original report

Prevalence of Homologous Recombination–Related Gene Mutations Across Multiple Cancer Types

abstract

Purpose The prevalence of homologous recombination DNA damage repair (HR-DDR) deficiencies among all tumor lineages is not well characterized. Therapy directed toward homologous recombination DDR deficiency (HRD) is now approved in ovarian and breast cancer, and there may be additional opportunities for benefit for patients with other cancers. Comprehensive evaluations for HRD are limited in part by the lack of a uniform, cost-effective method for testing and defining HRD.

Methods Molecular profiles of 52,426 tumors were reviewed to identify pathogenic mutations in the HR-DDR genes *ARID1A*, *ATM*, *ATRX*, *BAP1*, *BARD1*, *BLM*, *BRCA1/2*, *BRIP1*, *CHEK1/2*, *FANCA/C/D2/E/F/G/L*, *MRE11A*, *NBN*, *PALB2*, *RAD50*, *RAD51*, *RAD51B*, or *WRN*. From solid tumors submitted to Caris Life Sciences, molecular profiles were generated using next-generation sequencing (NGS; average read depth, 500×). A total of 17,566 tumors were sequenced with NGS600 (n = 592 genes), and 34,860 tumors underwent hotspot Illumina MiSeq platform testing (n = 47 genes).

Results Of the tumors that underwent NGS600 testing, the overall frequency of HR-DDR mutations detected was 17.4%, and the most commonly mutated lineages were endometrial (34.4%; n = 1,475), biliary tract (28.9%; n = 343), bladder (23.9%; n = 201), hepatocellular (20.9%; n = 115), gastroesophageal (20.8%; n = 619), and ovarian (20.0%; n = 2,489). Least commonly mutated lineages included GI stromal (3.7%; n = 108), head and neck (6.8%; n = 206), and sarcoma (9.3%; n = 592). *ARID1A* was the most commonly mutated gene (7.2%), followed by *BRCA2* (3.0%), *BRCA1* (2.8%), *ATM* (1.3%), *ATRX* (1.3%), and *CHEK2* (1.3%).

Conclusions HR-DDR mutations were seen in 17.4% of tumors across 21 cancer lineages, providing a path to explore the role of HRD-directed therapies, including poly-ADP ribose polymerase inhibitors, DNA-damaging chemotherapies, and newer agents such as ATR inhibitors.

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INTRODUCTION

In the 1990s, *BRCA1* and *BRCA2* were demonstrated to encode genes that play a key role in homologous recombination DNA damage repair (HR-DDR) and together are considered the gatekeepers of genomic integrity. Germline mutations in one or both of these genes place patients at heightened risk for development of breast,[1-6] ovarian,[1-6] prostate,[7-9] melanoma,[7,10] and pancreatic cancers[7,10-12] during their lifetime. It has become apparent that BRCA interacts with a number of other DNA repair proteins to form a complex system for DDR, including ATM, RAD51, PALB2, MRE11, RAD50, NBN, and the Fanconi anemia proteins.[13,14] Recent evidence suggests mutations in *PALB2*, *ATM*, and the genes responsible for the MRN complex, *RAD50*, *MRE11*, and *NBN*, play a role in hereditary cancers.[15,16] For example, *PALB2* mutation carriers have a lifetime risk of breast cancer development of approximately 50%,[17,18] and *ATM* mutation carriers are at higher risk for development of breast,[19,20] pancreatic,[21,22] and prostate cancers.[23,24]

Homologous recombination (HR) pathway mutations can also predict response to anticancer therapies. In germline *BRCA1/2* mutation carriers, exposure to platinum chemotherapy led to improved objective response rates in advanced triple-negative breast cancer versus taxanes (68% *v* 33%),[25] and overall survival in pancreatic cancer versus other nonplatinum chemotherapy (22 months *v* 9 months).[26] MyChoice HR-DDR deficiency (HRD) score-high triple-negative breast cancer responded better to platinum-based neoadjuvant therapy, with pathologic complete response (CR) rates of 27.5% versus 0% in the HR-DDR–proficient cohort.[27] The MyChoice HRD score is frequently used to identify patients with HRD. It is a proprietary diagnostic test to assess a HRD phenotype, including an evaluation of loss of heterozygosity, telomeric allelic imbalance, and large-scale state transitions.

