive mutations can retain some of the functional properties of wt-p53, and more importantly, experimental evidence shows that they often associate with GOF activities.
LUAD*	*TP53*	Ma et al.	2016	Prognostic and Predictive Effect of *TP53* Mutations in Patients with Non–Small Cell Lung Cancer from Adjuvant Cisplatin–Based Therapy Randomized Trials: A LACE-Bio Pooled Analysis	To clarify the putative prognostic and predictive value of *TP53* mutation for ACT, we performed a pooled analysis of four randomized trials of cisplatin-based adjuvant chemotherapy (ACT) versus observation (OBS).	In patients who received ACT, *TP53* mutation tended to be associated with shorter survival than wild-type *TP53*.
LUAD*	*TP53*	Gu et al.	2016	*TP53* mutation is associated with a poor clinical outcome for non-small cell lung cancer: Evidence from a meta-analysis	To investigate the prognostic significance of *TP53* gene mutations in patients with NSCLC.	*TP53* gene alteration may be an indicator of a poor prognosis in patients with NSCLC. When the *TP53* mutation group (n=1,406) was compared with the wild-type group (lacking *TP53* mutations; n=1,965), the wild-type group was associated with a significantly higher overall survival rate [hazard ratio (HR), 1.26; 95% confidence interval (CI) 1.12-1.41, P<0.0001].

LUAD*	*TP53*	Canale et al	2017	Impact of *TP53* Mutations on Outcome in *EGFR*-Mutated Patients Treated with First-Line Tyrosine Kinase Inhibitors	To analyze the impact of *TP53* mutations on response to first-line tyrosine kinase inhibitors (TKI) in patients with *EGFR* mutated non–small cell lung cancer (NSCLC).	*TP53* mutations, especially exon 8 mutations, reduce responsiveness to TKIs and worsen prognosis in *EGFR* mutated NSCLC patients, mainly those carrying exon 19 deletions.
LUAD*	*TP53*	Zhao et al.	2020	*TP53* somatic mutations are associated with poor survival in non-small cell lung cancer patients who undergo immunotherapy	To investigate the association between *TP53* somatic mutations and immunotherapeutic outcomes in non-small cell lung cancer (NSCLC) patients.	Truncating *TP53* mutations correlate with poor immunotherapy outcomes in NSCLC patients with low TMB. truncating *TP53* mutations are an independent predictor of immunotherapeutic outcomes. Moreover, among NSCLC patients with lower tumor mutation burden (TMB), those with *TP53* truncating mutations showed significantly lower OS than those with wild-type *TP53* [hazard ratio (HR) = 1.40, confidence interval (CI) = 1.13-1.73; P = 0.002].
LUAD	*TP53*	Saleh et al.	2021	Comprehensive Analysis of *TP53* and *KEAP1* Mutations and Their Impact on Survival in Localized- and Advanced-Stage NSCLC	To systematically analyze the impact of different *TP53* mutations on OS.	Truncating *TP53* mutations were independent negative prognostic markers in multivariable analysis (hazard ratio [HR]*TP53* truncating= 1.43, 95% confidence interval [CI]: 1.07–1.91, p=0.015). Consistently, these mutations were associated with shorter disease-free survival. In 4779 patients with advanced-stage (UICC IIIB–IV) tumors, *TP53* mutations did not predict outcome in univariable analysis.
LUAD*	*TP53, EGFR, KRAS* co-occurrence	Shepherd et al.	2017	Pooled Analysis of the Prognostic and Predictive Effects of *TP53* Comutation Status Combined with *KRAS* or *EGFR* Mutation in Early-Stage Resected Non–Small-Cell Lung Cancer in Four Trials of Adjuvant Chemotherapy	To explore the prognostic and predictive roles of *TP53/KRA*S and *TP53/EGFR* comutations in randomized trials of adjuvant chemotherapy versus observation.	For *TP53/KRAS* mutation status (WT/WT, KRASMUT, TP53MUT, Double mutant) no prognostic effect was observed (P = .61). No prognostic effect of dual *TP53/KRAS* mutation compared with the WT/WT cohort or with cohorts with only one mutation. *TP53/EGFR* comutation in adenocarcinoma was neither prognostic (P = .83), nor significantly predictive (P = .86).
LUAD*	*TP53, EGFR* co-ocurrance	Labbéa et al	2017	Prognostic and predictive effects of *TP53* co-	To study the clinical outcomes of patients with	Patients with dual TP53/EGFR mutations, especially missense mutations, had

