Homologous Recombination Deficiency as a Novel Therapeutic Target in Various Solid Tumors

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Abstract

Background: Homologous recombination deficiency (HRD) is related to tumorigenesis. Currently, the possibility of HRD as a prognostic biomarker to immune check point inhibitors is unknown.

Methods: The status of homologous recombination deficiency (HRD) was assessed with the next-generation sequencing (NGS) TruSight^{TM} Oncology 500 assay in 501 patients with advanced solid tumor including gastrointestinal (GI), genitourinary (GU), or rare cancer. Results: Among the 501 patients, HRD was observed as follows: 74.7% (347/501) patients; GU cancer (92.0%, 23 of 25), colorectal cancer (CRC) (86.1%, 130 of 151), hepatocellular carcinoma (HCC) (83.3%, 10 of 12), pancreatic cancer (PC) (76.2%, 32 of 42), biliary tract cancer (BTC) (75.0%, 36 of 48), sarcoma (65.0%, 39 of 60), melanoma (52.4%, 11 of 21), other GI cancers (50.0%, 11 of 22), and rare cancer (50.0%, 2 of 4). Sixty-five of the 501 patients had received immune checkpoint inhibitors (ICIs) during the course of the disease. Tumor types of 65 patients treated with ICIs are as follows: melanoma (95.2%, 20 of 21), HCC (33.3%, 4 of 12), rare cancer (25.0%, 1 of 4), GC (12.2%, 14 of 116), BTC (10.4%, 5 of 48), and sarcoma (5.0%, 3 of 60). Patients without HRD exhibited an objective response rate (ORR) of 33.3% (4 of 12), and patients with HRD exhibited an ORR of 34.0% (18 of 53). There was no significant difference in ORR between patients with and without HRD (p = 0.967). Progression-free survival (PFS) was 6.5 months (95% CI: 0.000 – 16.175) in patients without HRD and 4.1 months (95% CI: 2.062 – 6.138) in patients with HRD, revealing no statistical significance (p = 0.441).

Conclusion: Herein, we reported the status of HRD using a cancer-panel for various solid tumor patients in routine clinical practice and demonstrated that HRD as a single biomarker was not sufficient to predict efficacy of ICIs in solid tumor patients.

Background

After immune checkpoint inhibitors (ICIs) were introduced for treatment of solid tumors, they exhibited improved survival and treatment outcomes compared to traditional non-immune anti-cancer therapies, especially for patients with advanced melanoma, non-small-cell lung cancer (NSCLC), urothelial cancer (UC), renal cell carcinoma (RCC), or other cancer types [1–7]. However, only some patients achieved a response to ICIs. This indicates the need for further development of immune-relevant biomarkers to identify patients who might benefit from immunotherapy.

The DNA damage repair (DDR) system is essential to maintain the integrity of the genome in organisms. Genomic alteration due to failure to repair DDR causes tumor initiation. The homologous recombination (HR) pathway has a substantial influence on genomic integrity and germline mutations in this pathway and is related to tumorigenesis [8–10]. HR is one of the major repair mechanisms of DNA double-strand breaks. Homologous recombination deficiency (HRD) is a DNA repair deficiency related to tumorigenesis and causes increased sensitivity to platinum-based chemotherapy and PARP inhibitors [11]. The concept of therapy-directed HRD is approved in ovarian and breast cancers. The mutation in the HR pathway

related to *BRCA1/2* was used to predict better objective response rates to platinum-based chemotherapy in advanced triple-negative breast cancer [12].

Recently, targeted cancer gene panel assay or NGS for HRD have been performed in clinical settings. These panels assess genomic profiles including Tumor Mutational Burden (TMB), Microsatellite Instability (MSI), and HRD. To date, the clinical significance of gene mutations related to HRD has not been studied well across various solid tumors. Herein, we analyzed the status of HRD using cancer panels for various solid tumor patients in routine clinical practice and determined the value of HRD as a biomarker of response to ICIs.