On exposure to another class of DNA-damaging agents, poly-ADP ribose polymerase (PARP) inhibitors, patients with germline or somatic deleterious mutations in the HR-DDR pathway have also achieved favorable responses. Olaparib is now approved by the US Food and Drug Administration (FDA) for patients with ovarian cancer with germline *BRCA1* or *BRCA2* mutations in the advanced setting, after the results of a phase II clinical trial demonstrated a response rate of 34% with a median duration of response of 7.9 months,[28] as well as for recurrent ovarian cancer as maintenance therapy, on the basis of the results of the SOLO-2 and Study 19 trials demonstrating an improvement in progression-free survival (PFS) of 19.1 months in patients with germline mutated *BRCA* versus 5.5 months with placebo,[29] and 8.4 months versus 4.8 months regardless of *BRCA* mutation status.[30] In advanced breast cancer, patients with germline *BRCA* mutations were recently found to achieve superior PFS when treated with olaparib versus standard of care therapy (7.0 months *v* 4.2 months) in the phase III Olympiad (Assessment of the Efficacy and Safety of Olaparib Monotherapy Versus Physicians Choice Chemotherapy in the Treatment of Metastatic Breast Cancer Patients With Germline *BRCA1/2* Mutations) trial, leading to FDA approval of olaparib for this indication in January 2018.[31] Rucaparib, another PARP inhibitor, has also been approved for treatment of patients with advanced ovarian cancer with germline or somatic *BRCA1/2* mutations, on the basis of the combined analysis of the Study 10 and ARIEL2 phase II trials that showed an objective response rate of 54% and a median duration of response of 9.2 months with monotherapy.[32,33] In addition, in patients with recurrent ovarian cancer treated with maintenance niraparib, prolonged PFS was seen not only in the germline *BRCA1/2* mutation cohort (21.0 months *v* 5.5 months) but also in the nongermline *BRCA1/2* mutation cohort with high MyChoice HRD scores (12.9 months *v* 3.8 months),[34] leading to FDA approval of niraparib as maintenance treatment.

Looking more broadly at PARP inhibitor therapy responsiveness across multiple mutations within the HR-DDR pathway, in a study by Mateo et al,[35] patients with advanced prostate cancer with germline or somatic HRD have achieved an 88% response rate with olaparib monotherapy (HRD identified in 16 of 49 patients), compared with 33% in the overall cohort. In this study, three patients had germline *BRCA2* mutations, three patients had germline *ATM* mutations, and the remaining responders had tumor expression of a deleterious mutation (including *PALB2*, *BRCA2*, *BRCA1*, *CHEK2*, *FANCA*, and *ATM*).[35] All germline mutation carriers except for one patient with *ATM* mutation responded to therapy.

Despite the exciting therapeutic potential of DNA-damaging agents in patients with broader evidence of HRD, the prevalence of HRD among all tumors is largely unknown. Comprehensive evaluations of solid tumors for HRD have been limited by the lack of a uniform method for testing and defining HRD. Furthermore, thorough testing with whole-exome sequencing is expensive, making large-scale evaluations impractical. The aim of our study was to determine the prevalence of HR-DDR pathogenic or presumed pathogenic mutations detected on tumor next-generation sequencing (NGS) testing across multiple cancer lineages, using commercially available DNA sequencing (NGS or Sanger sequencing panel testing, multiplatform profiling; Caris Life Sciences [Caris], Irving, TX) to better define the proportion of patients who may benefit from such therapy.

