VHL mutations predict survival of patients with renal cell carcinoma in response to the therapy of immune checkpoint inhibitors

Jun Zhang (✉ junzhang10977@sjtu.edu.cn)

Ruijin Hospital: Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

<https://orcid.org/0000-0002-7973-8416>

Liqin Zhao

Ruijin Hospital: Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Junwei Wu

Ruijin Hospital: Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Zhenhua Wu

Shanghai Cancer Hospital: Fudan University Shanghai Cancer Center

Qing Qu

Ruijin Hospital: Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

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Abstract

Purpose: To identify a predictive biomarker of immune checkpoint inhibitor (ICI) therapies for renal cell carcinoma (RCC).

Methods: Survival analysis of mutations in a panel of 468 cancer-related genes in a cohort of 151 RCC patients who underwent the ICI treatment using Cox regression model univariate or multivariate analysis in publicly available datasets.

Results: We found that *VHL* mutations were the only promising independent predictor for overall survival (OS) (HR=0.44, 95% CI=0.25-0.77 and $P=0.004$). More specifically, compared with 26 months of survival in the wildtype patients, metastatic RCC patients carrying truncated *VHL* mutations had a significantly longer survival time of nearly 70 months (HR=0.45, 95% CI=0.25-0.82 and $P=0.008$) in the presence of the ICI therapy. This survival benefit was also observed in another cohort of 35 patients with clear cell RCC from Dana-Farber Cancer Institute (DFCI): compared with 29 months in the wildtype patients, patients with *VHL* truncated mutations also had a longer median OS of 33 months (HR=0.59, 95% CI=0.24-1.44 and $P=0.243$). These observed survival benefits were independent of *VHL* expression and tumor infiltration immune cells in The Cancer Genome Atlas Kidney Renal Clear Cell Carcinoma (TCGA KIRC).

Conclusion: *VHL* mutational inactivation may have an effect on the RCC response to ICI therapy, likely by the upregulation of PD-L1 via attenuating degradation of HIF-1 α . To our knowledge, this is the first report of *VHL* mutations as an independent predictive biomarker for the ICI therapy in RCC, which, once validated by larger clinical trials, may help improve clinical decision-making in individualized treatment of RCC patients.

Introduction

RCC is one of the most common malignancies in the urinary system. Over 400,000 new patients suffer from RCC along with over 175,000 cancer-related deaths worldwide in 2018 ^[1]. Due to intrinsic resistance to the chemo-radiotherapy, the mainstay therapy for metastatic RCC (mRCC) has been limited to traditional therapies, such as cytokines or VEGF/mTOR targeting therapies. While these therapies have improved outcomes compared to the chemo-radiotherapy, they are still limited by toxicities or drug resistance that usually occur within the first year after the treatment. As a result, the 5-year over survival of mRCC has remained < 8% for the past decades ^[2-4]. The immune checkpoint inhibitor (ICI) treatment, including anti-bodies targeting programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1) or cytotoxic T-lymphocyte-associated protein 4 (CTLA4), provide survival benefits only to a minority of mRCC with a durable anti-tumor effect; there is no obvious improvement of progression-free survival (PFS) in unselected mRCC patients, compared to the mTOR inhibitor therapy ^[3, 5], making it critical to identify sensitive mRCC patients who can benefit from the ICI treatments.

Recently, PD-L1 expression levels, defect mismatch repair (dMMR) or microsatellite instability high (MSI-H), tumor mutation burden (TMB) have positively demonstrated some predictive effects of the ICI

treatment on various types of cancer [6–8]. However, to the best of our knowledge, there are few reliable predictive biomarkers for the indications of ICI treatment of mRCC, including TMB that has been proven to be incapable of providing potential survival benefits [8]. As for PD-L1, which is expressed in less than 1% of the tumors in 75% of RCC patients, its role in predicting the treatment response is obviously trivial [9]. Hence, it is pivotal to identify alternative predictive biomarkers for the ICI treatment of mRCC. To this end, we analyzed the existing mutation profiles in tumors for the prediction of the ICI treatment outcomes, because mutations in several genes have been demonstrated to predict outcomes of cancer patients effectively [10]. Hence, in the present study, by utilizing the publicly available mutation data on various cancers, we aimed to find mutations that may serve as potential predictors for overall survival (OS) in RCC patients.