				mutation in patients with *EGFR*-mutated non-small cell lung cancer (NSCLC)	EGFR-driven NSCLC, based on their TP53 mutational status.	marginally lower response rates and shorter PFS when treated with EGFR TKI therapy.
LUAD*	*TP53, KRAS* co-ocurrance	Tomasini et al.	2019	Effect of Coexisting *KRAS* and *TP53* Mutations in Patients Treated With Chemotherapy for Nonesmall-cell Lung Cancer	To study the clinical outcomes of patients tested for *KRAS* and *TP53* mutations and treated with chemotherapy for any stage NSCLC at the Princess Margaret Cancer Centre.	There was no significant difference in DFS/PFS between the 4 groups (*KRAS/TP53* co-mutations, *TP53* mutated, *KRAS* mutated, no *KRAS/TP53* mutated). However, OS was longer for patients with *TP53* and *KRAS* wild-type NSCLC who received chemotherapy for any stage compared with patients with *KRAS, TP53* mutation, or double mutant tumors.
LUAD*	*TP53, EGFR, KRAS* co-ocurrance	Zheng et al.	2020	Coexisting *EGFR* and *TP53* Mutations in Lung Adenocarcinoma Patients Are Associated With COMP and ITGB8 Upregulation and Poor Prognosis	To determine the gene expression, SNP, and co-mutation participating in the initiation and progression of lung AC to examine suitable options for a novel targeted therapy.	EGFRL858R with co-mutation *TP53* was significant prognostic determinant versus that with co-wild TP53 (hazard ratio, 2.77, P = 0.012). Mutations in KRAS were not related to prognosis.
PDAC**	*TP53, KRAS, CDKN2A, SMAD4*	Quian et al.	2017	Association of Alterations in Main Driver Genes With Outcomes of Patients With Resected Pancreatic Ductal Adenocarcinoma	To evaluate the alterations of the 4 main driver genes in pancreatic adenocarcinoma and patient outcomes after cancer resection.	Patients with KRAS mutant tumors had worse DFS (median [IQR], 12.3 [6.7 -27.2] months) and OS (20.3 [11.3-38.3] months) compared with patients with KRAS wild-type tumors (DFS, 16.2 [8.9-30.5] months; OS, 38.6 [16.6-63.1] months) and had 5-year OS of 13.0% vs 30.2%. Patients whose tumors lacked *CDKN2A* expression had worse DFS (median, 11.5 [IQR, 6.2-24.5] months) and OS (19.7 [10.9-37.1] months) compared with patients who had intact *CDKN2A* (DFS, 14.8 [8.2-30.5] months; OS, 24.6 [14.1-44.6] months). The molecular status of *SMAD4* was not associated with DFS or OS. TP53 was associated with shorter DFS (HR, 1.33;95%CI, 1.02-1.75; P = .04) but was not associated with OS (HR, 1.18; 95% CI, 0.91-1.53; P = .23).Patients had