Methods

Patients

Patients with pathologic confirmation of advanced gastrointestinal, GU, or rare cancers at Samsung Medical Center between Oct 2019 and Mar 2020 (n = 501), were prospectively tested for molecular aberrations, including TMB, with the TruSight™ Oncology 500 assay. All study participants provided written informed consent before study entry. The following clinicopathologic characteristics were collected for all patients: age, sex, primary tumor site, number of metastatic sites, site of metastasis, treatment, and survival. The study protocol was approved (#2020-11-151) by the Institutional Review Board of Samsung Medical Center (Seoul, Korea) and was conducted in accordance with the ethical principles of the Declaration of Helsinki and the Korea Good Clinical Practice guidelines. All patients provided written informed consent before enrollment. Written informed consent included disclosure of information, competency to make a decision, and voluntary nature of the decision for the purpose, benefit, and potential risk of this study.

Tumor samples

Samples for analysis were collected from 501 solid tumors and prepared as formalin-fixed paraffin-embedded (FFPE) material. The samples were gathered through biopsy at diagnosis, surgical specimen, or repeat biopsy at the time of disease progression; all were obtained before immunotherapy. The types of samples used in the analysis were as follows: biopsied samples (n = 320, 63.9%) and surgically resected samples (n = 181, 36.1%).

TruSight™ oncology 500assay

Forty (40) ng of DNA were quantified with the Qubit dsDNA HS Assay (Thermo Fisher Scientific) on the Qubit 2.0 Fluorometer (Thermo Fisher Scientific) and then sheared using a Covaris E220 Focused-ultrasonicator (Woburn, MA, USA) and the 8 microTUBE–50 Strip AFA Fiber V2 following the manufacturer's instructions. Treatment time was optimized for FFPE material. The treatment settings were as follows: peak incident power (W): 75; duty factor: 15%; cycles per burst: 500; treatment time (s): 360; temperature (°C): 7; and water level: 6. For DNA library preparation and enrichment, the TruSight™

Oncology 500 Kit (Illumina) was used following the manufacturer's instructions. Post-enriched libraries were quantified, pooled, and sequenced on a NextSeq 500 (Illumina Inc., San Diego, CA, USA). The quality of the NextSeq 500 (Illumina) sequencing runs was assessed with the Illumina Sequencing Analysis Viewer (Illumina). Sequencing data were analyzed with the TruSight™ Oncology 500 Local App Version 1.3.0.39 (Illumina), a comprehensive tumor profiling assay designed to identify known and emerging tumor biomarkers, including small variants, splice variants, and fusions. Importantly, the TruSight™ Oncology 500 measures homologous recombination deficiency (HRD). The HRD-related genes were as follows: *ARID1A, ATM, ATRX, BAP1, BARD1, BLM, BRCA1, BRCA2, BRIP1, CHEK1, CHEK2, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCL, MRE11A, NBN, PALB2, PTEN, RAD50, RAD51*, and *RAD51B.* Homologous recombination deficiency was diagnosed if there was at least one HR-related gene mutation.

Statistical analyses and disease evaluation

Descriptive statistics are reported as proportion and median. Data are presented as number (%) for categorical variables. Correlations between status of HRD and clinicopathologic features were analyzed by t-test, Fisher's exact test, or one-way analysis of variance (ANOVA), as appropriate. Response categories were assessed according to RECIST 1.1. A Cox regression model was used to analyze the associations of suspecting factors, including HRD and disease progression after ICI treatment. The Mann-Whitney test was used to compare the difference between HRD and non-HRD. Kaplan-Meier estimates and log rank tests were used in analysis of all time to event variables, and 95% confidence interval for the median time to event was computed.

Results

Patient characteristics

Table 1 presents the clinical characteristics of the 501 patients included in this study. The median age of the patients was 59.7 years (range, 21–86), and the majority were male (60.3%). The median age of males was 61 years, while that of female was 58 years. The most frequent tumor type was colorectal cancer (n = 151, 30.1%), followed by gastric cancer (n = 116, 23.2%), sarcoma (n = 60, 12.0%), pancreatic cancer (n = 42, 8.4%), genitourinary (GU) cancer (n = 25, 5.0%), other gastrointestinal (GI) tract cancer (n = 22, 4.4%), melanoma (n = 21, 4.2%), hepatocellular carcinoma (HCC) (n = 12, 2.4%), and rare cancer (n = 4, 0.8%). Among the 501 patients, 65 had been treated with immune checkpoint inhibitors (ICIs). Figure 1 shows the distribution of TMB, MSI, and HR deficiencies. All seven patients with MSI were TMB-high and HR-deficient. On the other hand, of 375 patients with HR deficiency, only 54 were confirmed to be TMB-high or MSI.