METHODS

Study Design

Approval for this study was obtained from the Georgetown University Institutional Review Board. In collaboration with Caris, we surveyed

their entire DNA sequencing database for solid tumors that underwent extended NGS or Sanger sequencing panel testing between July 2013 and September 2017. Tumor biopsy specimens were submitted to Caris from across the world. We defined HRD on tumor NGS testing as a mutation in the following genes, each of which has some activity within the HR-DDR pathway[36-45] and has been included previously in HR-DDR biomarker clinical trials: *ARID1A*, *ATM*, *ATRX*, *BAP1*, *BARD1*, *BLM*, *BRCA1/2*, *BRIP1*, *CHEK1/2*, *FANCA/C/D2/E/F/G/L*, *MRE11A*, *NBN*, *PALB2*, *RAD50*, *RAD51*, *RAD51B*, or *WRN*. Each of these genes is evaluated as part of the targeted NGS platform offered by Caris. Only tumor tissue was sequenced and was not supplemented by germline testing. Frequencies of each mutation were determined for the total cohort, as well as for each cancer lineage (biliary tract, bladder, breast, cervix, colorectal [CRC], endometrial, gastroesophageal [GE], gastrointestinal stromal [GIST], glioma, head and neck, hepatocellular [HCC], melanoma, neuroendocrine/small cell lung, non–small-cell lung [NSCLC], ovarian, pancreas, prostate, renal, sarcoma, thyroid, and unknown primary).

NGS Testing Platforms

HR-DDR mutation analysis from solid tumor biopsy specimens was determined by NGS at Caris, a Clinical Laboratory Improvement Amendments–certified laboratory. DNA was extracted, purified, and quantified from formalin-fixed, paraffin-embedded solid tumor specimens according to regulated processes at Caris. For NGS600, a custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies, Santa Clara, CA). While 17,566 tumors were sequenced with NGS600, 34,860 tumors underwent hotspot Illumina MiSeq platform testing (Illumina TruSeq Amplicon Cancer Hotspot panel, evaluating 47 genes including the HR-DDR genes *ATM*, *BRCA1*, and *BRCA2*; Illumina, San Diego, CA). All

Table 1. Next-Generation Sequencing Testing by Lineage

	Total No.				No. (NGS600 only)			
Cancer Types	**All**	**Primary**	**Metastatic**	**Unknown**	**All**	**Primary**	**Metastatic**	**Unknown**
Ovarian	9,630	3,459	5,033	1,138	2,489	989	1,500	0
NSCLC	8,119	4,375	3,032	712	3,245	1,855	1,390	0
CRC	6,650	3,328	2,737	585	2,454	1,296	1,158	0
Breast	5,910	2,525	2,709	676	1,625	703	921	1
Endometrial	5,540	3,101	1,895	544	1,475	877	598	0
Pancreas	2,162	901	1,038	223	833	378	455	0
Melanoma	1,889	596	1,029	264	670	203	467	0
Glioma	1,830	1,670	12	148	854	850	4	0
Sarcoma	1,778	1,044	527	207	592	420	167	5
Gastroesophageal	1,532	1,007	461	64	619	421	198	0
Unknown primary	1,531	313	1,012	206	488	158	327	3
Neuroendocrine/SCLC	1,498	568	723	207	449	186	262	1
Biliary tract cancer	870	507	298	65	343	218	125	0
Cervix	824	392	344	88	227	125	102	0
Prostate	687	279	362	46	312	133	179	0
Head and neck	684	322	266	96	206	102	104	0
Hepatocellular carcinoma	328	194	99	35	115	71	44	0
Bladder	283	172	111	0	201	115	86	0
Renal	251	136	115	0	199	112	87	0
GIST	226	122	68	36	108	76	31	1
Thyroid	204	91	85	28	62	32	30	0
Total	52,426				17,566			

Abbreviations: CRC, colorectal cancer; GIST, GI stromal; NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.