PDAC**	*TP53, KRAS, CDKN2A, SMAD4*	McIntyre et al	2020	Alterations in Driver Genes Are Predictive of Survival in Patients With Resected Pancreatic Ductal Adenocarcinoma	To investigate the associations of the 4 driver genes and other commonly altered genes, including those involved in DNA repair pathways, with clinical and pathologic out-comes for those who underwent surgical resection for PDAC at Memorial Sloan Kettering (MSK).	worse DFS and OS if they had a greater number of altered driver genes. Compared with patients with 0 to 2 altered genes, those with 4 altered genes had worse DFS (HR, 1.79 [95% CI, 1.24-2.59; P = .002]) and OS (HR, 1.38 [95% CI, 0.98-1.94; P = .06]). Five-year OS was 18.4% for patients with 0 to 2 gene alterations, 14.1% for those with 3 alterations, and 8.2% for those with 4 alterations. Alterations in *KRAS* and *TP53* were associated with worse overall survival (OS) in comparison to wild type (median for *KRAS*, 38.8 months [95% CI, 33.0-45.5 months] vs 91.0 months [95% CI, 34.8 months to not available (NA)]; P = .043; median for TP53, 37.4 months [95% CI, 32.1-42.8 months] vs 65.0 months [95% CI, 33.0 months to NA]; P = .035). TP53 truncating mutations (median, 39.6 months [95% CI, 32.4-75.2 months] vs 33.9 months [95% CI, 24.0-39.0 months]; P = .020) and those associated with loss of heterozygosity (median, 26.6 months [95% CI, 21.6-44.2 months] vs 39.2 months [95% CI, 34.5-49.1 months]; P = .048) had decreased OS. TP53 alterations were independently associated with OS in a multivariate analysis (hazard ratio, 1.54; 95% CI, 1.01-2.33; P = .042). Individuals with germline alterations in homologous recombination deficiency (HRD) genes had improved OS in comparison with those without them (median, not reached vs 37.0 months; 95% CI, 33.0-49.8 months; P = .035).

PDAC**	*TP53*	Maddalena et al.	2021	*TP53* missense mutations in PDAC are associated with enhanced fibrosis and an immunosuppressive microenvironment	To elucidate the impact of missense *TP53* mutations on the immune TME of PDAC.	Missense p53 mutations may contribute to worse PDAC prognosis by promoting a more vigorous fibrotic tumor microenvironment and impeding the ability of the immune system to eliminate the cancer cells.
PDAC**	*TP53*	Sijde et al.	2021	Circulating *TP53* mutations are associated with early tumor progression and poor survival in pancreatic cancer patients treated with FOLFIRINOX	To identify circulating tumor DNA (ctDNA) mutations that associate with tumor progression during FOLFIRINOX chemotherapy, and overall survival (OS).	The combination of the *TP53* mutation and *TP53* germline variant was associated with shorter survival (median OS 4.4 months, 95% CI 2.6–6.2 months) compared with patients without any TP53 alterations (median OS 13.0 months, 95% CI 8.6–17.4 months).
PDAC**	*TP53*	Liu et al	2021	A novel *TP53*-associated nomogram to predict the overall survival in patients with pancreatic cancer	To explore the gene alterations in tumorigenesis and cancer development of PC based on comprehensive bioinformatic analysis	*TP53* mutation was indicated to be the only robust and survival-related mutation type. *TP53*-mutated patients showed significantly worse overall survival than *TP53*-wild patients in included cohorts.

*Lung Adenocarcinoma

**Pancreatic Ductal Adenocarcinoma

Table 2. Demographic and Tumor Characteristics of Patients with Lung Adenocarcinoma from the TCGA, MSK-IMPACT and the Combined Cohorts

Characteristic	Cohort		
	TCGA	**MSK-Impact**	**Combined**
Demographic	n=575	n=4,176	N=4,751
Age at diagnosis, median (IQR), years	66.00(59.00-73.00)	66.27(58.53-73.02)	66.25(58.63-73.00)
Sex, n(%)			
Female	275 (47.83)	2,586(61.93)	2,861(60.22)
Male	247 (42.96)	1,589(38.05)	1,836(38.64)
Race/Ethnicity, n(%)			
White/Caucasian	396 (68.87)	3,281(78.57)	3,677(77.39)
Black or African American	50 (8.70)	194(4.65)	244(5.14)
Asian	8 (1.39)	427(10.23)	435(9.16)
Tumor			
TP53 mutation type, n(%)			
Wild-type	271 (47.13)	2,153 (51.56)	2,424 (51.02)
Missense	197 (34.26)	1,352 (32.38)	1,549 (32.60)
Truncating	107 (18.61)	671 (16.07)	778 (16.38)
Tumor stage (AJCC),n(%)			
T1	68 (11.83)	--	--
T1a	47 (8.17)	--	--
T1b	53 (9.22)	--	--
T2	172 (29.91)	--	--
T2a	83 (14.43)	--	--
T2b	28 (4.87)	--	--
T3	47 (8.17)	--	--
T4	21 (3.65)	--	--
Lymph node stage (AJCC),n(%)			
N0	333 (57.91)	--	--
N1	100 (17.39)	--	--
N2	76 (13.22)	--	--
N3	2 (0.35)	--	--
Metastatic disease, n(%)			
No	355(61.74)	735(17.60)	1090(22.94)
Yes	25(4.35)	3441(82.40)	3466(72.95)
Prognostic stage group (AJCC),n(%)			
I	5 (0.87)	--	--
IA	133 (23.13)	--	--
IB	140 (24.35)	--	--
II	1 (0.17)	--	--
IIA	52 (9.04)	--	--
IIB	75 (13.04)	--	--
IIIA	73 (12.70)	--	--
IIIB	13 (2.26)	--	--
IV	26 (4.52)	--	--