Table 1
Characteristics of 501 patients with various solid tumors

	All patients (N = 501)
Age (yr)	
Median (range)	59.7 (21–86)
Sex	
Male	302 (60.3%)
Female	199 (39.7%)
Tumor type	
Colorectal cancer	151
Gastric cancer	13
Sarcoma	60
Biliary tract cancer	46
Pancreatic cancer	42
Genitourinary cancer	25
Other GI tract cancer	22
Melanoma	21
Hepatocellular carcinoma	12
Rare cancer	4
TMB	
TMB low	443 (88.4%)
TMB high	58 (11.6%)
Microsatellite	
Instability	
Non-MSI	494 (98.6)
MSI	7 (1.4)
PD-L1	
Positive	101 (20.2)
Negative	124 (24.8)

	All patients (N = 501)
HR	
Deficiency	375 (74.9%)
Non-deficiency	126 (25.1%)
Receiving ICIs	
Yes	65 (13.0%)
No	436 (87.0%)

Frequency of tumors with HRD according to type

Tumors with HRD were observed in 375 of 501 patients irrespective of type. Table 2 presents the status of the HRD and the ratio of patients who received ICI treatment according to tumor type. HR deficiency was observed in 74.9% of patients with various solid tumors including GU cancer (92.0%, 23 of 25), CRC (86.1%, 130 of 151), HCC (83.3 %, 10 of 12), pancreatic cancer (76.2%, 32 of 42), biliary tract cancer (75.0%, 36 of 48), gastric cancer (69.0%, 80 of 116), sarcoma (65.0%, 39 of 60), melanoma (57.1%, 12 of 21), other GI tract cancer (AOV cancer, appendiceal cancer, cecal cancer, duodenal cancer, GIST) (50.0%, 11 of 22), and rare cancer (50.0%, 2 of 4). Figure 1 presents the distribution relationship with other biomarkers. All MSI were TMB high and HR deficiency. However, some TMB high have no HR deficiency. Figure 2 shows the percentage of confirmed HRD for each tumor type listed in order of high frequency rate. The tumor with the highest frequency of HRD was GU cancer with 92.0% and the lowest frequency was other GI tract cancer (AOV cancer, appendiceal cancer, cecal cancer, duodenal cancer, and GIST) and rare cancer at 50.0%.

Table 2
Prevalence of HRD and use of ICIs according to tumor type

Tumor type	HR deficiency	ICIs
Colorectal cancer (151)	130 (86.1%)	4 (2.6%)
Gastric cancer (116)	80 (69.0%)	14 (12.1%)
Sarcoma (60)	39 (65.0%)	3 (5.0%)
Biliary tract cancer (48)	36 (75.0%)	5 (10.4%)
Pancreatic cancer (42)	32 (76.2%)	1 (2.4%)
Genitourinary cancer (25)	23 (92.0%)	12 (48.0%)
Other GI tract cancer[a] (22)	11 (50.0%)	1 (4.5%)
Melanoma (21)	12 (57.1%)	20 (95.2%)
Hepatocellular carcinoma (12)	10 (83.3%)	4 (33.3%)
Rare cancer[b] (4)	2 (50.0%)	1 (25.0%)
Total 501	375 (74.9%)	65 (13.0%)
[a] AOV cancer, appendiceal cancer, cecal cancer, duodenal cancer, and GIST		
[b] Adrenocortical cancer and MUO (malignancy of unknown primary)		

Correlations between HRD and disease progression in 65 patients treated with ICIs

Sixty-five patients treated with ICIs ad diagnoses as follows: melanoma (95.2%, 20 of 21), HCC (33.3%, 4 of 12), rare cancer (25.0%, 1 of 4), GC (12.2%, 14 of 116), BTC (10.4%, 5 of 48), and sarcoma (5.0%, 3 of 60) (Table 2). We analyzed the correlation between HRD and efficacy to ICIs. Patients with HRD exhibited an objective response rate (ORR) of 27.3% (3 of 11), while patients without HRD achieved an ORR of 39.0% (16 of 41).