Materials And Methods

Data information

We downloaded all the clinical information, mutations and RNA sequencing data of three independent datasets from the publicly available cBioPortal database (<https://www.cbioportal.org>), which included the Memorial Sloan Kettering Cancer Center Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) dataset (https://www.cbioportal.org/study/summary?id=tmb_mskcc_2018), The Cancer Genome Atlas Kidney Renal Clear Cell Carcinoma (TCGA KIRC Provisional) dataset (https://www.cbioportal.org/study/summary?id=kirc_tcga), and the DFCI (Dana-Farber Cancer Institute) ccRCC dataset (https://www.cbioportal.org/study?id=ccrcc_dfci_2019).

Initial analysis

In the initial analysis of ICI-related gene mutation data, we used the MSK-IMPACT dataset, considering all somatic mutation data that included missense, frame-shift, nonsense, nonstop, splice site, and translation start site changes, except for the synonymous mutations of the target panel containing 468 cancer-related genes [11]. These data were derived from the MSK-IMPACT assay that was applied to all the 151 mRCC patients who received ICI therapies. We first divided the 151 mRCC patients into three groups according to the 468 genes panel tested: patients having non-mutated genes, patients with certain genes (e.g., *VHL*) with mutations, and patients with other genes with mutations, and then we performed univariate Cox proportional hazards regression analysis for these three groups to evaluate the association between gene mutation status and OS of mRCC patients. We also generated Kaplan-Meier survival curves to visualize the results. Those genes with a *P* value <0.05 for a mutation rate >0.05 were considered the candidates for the multivariate Cox regression analysis with adjustment for age, sex, TMB, and ICI regimes. Subsequently, we selected the patients with those genes that had truncated mutations (frameshift insertion, frameshift deletion, nonsense, splice-site) to be compared with those patients with the wildtype genes, and we then performed log-rank test to compare the differences in OS between these two groups of patients.

Replication

To replicate the findings of those ICI-related genes present in mRCC patients included in the above-mentioned patient cohorts, we used another DFCI cohort that had 35 metastatic ccRCC patients who had the similar genes detected for mutations and also received ICI therapies. To compare the survival between the patients carrying the genes with truncated mutations and the patients with wildtype genes, we performed the log-rank test to compare the differences in OS between two groups.

Further exploration

To ascertain whether those mutational genes present in the above-mentioned MSK-IMPACT cohort may affect the survival of RCC patients without ICI therapies, we analyzed the TCGA KIRC dataset from 451 ccRCC patients who had somatic mutation data but only received traditional treatments, including surgery, chemo-radiotherapy, cytokines and target therapy. Then, we compared OS among patients carrying all genes with mutations, patients carrying genes with truncated mutations, and patients carrying wildtype genes.

We further evaluated the effect of *VHL* expression on OS in ccRCC patients by using the TCGA-KIRC dataset by using the median *VHL* mRNA expression value obtained from RNA sequencing data as the cutoff value to divide the 534 ccRCC patients into two groups of low and high *VHL* mRNA expression, and we used the log-rank tests to compare the differences in OS between the two groups.

We also compared the differences in tumor-infiltrated immune cells between the groups of the patients carrying *VHL* mutational genes and patients carrying *VHL* wildtype genes in the 451 patients from the TCGA KIRC dataset by calculating the gene expression matrix using CIBERSORT online tools (<https://cibersort.stanford.edu/>)^[12].

All the statistical analyses were performed by using R language (version 3.5.1), and *P* values were two-sided with a significance level of 0.05.

Results

The analysis flowchart is shown in **Figure 1**. In the initial analysis, we used a cohort of 151 metastatic or unresectable RCC patients who underwent the ICI therapy (122 received anti PD-1/PD-L1 monotherapy, and the remaining 29 received a combination of anti CTLA-4 and anti PD-1/PD-L1 therapies). Clinical characteristics of the patients are presented in **Table 1**. The univariate Cox regression analyses of the three groups (i.e., patients with non-mutated genes, patients with certain genes with mutations, and patients with other genes with mutations) showed that mutations in 21 genes were significantly associated with OS, but the patients with the highest *VHL* mutation rate (70%) had the largest HR of 1.91, compared with the patients with mutations in other 20 genes (2%) (**Supplement Table 1**). Therefore, subsequent analyses focused on the *VHL* mutations.