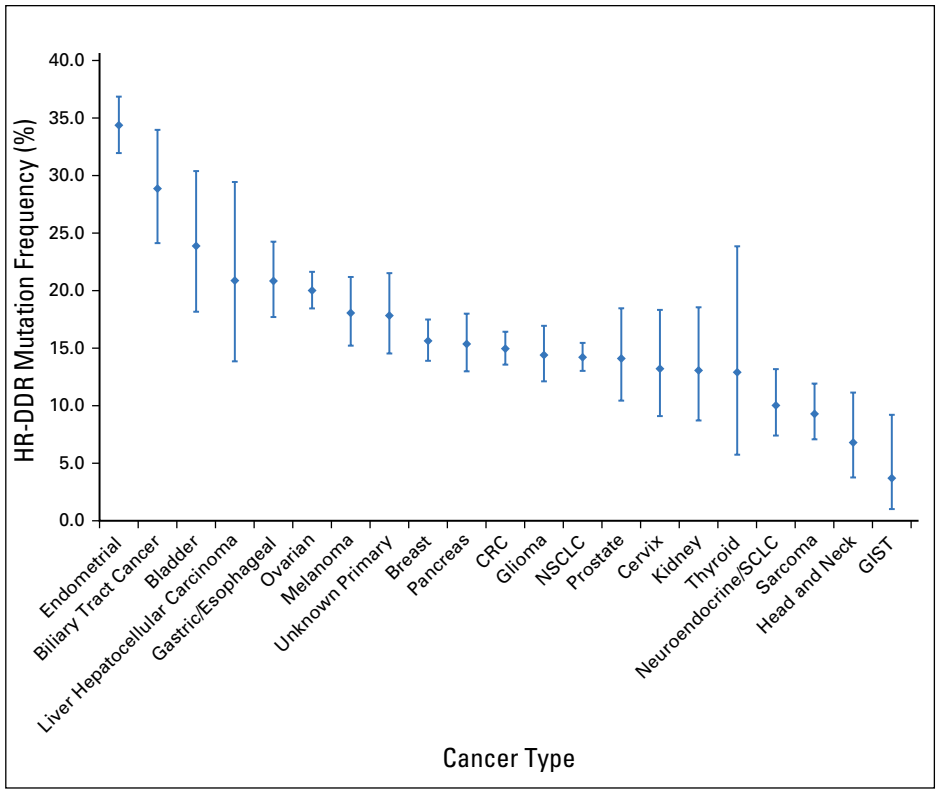

Fig 1. Total HR-DDR mutation frequency by lineage, NGS600 testing platform only (N = 17,566). Bars represent the upper and lower 95% CIs. CRC, colorectal cancer; GIST, GI stromal; HR-DDR, homologous recombination DNA damage repair; NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.

variants were detected with > 99% confidence on the basis of allele frequency and amplicon coverage, with an average sequencing depth of coverage of > 500 and with an analytic sensitivity of 5%. Tumor enrichment was achieved by harvesting targeted tissue by manual microdissection performed on all cases before molecular testing.

The pathogenicity of gene variants identified were interpreted by board-certified molecular geneticists and categorized as pathogenic, presumed pathogenic, variant of unknown significance, presumed benign, or benign, according to American College of Medical Genetics and Genomics standards on the basis of the level of evidence of published studies on the identified variants.[46,47] Only pathogenic or presumed pathogenic mutations were considered deleterious; variants of unknown significance and variants that have not been previously reported in individuals affected by cancer in the literature were excluded. Variants that have not been interpreted by a molecular geneticist were excluded. Deleterious mutations reported included frameshift mutations, premature stop codons, mutations shown to disrupt natural splicing, as well as point mutations; deleterious mutations included those that have and have not been reported as causal for hereditary cancers.

Statistical Analysis

The proportion of pathogenic or presumed pathogenic mutations identified from all tumor specimens tested for each specific mutation were calculated for the total cohort and for each cancer lineage investigated. Sequencing tests with indeterminate results due to low depth of coverage were excluded from the total number for percentage calculation. The total frequency of HR-DDR mutations in the complete cohort and per cancer lineage was calculated by dividing the number of tumors carrying at least one mutation by the total number of tumors tested, to avoid counting tumors carrying more than one HR-DDR mutation multiple times. The 95% CIs were computed using the Pearson-Klopper exact method using R (https://www.r-project.org/), and the graphics were generated by SPSS Statistics, version 24 (IBM, Armonk, NY).