Abbreviations: IQR, interquartile range; AJCC, American Joint Committee on Cancer

[a]Percentages may not sum to 100% due to rounding or missing values.

Table 3. Univariate and Multivariable Analyses of Associations between *TP53* Mutations and Overall Survival of Patients with Lung Adenocarcinoma, Stratified by Co-occurring *EGFR* and *KRAS* Mutations

	Characteristic	Combined cohort	
		HR (95% CI)	***p-value***
Univariate Analysis	Sex		
	Female	--	
	Male	1.36(1.24-1.49)	**<0.001**
	TP53 mutation type		
	Wild-type	--	
	Missense	1.16(1.06-1.27)	**0.002**
	Truncating	1.35(1.20-1.51)	**<0.001**
Multivariate analysis	Sex		
	Female	--	
	Male	1.34(1.22-1.47)	**<0.001**
	TP53 mutation type		
	Wild-type	--	
	Missense	1.12(1.00-1.24)	**0.044**
	Truncating	1.33(1.17-1.51)	**<0.001**
***KRAS/EGFR* wild-type**			
Univariate Analysis	Sex		
	Female	--	
	Male	1.35(1.18-1.55)	**<0.001**
	TP53 mutation type		
	Wild-type	--	
	Missense	1.21(1.05-1.39)	**0.010**
	Truncating	1.35(1.14-1.60)	**<0.001**
Multivariate analysis	Sex		
	Female	--	
	Male	1.36(1.18-1.58)	**<0.001**
	TP53 mutation type	--	
	Wild-type	--	
	Missense	1.24(1.05-1.46)	**0.013**
	Truncating	1.41(1.16-1.72)	**<0.001**

	***EGFR* mutated**		
Univariate Analysis	Sex		
	Female	--	
	Male	1.54(1.28-1.86)	**<0.001**
	TP53 mutation type		
	Wild-type	--	
	Missense	1.35(1.13-1.62)	**0.001**
	Truncating	1.65(1.34-2.04)	**<0.001**
Multivariate analysis	Sex		
	Female	--	
	Male	1.55(1.27-1.89)	**<0.001**
	TP53 mutation type	--	
	Wild-type	--	
	Missense	1.56(1.24-1.96)	**<0.001**
	Truncating	1.86(1.43-2.42)	**<0.001**
	***KRAS* mutated**		
Univariate Analysis	Sex		
	Female	--	
	Male	1.22(1.05-1.43)	**0.011**
	TP53 mutation type		
	Wild-type	--	
	Missense	1.05(0.88-1.25)	0.600
	Truncating	1.17(0.93-1.49)	0.185
Multivariate analysis	Sex		
	Female	--	
	Male	1.11(0.94-1.32)	0.216
	TP53 mutation type	--	
	Wild-type	--	
	Missense	0.90(0.74-1.09)	0.259
	Truncating	1.10(0.85-1.42)	0.467

Abbreviations: HR, hazard ratio; CI, confidence interval

[a]Cox proportional hazards regression model adjusted for TP53 mutation type, age at diagnosis, sex, race/ethnicity, metastatic disease at the time of diagnosis and cohort to which the patient belonged

*Statistically significant p-values at the alpha=0.05 level are bolded.