Table 1

Clinicopathological characteristics of the MSK-IMPACT RCC cohort underwent ICI treatment

Characteristics	No. of Cases (%)
All subjects	151 (100)
Age at diagnosis	
<30	3 (2.0)
31-50	25 (16.6)
50-60	54 (35.8)
61-70	51 (33.8)
>71	18 (11.9)
Sex	
Female	42 (27.8)
Male	109 (72.2)
ICI Regime	
PD-1/PD-L1	122 (80.8)
Combo	29 (19.2)
Mean of TMB (/Mb)	4.25
Cell subtype	
Chromophobe Renal Cell Carcinoma	5 (3.3)
FH-Deficient Renal Cell Carcinoma	2 (1.3)
Papillary Renal Cell Carcinoma	5 (3.3)
Renal Cell Carcinoma	4 (2.6)
Renal Clear Cell Carcinoma	121 (80.1)
Renal Clear Cell Carcinoma with Sarcomatoid Features	1 (0.7)
Renal Mucinous Tubular Spindle Cell Carcinoma	1 (0.7)
Translocation-Associated Renal Cell Carcinoma	3 (2.0)
Unclassified Renal Cell Carcinoma	9 (6.0)

Abbreviations: MSK-IMPACT, Memorial Sloan Kettering Cancer Center Integrated Mutation Profiling of Actionable Cancer Targets; RCC, renal cell carcinoma; ICI, immune checkpoint inhibitors; Combo, anti CTLA-4 combined with anti PD-1/PD-L1; TMB, tumor mutation burden; /Mb, per Mega bases.

In **Figure 2**, we present survival curves of patients without mutations in any genes, patients with *VHL* mutations, and patients with mutations in genes other than *VHL*. In the following analysis, we merged the group of patients without mutations or with the *VHL* wildtype and the group of patients with mutations in genes other than *VHL* as one group to be compared with those with *VHL* mutations. In the univariate and multivariate Cox regression analyses for the association between the *VHL* mutation status and OS, we found that the *VHL* mutation status (70.2%, 106/151) was an independent predictor for OS in this patient cohort (HR = 0.52, 95% CI = 0.36-0.77, $P = 0.001$ and HR = 0.44, 95% CI = 0.25-0.77, $P = 0.004$ in univariate and multivariate analyses, respectively) (**Table 2**). Specifically, RCC patients with *VHL* mutations showed a significantly longer OS of 50 months, compared with 26 months in patients with the wildtype *VHL* or mutations in other genes (**Figure 3A**). The detailed clinical information on the group of patients with *VHL* mutations and the other group is presented in **Table 3**. It appeared that *VHL* mutations occurred more often in ccRCC (102/121, 84.3%) than in non-ccRCC ($n=4/30$, 13.3%) ($P < 0.001$), and the *VHL* mutation group had a relatively higher mean TMB (4.70 ± 2.46) than that (3.20 ± 3.54) of the *VHL* wildtype group ($P = 0.003$). We then focused on truncated mutations, because they were considered functionally important mutations^[13]. We found that RCC patients ($n = 65$) with *VHL* truncated mutations showed a significantly longer median OS of nearly 70 months, compared with 26 months in patients ($n = 45$) with the wildtype *VHL* (HR = 0.45, 95% CI = 0.25-0.82 and $P = 0.008$) (**Figure 3B**).