Progression-free survival (PFS) after ICIs was 6.5 months (95% CI: 0.000–16.175) in patients without HRD and 4.1 months (95% CI: 2.062–6.138) in patients with HRD. This difference was not significant (p = 0.441) (Fig. 3).

Additionally, we conducted univariate and multivariate analyses for PFS after ICIs (Table 3). Univariate analysis revealed that smoking, HRD, and tumor mutational burden (TMB) were significant prognostic factors for PFS after ICIs. However, in multivariate analysis, TMB was the only meaningful prognostic factor.

Table 3
Univariate and multivariate analyses for PFS

Variable	Cases	Univariate	
		OR (95.0% CI)	P
Age			
< 65	37		
≥ 65	28	0.618 (0.319–1.199)	0.154
Sex			
Male	39		
Female	26	2.765 (1.225–7.574)	0.051
Smoking			
No	38		
Yes	27	2.167 (0.760–6.174)	0.148
HRD			
0 (non-deficiency)	12		
1 (deficiency)	53	2.617 (1.095–6.254)	0.030
TMB			
Low	50		
High	15	0.507 (0.198–1.294)	0.155
Microsatellite instability			
Non-MSI	61		
MSI	4	0.659 (0.122–3.550)	0.627
PD-L1 by IHC			
Negative	16		
Positive	15	0.493 (0.205–1.185)	0.114
Not available	34	0.720 (0.351–1.478)	0.370
Variable	Cases	Multivariate	
		OR (95.0% CI)	*P*

CI Confidence interval, *OR* Odds ratio

Variable	Cases	Univariate	
		OR (95.0% CI)	P
Smoking			
No	38		
Yes	27	2.321 (0.835–6.455)	0.107
HRD			
0 (non-deficiency)	12		
1 (deficiency)	53	2.104 (0.930–4.759)	0.074
TMB			
Low	50	0.349 (0.157–0.773)	0.010
High	15		

CI Confidence interval, *OR* Odds ratio

Discussion

In the present study, we investigated whether HRD is a useful biomarker to predict response to ICIs. In addition, we assessed the status of HRD using cancer panels for various solid tumor patients in routine clinical practice. In 501 patients with various solid tumors, HRD was observed as follows: 74.7% (347/501) patients; GU cancer (92.0%, 23 of 25), colorectal cancer (CRC) (86.1%, 130 of 151), hepatocellular carcinoma (HCC) (83.3%, 10 of 12), pancreatic cancer (PC) (76.2%, 32 of 42), biliary tract cancer (BTC) (75.0%, 36 of 48), sarcoma (65.0%, 39 of 60), melanoma (52.4%, 11 of 21), other GI cancer (50.0%, 11 of 22), and rare cancer (50.0%, 2 of 4). In 65 patients treated with ICIs, there were no significant differences for ORR and PFS between patients with and without HRD ($p = 0.967$ and $p = 0.441$, respectively). These findings suggest that HRD as a single biomarker is not sufficient to predict the efficacy of ICIs in solid tumor patients.

We analyzed HRD in 501 tumor samples using the NGS panel. The overall frequency of HRD was 74.7% (347/501). This finding was not consistent with other studies about HRD. A previous study reported that the prevalence of HR-DDR mutations was 17.4% in multiple tumor types [13]. This discordance might be caused by different studied genes including different NGS panels and different genes defining HRD. Detection of HRD by the NGS panel has limitations. There is no established definition to assess HRD. Therefore, there are many different results among published papers about the prevalence of HRD. Furthermore, this difference might be caused by discrepancy between measurement of HRD with whole exome sequencing and NGS panels.

There are a few limitations to this study. First, it was a retrospective study, and clinically heterogeneous populations were subject to potential biases. Second, only the Asian population was assessed in the study, so differences in genomic profiles and clinical features between Western and Eastern patients with solid tumors were not considered. Last, this study included a relatively small proportion of patients who had been treated with ICIs, making it difficult to draw definite conclusions regarding biomarkers.

HRD is a potential candidate predictor of response to ICIs, but the prevalence of HRD has not been investigated across tumor types. The present analysis produced useful information on the prevalence of HRD in various solid tumors under routine clinical practice and demonstrated that HRD as a single biomarker was not sufficient to predict the efficacy of ICIs in solid tumor patients.