Table 4. Demographic and Tumor Characteristics of Patients with Pancreatic Ductal Adenocarcinoma from the TCGA, MSK-IMPACT and the Combined Cohorts

Characteristic	Cohort		
	TCGA	**MSK-Impact**	**Combined**
Demographic	n=141	n=376	N=517
Age at diagnosis, median (IQR), years	65.00(57.00-74.00)	65.04(56.04-72.05)	65.04(56.04-72.05)
Sex, n(%)			
Female	65(46.10)	177(47.07)	242(46.81)
Male	76 (53.90)	199(52.93)	275(53.19)
Race/Ethnicity, n(%)			
White/Caucasian	119(84.40)	300(79.79)	419(81.04)
Black or African American	5(3.55)	10(2.66)	15 (2.90)
Asian	8(5.67)	12(3.19)	20 (3.87)
Hispanic/Latino	5(3.55)	16(4.26)	21 (4.06)
Clinical/Tumoral			
TP53 mutation type, n(%)			
Wild-type	45 (31.91)	113(30.05)	158(30.56)
Missense	59(41.84)	181(48.14)	240(46.42)
Truncating	37 (26.24)	82(21.81)	119(23.02)
Anatomic site,n(%)			
Head	111(78.72)	--	--
Body or Tail	23(16.31)	--	--
Unspecified	7 (4.96)	--	--
Tumor stage (AJCC),n(%)			
T1	5(3.55)	--	--
T2	15(10.64)	--	--
T3	117(82.98)	--	--
T4	3(2.13)		--
Lymph node stage (AJCC),n(%)			
N0	35(24.82)	--	--
N1	105(74.47)	--	--
Metastatic disease, n(%)			
No	33(23.40)	225(59.84)	258(49.90)
Yes	107(75.89)	151(40.16)	258(49.90)
Prognostic stage group (AJCC),n(%)			
IA	4(2.84)	--	--
IB	8(5.67)	--	--
IIA	21(14.89)	--	--
IIB	100(70.92)	--	--
III	4 (2.84)	--	--
IV	3(2.13)	--	--
Histologic Grade,n(%)			
G1-Well differentiated	4(2.84)	--	--
G2-Moderately differentiated	71(50.35)	--	--
G3-Poorly differentiated	66(46.81)	--	--

Abbreviations: IQR, interquartile range; AJCC, American Joint Committee on Cancer

[a]Percentages may not sum to 100% due to rounding or missing values.

Table 5. Univariate and Multivariable Analyses of Associations between *TP53* Mutations and Overall Survival of Patients with Pancreatic Ductal Adenocarcinoma

	Characteristic	Combined cohort	
		HR (95% CI)	***p-value***
Univariate Analysis	*TP53* mutation type		
	Wild-type	--	
	Missense	1.47(1.07-2.02)	**0.016**
	Truncating	0.96(0.68-1.37)	0.065
	Metastatic disease		
	No	--	
	Yes	2.35(1.64-3.36)	**<0.001**
Multivariate analysis	*TP53* mutation type		
	Wild-type	--	
	Missense	1.47(1.00-2.17)	0.051
	Truncating	1.11(0.70-1.76)	0.654
	Metastatic disease		
	No	--	
	Yes	2.58(1.74-3.81)	**<0.001**

Abbreviations: HR, hazard ratio; CI, confidence interval

[a]Cox proportional hazards regression model adjusted for TP53 mutation type, age at diagnosis, sex, race/ethnicity, metastatic disease at the time of diagnosis and cohort to which the patient belonged

*Statistically significant p-values at the alpha=0.05 level are bolded.