Table 2

Univariate and multivariate analysis of factors associated with overall survival in RCC underwent ICI treatment

Variables		Events/ Subjects	Univariate analysis			Multivariate analysis		
			HR	95% CI	<i>P</i> value	HR	95% CI	<i>P</i> value
Age (group in years)			1.00	0.76-1.32	0.987	1.00	0.76-1.32	0.994
Sex	Female (reference)	21/42	1.00			1.00		
	Male	37/109	0.68	0.40-1.17	0.163	0.72	0.42-1.25	0.294
ICI regime	Combo (reference)	8/29	1.00			1.00		
	PD-1/PDL-1	50/122	1.39	0.96-2.03	0.085	1.41	0.96-2.06	0.077
TMB (/Mb)			0.94	0.85-1.05	0.273	0.97	0.89-1.06	0.532
VHL	Wildtype (reference)	24/45	1.00			1.00		
	Mutations	34/106	0.52	0.36-0.77	0.001*	0.44	0.25-0.77	0.004*

Abbreviations: RCC, renal cell carcinoma; ICI, immune checkpoint inhibitors. HR, hazards ratio; CI, confidence interval; TMB, tumor mutation burden; /Mb, per Mega bases; Combo, anti CTLA-4 combined with anti PD-1/PD-L1; *VHL*, von Hippel-Lindau tumor suppressor; * The results were in **bold**, if the *P* value was less than 0.05.

Table 3

Clinicopathological features of patients with a different *VHL* mutation status in the MSK-IMPACT RCC cohort

Characteristics	All patients	Patients with <i>VHL</i> mutations (%)	<i>P</i> value
All subjects	151	106 (100)	
Age (group in years)			0.801
<30	3	0 (0.0)	
31-50	25	18 (68.0)	
50-60	54	38 (70.4)	
61-70	51	38 (74.5)	
>71	18	12 (66.7)	
Sex			0.538
Male	109	81 (74.3)	
Female	42	25 (59.5)	
ICI regime			1.000
PD-1/PDL-1	122	85 (69.7)	
Combo	29	21 (53.8)	
Mean of TMB $\pm$ SD	151	4.70 $\pm$ 2.46 / 3.20 $\pm$ 3.54*	0.003**
Subtype			<0.001**
Renal clear cell carcinoma	121	102 (84.3)	
Non-clear cell renal cell carcinoma	30	4 (13.3)	

Abbreviations: MSK-IMPACT, Memorial Sloan Kettering Cancer Center Integrated Mutation Profiling of Actionable Cancer Targets; RCC, renal cell carcinoma; ICI, immune checkpoint inhibitors; Combo, anti CTLA-4 combined with anti PD-1/PD-L1; TMB, tumor mutation burden; SD, standard deviation. * For those who had the *VHL* wildtype.

The results were in **bold, if the *P* value was less than 0.05.

To further ascertain whether *VHL* mutations affected OS in RCC treated with traditional therapies, including surgery, chemo-radiotherapy, cytokines and target therapy, another 451 ccRCC patients from the TCGA KIRC dataset were divided into two groups by *VHL* mutation status with detailed information presented in **Supplemental Table 2**. The *VHL* mutation group (n= 225) showed no survival benefit, compared with the wildtype group (n = 226) (**Figure 3C**). In addition, the *VHL* truncated mutation group (n = 126) also showed no significant survival benefit, compared with the wildtype group in this dataset

(**Figure 3D**). Furthermore, there was no association between the tumor infiltrated immune cells and the *VHL* mutation status in RCC in the TCGA KIRC dataset (**Supplemental Table 3**).

Additional analysis with the data from the TCGA KIRC dataset suggested that the *VHL* expression levels did not affect OS in ccRCC patients (n = 534) who did not receive any immunotherapies (**Figure 3E**), of whom 267 patients had lower *VHL* expression, while other 267 patients had higher *VHL* expression.

To replicate the findings in the MSK-IMPACT dataset that the *VHL* truncated mutation group had a longer median OS than the *VHL* wildtype group, who all received ICI therapies, we used the data from another DFCI cohort of 35 metastatic ccRCC patients, of whom those (n=16) with *VHL* truncated mutations also showed a longer median OS (33 months), compared with those (n=10) with the wildtype *VHL* (29 months), although it did not reach the statistical significance (HR = 0.59, 95% CI = 0.24-1.44 and *P* = 0.243) (**Figure 3F**), likely due to a much smaller sample size (n = 35) of this small dataset.