Conclusion

Herein, we reported the status of HRD using a cancer-panel for various solid tumor patients in routine clinical practice and demonstrated that HRD as a single biomarker was not sufficient to predict efficacy of ICIs in solid tumor patients.

Abbreviations

BTC: Biliary tract cancer

CRC: Colorectal cancer

DDR: DNA damage repair

FFPE: Formalin-fixed paraffin-embedded

GU: Genitourinary

HCC: Hepatocellular carcinoma

HRD: Homologous recombination deficiency

ICIs: Immune checkpoint inhibitors

MSI: Microsatellite instability

NGS: PC: Pancreatic cancer Next generation sequencing

ORR: Objective response rate

PFS: Progression free survival

RCC: Renal cell carcinoma

TMB: Tumor mutational burden

Declarations

Availability of data and materials

All data that can prove the conclusion of this article are included in the article.

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Ethics approval and consent to participate

All patients provided written informed consent before enrollment and this study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the Korea Good Clinical Practice guidelines.

Consent for publication

All authors agree to the publication of this work titked “ Homologlous recombination deficiency as a novel therapeutinc target in varius solid tumors” to *Journal of Experimental & Clinical Cancer Research.*

Competing interests

The authors have declared that no competing interest exists.

Contributions

HK and STK designed the concept of the study. HSK, JYH, JL, SHP, JOP, YSP, HYL, WKK were respnsible for data collection and analysis. KMK was reponsible for the pathological review. HK wrote the manuscript and HK and STK revised the manuscript. All authors read and approved the final manuscript

Disclosure

The authors have no conflicts of interest to declare.