RESULTS

We evaluated 52,426 solid tumor pathologic specimens that underwent extended NGS for HRD. The most common malignancies tested were ovarian (n = 9,630), NSCLC (n = 8,119), and CRC (n = 6,650), but substantial numbers of less common malignancies were also tested

Table 2. Homologous Recombination DNA Damage Repair Mutation Landscape by Lineage, NGS600 Testing Platform Only (N = 17,566): HRD Frequency ≥ 15%

HRD Frequency ≥ 15%, Gene	Cancer Type										
	Endometrial	Biliary Tract	Bladder	HCC	GE	Ovarian	Melanoma	Unknown Primary	Breast	Pancreas	CRC
Overall HRD, % (95% CI)	34.4 (31.9 to 36.9	28.9 (24.1 to 34.0)	23.9 (18.2 to 30.4)	20.9 (13.9 to 29.4)	20.8 (17.7 to 24.3)	20.0 (18.5 to 21.6)	18.1 (15.2 to 21.2)	17.8 (14.5 to 21.5)	15.6 13.9 to 17.5)	15.4 (13.0 to 18.0)	15.0 (13.6 to 16.4)
ARID1A	27.45	14.33	12.44	11.3	13.43	6.40	1.65	7.80	3.70	5.54	6.69
ATM	4.61	4.08	3.98	0.87	3.23	1.53	3.74	3.48	2.09	3.60	4.57
ATRX	3.13	0.29	0	0	0.32	0.16	1.80	0.82	0.49	0	0.73
BAP1	0.47	7.58	0.50	3.48	1.45	0.20	7.76	3.28	1.05	0.48	0.33
BLM	0.20	0	0	0	0.16	0	0.30	0.21	0.12	0	0.37
BRCA1	1.29	0.29	2.99	0	0.48	7.70	0.75	0.82	3.06	1.41	1.06
BRCA2	3.05	2.33	4.48	0	2.91	5.88	1.20	1.64	3.72	3.33	2.20
BRIP1	0.14	0	0.50	0	0.32	0.28	0.30	0	0.19	0.48	0.16
CHEK2	2.24	2.33	1.49	4.35	0.97	0.64	1.34	0.61	1.60	0.60	1.30
FANCC	0.07	0.29	0	0.87	0	0.12	0	0	0.12	0	0.12
MRE11A	0.34	0	0	0	0.16	0	0	0.21	0	0	0.29
NBN	0.75	0.29	0	0	1.13	0.28	0	0.21	0.06	0.12	0.69
PALB2	0.41	1.17	1.49	0	0.81	0.16	0.30	1.03	1.05	1.20	0.69
RAD50	0.27	0.29	0.50	0.87	0.16	0.12	0	0	0	0	0.20
WRN	0.34	0.29	0	0	0.16	0.16	0.15	0.21	0.12	0.12	0.29

NOTE. Data given as % unless otherwise indicated.

Abbreviations: CRC, colorectal cancer; GE, gastroesophageal; HCC, hepatocellular carcinoma.

including melanoma (n = 1,889), sarcoma (n = 1,778), and glioma (n = 1,830; Table 1). Molecular profiling was performed on the primary tumor in 47.9% of cases (n = 25,102), and on a metastatic site of disease in 41.9% (n = 21,956). In 10.2% (n = 5,368), the tissue source was unknown. Of the tumors that underwent NGS600 testing, the most common malignancies tested were NSCLC (n = 3,245), ovarian (n = 2,489), and CRC (n = 2,454; Table 1).