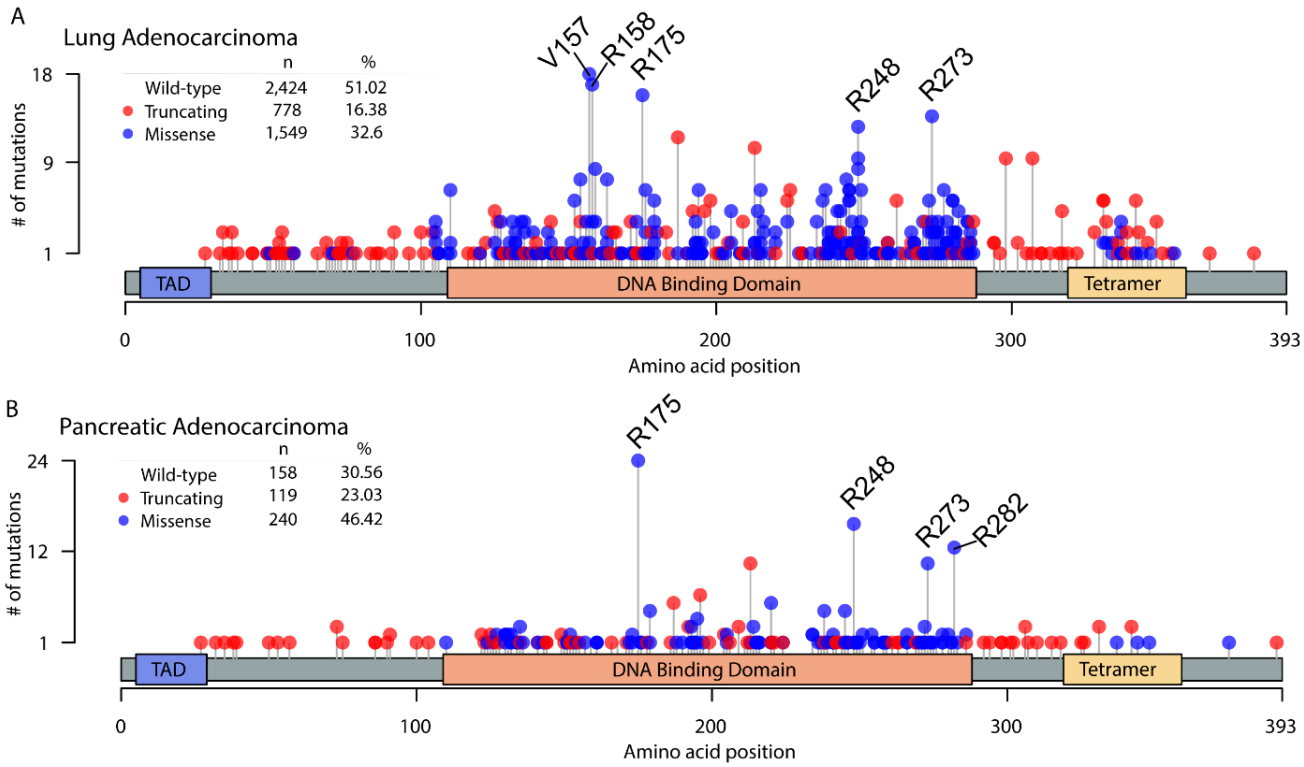

Figure 1. Lollipop plot showing the type and location of mutations in *TP53* observed in patients with lung adenocarcinoma (**A**) and pancreatic ductal adenocarcinoma (**B**) from the combined cohort; Transactivation Domains, TAD