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Figures

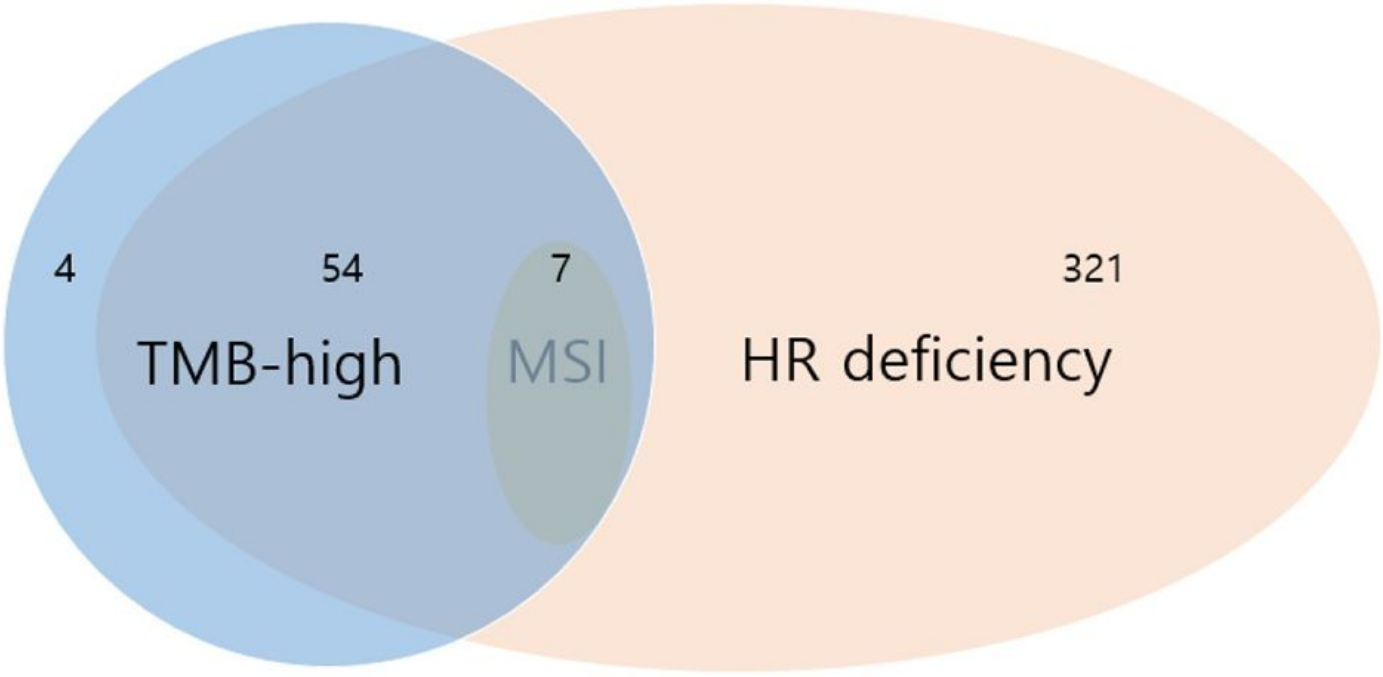

Figure 1

Distributions of TMB-high, MSI-high, and HRD

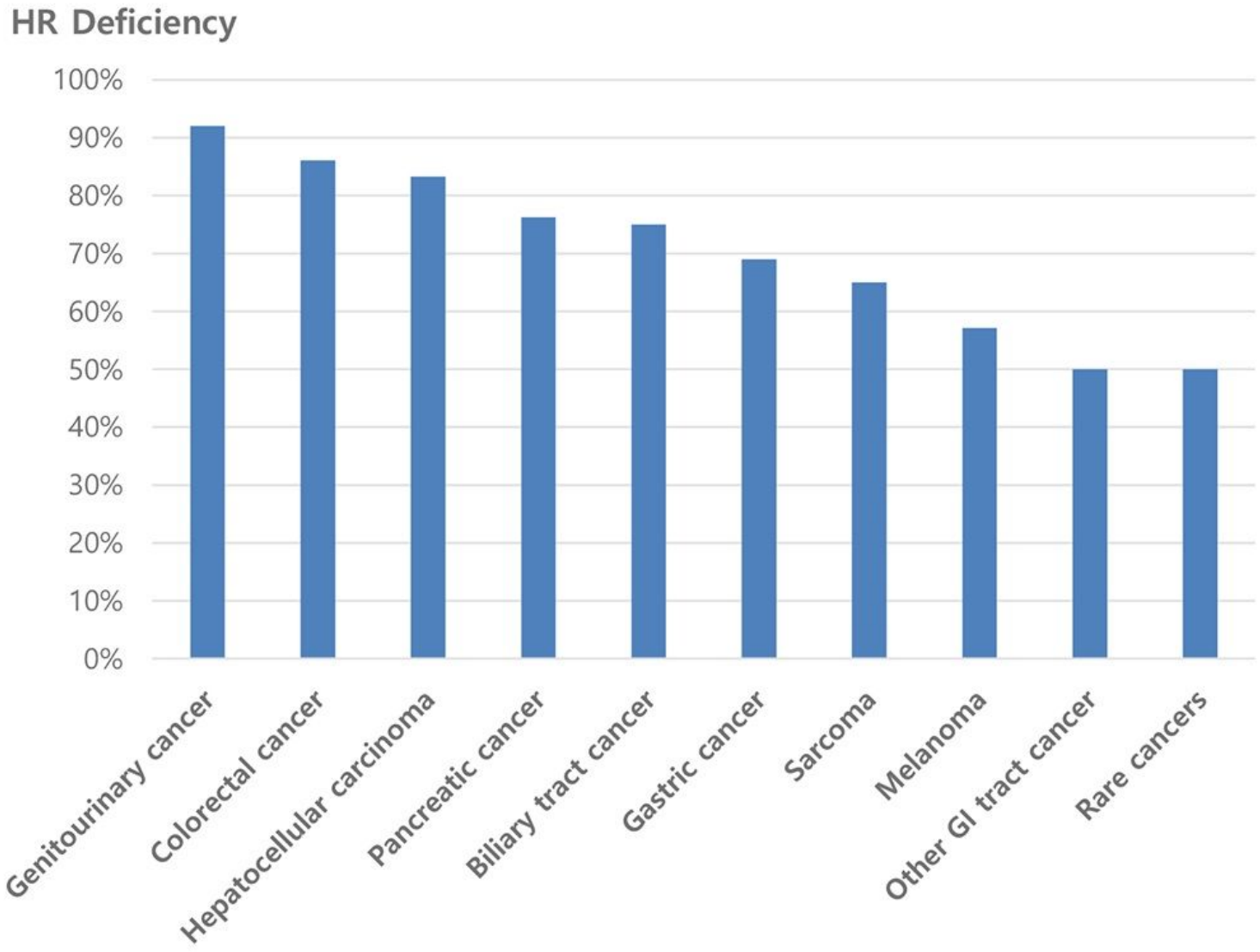