Discussion

VHL, as a tumor suppressor, is mutated in over 50% ccRCC that constitutes the majority of RCC. In the present study, the ccRCC accounted for 80.1% RCC in the MSK-IMPACT patient cohort. The *VHL* gene was mutated in 84.3% and 51% of the ccRCC patients in the MSK-IMPACT cohort and the TCGA KIRC dataset, respectively. Although *VHL* mutations were significantly associated with TMB in the dataset we used, the TMB was shown to have a little value in predicating the response to ICI treatment [8]. Some studies suggested that the impact of immune cell infiltration on the ICI treatment was not negligible, despite this notion was not well established yet [14–16]. However, we observed in the present study that the immune cell infiltration rate was not significantly different between tumors with mutational and wildtype *VHL*.

Truncated mutations have been considered the important functional mutations, usually leading to the loss of gene functions [13, 17]. It has been reported that inactivating mutations of *VHL* occurred in RCC may attenuate the degradation of HIF, thus leading to the hypoxia signal activation, which stimulates angiogenesis, cell growth and survival of renal cancer cells [18–20]. In addition, a previous study reported that the hypoxia induced the PD-L1 expression via upregulating HIF-1 α expression in tumor-infiltrating myeloid cells [21], while another study identified that overexpression of PD-L1 and PD-1 was induced in both human *in vitro* and murine models of hypoxia as well as by HIF-1 α transfection [22]. Because mutational inactivation of *VHL* may attenuate the degradation of HIF, it is reasonable to speculate that the mutational inactivation of *VHL* could lead to the accumulation of the HIF-1 α , thus leading to the PD-L1 up-expression in renal cancer cells, which then makes the cancer cells potentially targetable by the anti-PD-1/PD-L1 therapy. However, more experiments are needed to validate such a hypothesis.

In summary, we found in the present study that *VHL* mutations, the truncated mutations in particular as an independent predictor for OS, effectively predicted survival of mRCC patients in response to the ICI treatment. Considering other relevant findings from previous studies, we believe that the survival benefit associated with *VHL* mutations could be due to the upregulation of PD-L1 by *VHL* mutational inactivation

via HIF-1 α , a possible mechanism that may explain the efficacy of immunotherapies in treating RCC. However, because studies with a limited sample size were used in the present study, additional larger studies or clinical trials as well as functional investigations are needed to confirm these results.

Declarations

Funding

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Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Zhao LQ, Wu JW and Wu ZH. The first draft of the manuscript was written by Zhao LQ and Qu Q, Zhang J made revisions and approved the final version. All authors read and consented to the final manuscript.

Data Availability

All datasets used in this analysis were from the public database, the URLs were presented in the methodology section. The codes used in this analysis can be obtained upon reasonable requests through the corresponding author.

Ethics approval

The analysis was a post-hoc study with all the information was de-identified in the data. The Ruijin Hospital Research Ethics Committee has confirmed that no ethical approval is required.

References

1. Bray F, Ferlay J, Soerjomataram I et al (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries[J]. CA Cancer J Clin 68(6):394–424