HRD Frequency by Lineage

Evaluating results from the NGS600 platform alone, the cancer lineages with the highest frequencies of mutations in HR-DDR genes were endometrial (34.4%; 95% CI, 31.9 to 36.9; n = 1,475), biliary tract (28.9%; 95% CI, 24.1, 34.0; n = 343), bladder (23.9%; 95% CI, 18.2, 30.4; n = 201), hepatocellular (20.9%; 95% CI, 13.9, 29.4; n = 115), GE (20.8%; 95% CI, 17.7, 24.3; n = 619), and ovarian (20.0%; 95% CI, 18.5, 21.6; n = 2,489). Notable additional lineages with a significant proportion of tumors that tested positive for HR-DDR deficiency by NGS included melanoma (18.1%; 95% CI, 15.2, 21.2; n = 670), breast (15.6%; 95% CI, 13.9, 17.5; n = 1,625), pancreatic (15.4%; 95% CI, 13.0, 18.0; n = 833), and CRC (15.0%; 95% CI, 13.6, 16.4; n = 2,454). Least commonly mutated lineages included GIST (3.7%; 95% CI, 1.0, 9.2; n = 108), head and neck (6.8%; 95% CI, 3.8, 11.1; n = 206), and sarcoma (9.3%; 95% CI, 7.1, 11.9; n = 592; Fig 1). Within the tumor lineages, the frequencies of mutations varied (Tables 2 and 3; Fig 2).

HR Gene Mutation Frequency

Overall, pathogenic mutations within the homologous recombination pathway were seen in 17.4% of the 17,566 tumors tested with NGS600, and 8.3% of the 52,426 solid tumors overall (including 34,860 tumors that were evaluated for *ATM*, *BRCA1*, and *BRCA2* mutations only on the Hotspot panel). *ARID1A* was the most commonly mutated gene at 7.2%, followed by

Table 3. Homologous Recombination DNA Damage Repair Mutation Landscape by Lineage, NGS600 Testing Platform Only (N = 17,566): HRD Frequency < 15%

HRD Frequency < 15%, Gene	Cancer Type									
	Glioma	NSCLC	Prostate	Cervix	Renal	Thyroid	Neuroendo/ SCLC	Sarcoma	Head/ Neck	GIST
Overall HRD, % (95% CI)	14.4 (12.1 to 16.9)	14.2 (13.0 to 15.5)	14.1 (10.4 to 18.5)	13.2 (9.1 to 18.3)	13.1 (8.7 to 18.6)	12.9 (5.7 to 23.9)	10.0 (7.4 to 13.2)	9.3 (7.1 to 11.9)	6.8 (3.8 to 11.1)	3.7 (1.0 to 9.2)
ARID1A	1.52	4.61	0	4.85	4.52	0	4.04	1.01	2.43	0
ATM	1.76	3.48	4.50	1.32	1.51	3.23	1.34	1.35	0	0
ATRX	9.02	0.71	0	1.76	0	0	2.02	3.72	0	0.93
BAP1	0	0.77	0.32	2.64	7.04	0	0	0.51	0.49	0
BLM	0	0.15	0	0.44	0	0	0	0	0	0
BRCA1	0.35	0.83	1.42	0.44	0	1.61	0.67	1.18	2.43	0
BRCA2	0.23	2.03	6.76	2.64	0.50	3.23	0.89	0.68	0.97	0.93
BRIP1	0.47	0.19	0	0.44	0	0	0	0	0	0
CHEK2	1.17	1.23	1.92	1.32	0	3.23	1.11	1.18	0.49	1.85
FANCC	0	0.12	0.32	0	0.50	0	0.45	0.17	0	0
MRE11A	0	0.06	0	0	0	0	0	0	0	0
NBN	0.23	0.28	0.32	0	0	1.61	0	0	0	0
PALB2	0.23	0.65	0	0.44	0	0	0.22	0.17	0	0
RAD50	0	0	0.32	0	0	0	0.22	0	0	0
WRN	0.12	0.09	0	0	0	0	0	0	0	0

NOTE. Data given as % unless otherwise indicated.

Abbreviations: GIST, GI stromal; NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.