Figure 2

Prevalence of homologous recombination deficiency (HRD) in solid tumors

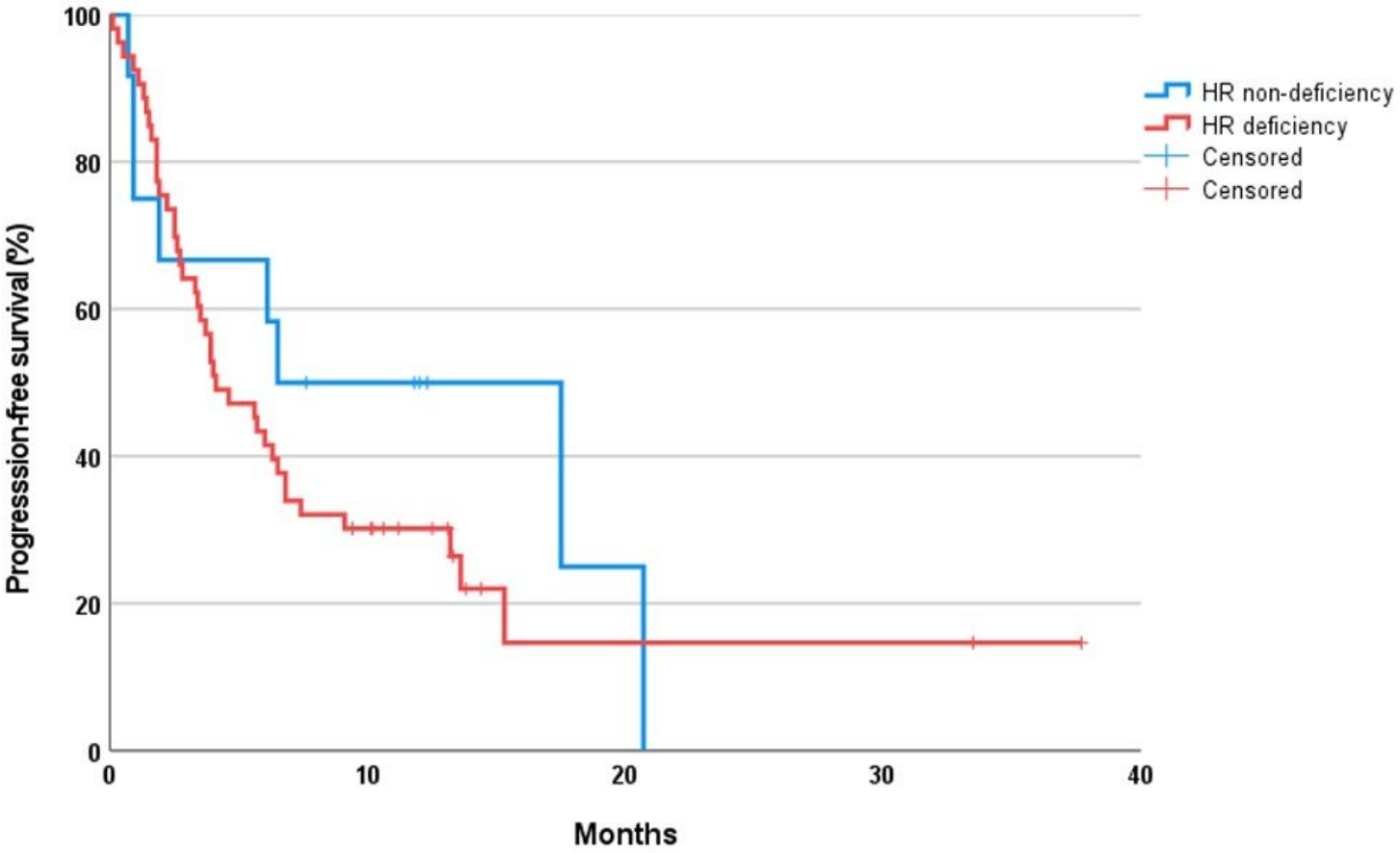

Figure 3

Progression-free survival (PFS) after ICIs according to HRD status (n = 65)