2. Fyfe G, Fisher RI, Rosenberg SA et al (1995) Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy[J]. *J Clin Oncol* 13(3):688–696
3. Escudier B, Sharma P, McDermott DF et al (2017) CheckMate 025 Randomized Phase 3 Study: Outcomes by Key Baseline Factors and Prior Therapy for Nivolumab Versus Everolimus in Advanced Renal Cell Carcinoma[J]. *Eur Urol* 72(6):962–971
4. Choueiri TK, Motzer RJ (2017) Systemic Therapy for Metastatic Renal-Cell Carcinoma[J]. *N Engl J Med* 376(4):354–366
5. Hammers HJ, Plimack ER, Infante JR et al (2017) Safety and Efficacy of Nivolumab in Combination With Ipilimumab in Metastatic Renal Cell Carcinoma: The CheckMate 016 Study[J]. *J Clin Oncol* 35(34):3851–3858
6. Le DT, Uram JN, Wang H et al (2015) PD-1 Blockade in Tumors with Mismatch-Repair Deficiency[J]. *N Engl J Med* 372(26):2509–2520
7. Carbone DP, Reck M, Paz-Ares L et al (2017) First-Line Nivolumab in Stage IV or Recurrent Non-Small-Cell Lung Cancer[J]. *N Engl J Med* 376(25):2415–2426
8. Samstein RM, Lee CH, Shoushtari AN et al (2019) Tumor mutational load predicts survival after immunotherapy across multiple cancer types[J]. *Nat Genet* 51(2):202–206
9. Motzer RJ, Escudier B, McDermott DF et al (2015) Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma[J]. *N Engl J Med* 373(19):1803–1813
10. Wang F, Zhao Q, Wang YN et al (2019) Evaluation of POLE and POLD1 Mutations as Biomarkers for Immunotherapy Outcomes Across Multiple Cancer Types[J]. *JAMA Oncol*,
11. Cheng DT, Mitchell TN, Zehir A et al (2015) Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology[J]. *J Mol Diagn* 17(3):251–264
12. Newman AM, Liu CL, Green MR et al (2015) Robust enumeration of cell subsets from tissue expression profiles[J]. *Nat Methods* 12:453
13. Miao D, Margolis CA, Gao W et al (2018) Genomic correlates of response to immune checkpoint therapies in clear cell renal cell carcinoma[J]. *Science* 359(6377):801–806
14. Stanton SE, Disis ML (2016) Clinical significance of tumor-infiltrating lymphocytes in breast cancer[J]. *J Immunother Cancer* 4:59
15. Badalamenti G, Fanale D, Incorvaia L et al (2018) Role of tumor-infiltrating lymphocytes in patients with solid tumors: Can a drop dig a stone?[J]. *Cell Immunol*, :103753
16. Uryvaev A, Passhak M, Hershkovits D et al (2018) The role of tumor-infiltrating lymphocytes (TILs) as a predictive biomarker of response to anti-PD1 therapy in patients with metastatic non-small cell lung cancer or metastatic melanoma[J]. *Med Oncol* 35(3):25
17. Hug N, Longman D, Caceres JF (2016) Mechanism and regulation of the nonsense-mediated decay pathway[J]. *Nucleic Acids Res* 44(4):1483–1495

18. Comprehensive molecular characterization (2013) of clear cell renal cell carcinoma[J]. Nature 499(7456):43–49
19. Latif F, Tory K, Gnarr J et al (1993) Identification of the von Hippel-Lindau disease tumor suppressor gene[J]. Science 260(5112):1317–1320
20. Gerlinger M, Horswell S, Larkin J et al (2014) Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing[J]. Nat Genet 46(3):225–233
21. Noman MZ, Desantis G, Janji B et al (2014) PD-L1 is a novel direct target of HIF-1alpha, and its blockade under hypoxia enhanced MDSC-mediated T cell activation[J]. J Exp Med 211(5):781–790
22. Cubillos-Zapata C, Avendano-Ortiz J, Hernandez-Jimenez E et al (2017) Hypoxia-induced PD-L1/PD-1 crosstalk impairs T-cell function in sleep apnoea[J].Eur Respir J, 50(4)

Figures

Figure 1

The analysis flowchart. Gene A represent any certain gene of 468 gene panel.

Figure 2

The status of *VHL* mutations affect the OS of RCC patients.

Figure 3

The association of *VHL* mutations and expression with OS of RCC patients under ICI therapies or traditional therapies.

A: The patients (n=106) with *VHL* mutations showed a significantly longer OS than the wildtype population (n=45) in MSK-IMPACT RCC cohort underwent ICI treatment.

- B:** The patients (n=65) with *VHL* truncated mutations showed a significantly longer OS than the wildtype population (n=45) in MSK-IMPACT RCC cohort underwent ICI treatment.
- C:** The patients (n=225) with *VHL* mutations showed no significantly benefit than the wildtype population (n=226) in TCGA KIRC dataset underwent traditional therapy.
- D:** The patients (n=126) with *VHL* truncated mutations showed no significantly benefit than the wildtype population (n=226) in TCGA KIRC dataset underwent traditional therapy.
- E:** The *VHL* gene expression showed no significantly association with OS of the patients (n=534) in the TCGA KIRC dataset underwent traditional therapy.
- F:** The patients (n=16) with *VHL* truncated mutations showed a longer OS than the wildtype patients (n=10) in the DFCI patient cohort underwent ICI treatment though the *P* value was not significant due to a smaller sample size.

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