BRCA2 and *BRCA1*, which were mutated in 3.0% and 2.8% of tumors tested, respectively. *BRCA1/2* mutations were seen predominately in ovarian and breast cancers, though pathogenic *BRCA2* mutations were seen in high frequencies among GI and nonovarian genitourinary malignancies, as well. Although *PALB2* mutations were less common overall and appreciated in only 0.6% of tumors tested, a significant proportion of *PALB2* mutations was found in bladder, breast, and GI malignancies. *ATM*, *ATRX*, and *CHEK2* mutations were each identified in 1.3% of the tumors tested. No pathogenic mutations were identified in *BARD1*, *CHEK1*, *FANCA/D2/E/F/G/L*, *RAD51*, or *RAD51B* (Table 4).

HR-DDR gene mutations were appreciated in both primary and metastatic lesions, though with different patterns. When the primary tumor was evaluated, *ARID1A* (7.7% of primary tumors tested [n = 9,305] *v* 6.6% of metastatic tumors tested [n = 8,208]), *ATM* (1.5% [n = 25,050] *v* 1.3% [n = 21,902]), and *ATRX* (1.7% [n = 9,304] *v* 0.9% [n =8,204]) mutations were seen in higher frequencies than when a metastatic lesion was evaluated. Conversely, *BRCA1* (3.3% of metastatic tumors tested [n = 15,636] *v* 2.4% of primary tumors tested [n = 17,638]), *BRCA2* (3.4% [n = 15,636] *v* 2.7% [n = 17,638]), and *BAP1* (1.4% [n = 8,234] *v* 0.9% [n = 9,320]) mutations were more common in metastatic lesions. The remaining mutated HR-DDR genes (*BLM*, *BRIP1*, *CHEK2*, *FANCC*, *MRE11A*, *NBN*, *PALB2*, *RAD50*, and *WRN*) had similar frequencies among primary and metastatic tissue evaluations.

Tumors With Multiple HR Gene Mutations

Of the 17,566 tumors that underwent extended molecular profiling with the NGS600 platform, 362 were found to carry more than one HR-DDR pathway mutation, including 112 endometrial (7.6%; n = 1,475), 75 CRC (3.1%; n = 2,454), 34 ovarian (1.4%; n = 2,489), 29 NSCLC (0.9%; n = 3,245), 23 GE (3.7%; n = 619), 20 breast (1.2%; n = 1,625), and 15 biliary tract (4.4%; n = 343) tumors. Most lineages had