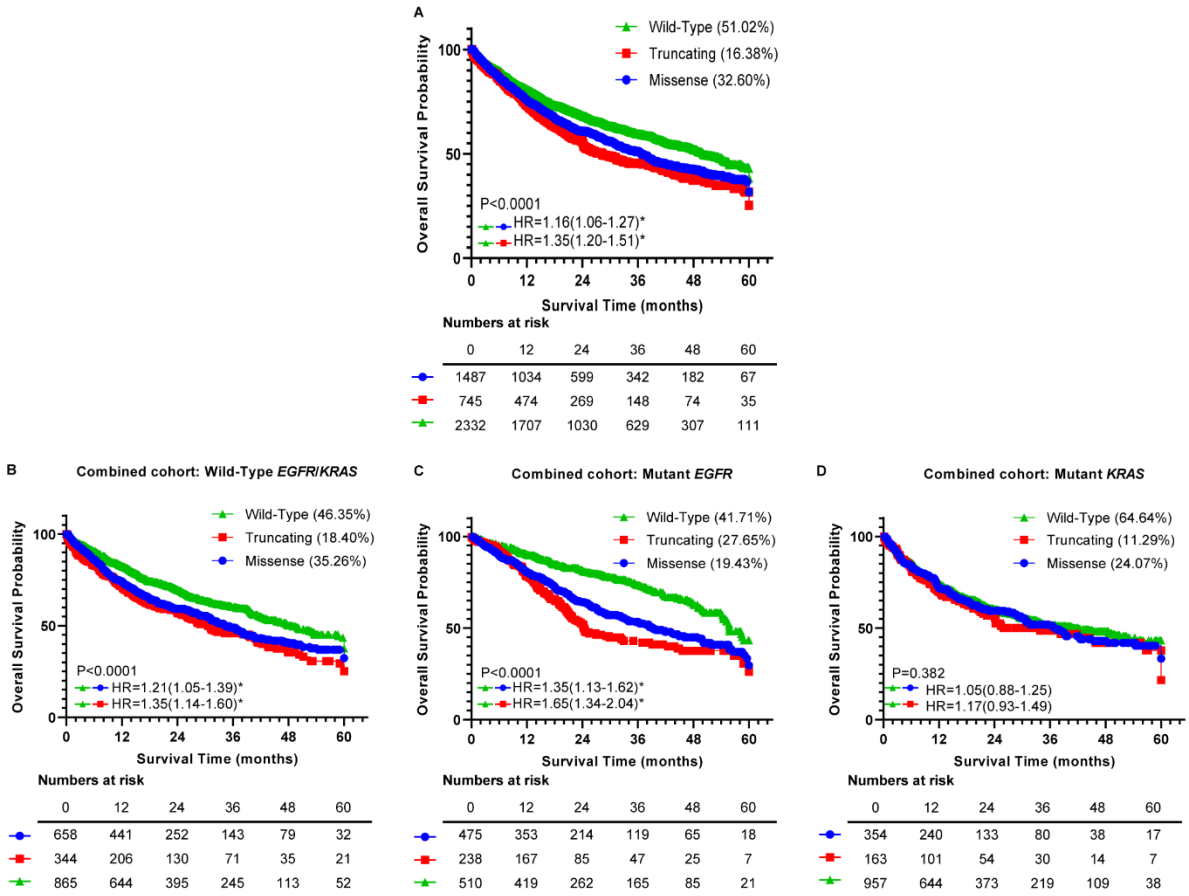

Figure 2. LUAD harboring truncating mutations in *TP53* have a worse overall survival compared with patients with wild-type*TP53***.** Kaplan-Meier survival curves for LUADs harboring different mutations in *TP53/KRAS/EGFR* genes from the combined **(A**) cohort; patients from the combined cohort were stratified into three different categories, *EGFR/KRAS* wild-type (**B**), *EGFR* mutated (**C**), and *KRAS* mutated (**D**); Lung Adenocarcinoma, LUAD; Hazard ratio, HR; P-value from log-rank test; *Hazard ratio statistically significant at the 0.05 level

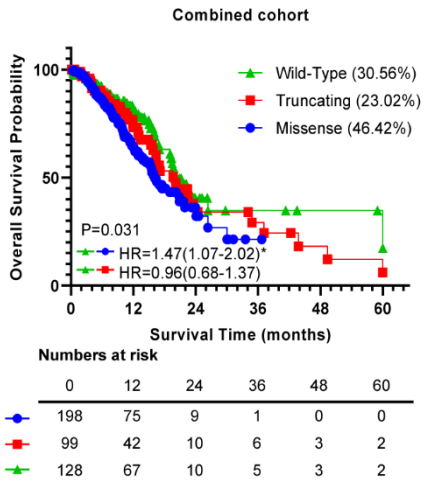

Figure 3. PDAC harboring with missense mutations in *TP53* have a worse overall survival compared with patients with wild-type*TP53*. Kaplan-Meier survival curves for PDACs harboring different mutations in *TP53* from the combined cohort; PDAC, pancreatic ductal adenocarcinoma; Hazard ratio, HR; P-value from log-rank test; *Hazard ratio statistically significant at the 0.05 level