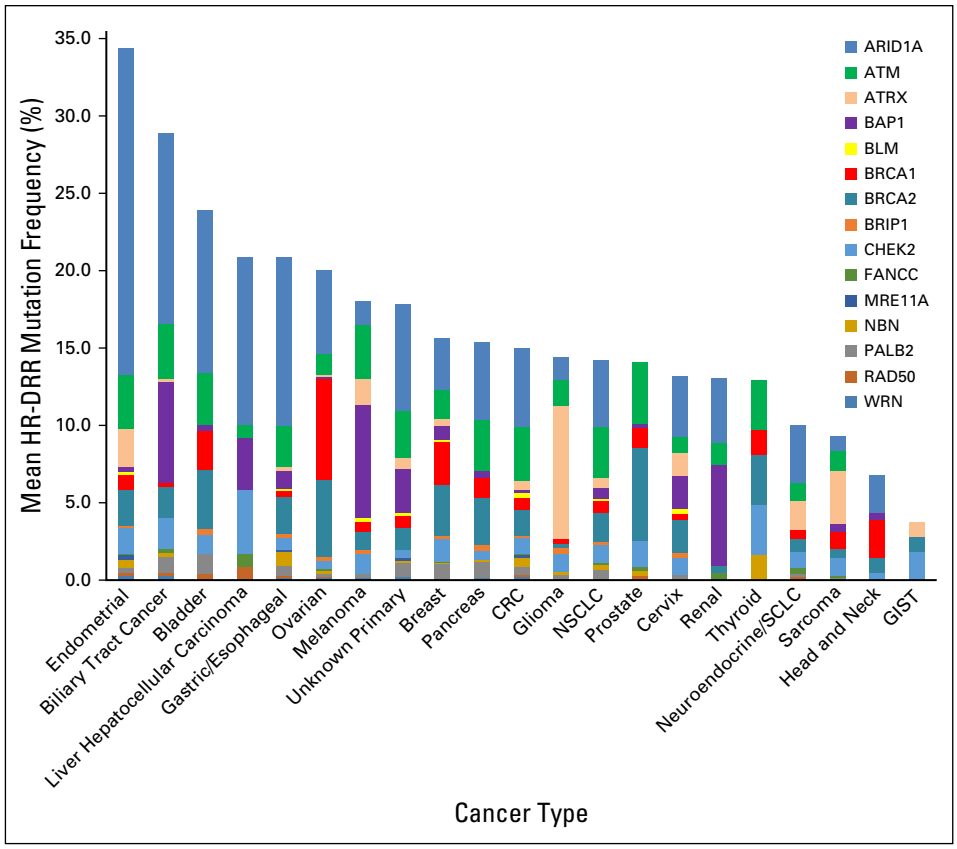

Fig 2. HR-DDR mutation landscape by lineage, NGS600 testing platform only (N = 17,566). Total bar height represents the overall frequency of HR-DDR–deficient tumors within each lineage; colored bar length represents the relative mutation frequency of individual genes in each cancer type. CRC, colorectal cancer; GIST, GI stromal; HR-DDR, homologous recombination DNA damage repair; NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.

at least one tumor with two or more HR-DDR pathway mutations, sparing GIST, head and neck, and thyroid cancers that did not have tumors with multiple HR gene mutations.

Table 4. Summary of Homologous Recombination DNA Damage Repair Mutations Identified*

Mutation	Overall, %	NGS600, %	Hotspot, %
ARID1A	7.2	7.2	N/A
BRCA2	3.0	2.7	3.3
BRCA1	2.8	2.0	3.7
ATM	1.3	3.0	0.4
ATRX	1.3	1.3	N/A
CHEK2	1.3	1.3	N/A
BAP1	1.1	1.1	N/A
PALB2	0.6	0.6	N/A
NBN	0.3	0.3	N/A
BRIP1	0.2	0.2	N/A
WRN	0.2	0.2	N/A
BLM	0.1	0.1	N/A
FANCC	0.1	0.1	N/A
RAD50	0.1	0.1	N/A
MRE11A	0.1	0.1	N/A

Abbreviation: N/A, not applicable.

*No mutations identified: *BARD1*, *CHEK1*, *FANCA/D2/E/F/G/L*, *RAD51*, *RAD51B*.

DISCUSSION

In this large-scale study of NGS molecular profiling of solid tumor samples across multiple cancer lineages, we confirmed HRD is common and was observed in 17.4% of the solid tumors evaluated by the NGS600 platform (spanning 21 cancer lineages). The most commonly mutated lineages included endometrial (34.4%), biliary tract (28.9%), bladder (23.9%), hepatocellular (20.9%), GE (20.8%), and ovarian (20.0%). Notable additional lineages with a significant proportion of tumors that tested positive for HRD by NGS included melanoma (18.1%), breast (15.6%), pancreatic (15.4%), and CRC (15.0%). The most commonly mutated HR-DDR genes included *ARID1A* (7.2%), *BRCA2* (3.0%), *BRCA1* (2.8%), *ATM* (1.3%), *ATRX* (1.3%), and *CHEK2* (1.3%). Additionally, 362 tumors of the 17,566 sequenced with the NGS600 platform harbored at least two HR-DDR pathway mutations. The clinical significance of multiple mutations is unknown.

Our study was a large assessment of HRD prevalence. It is also one of the few studies to assess HRD across multiple tumor types. Furthermore, we applied commercially available technology

Table 5. Published HRD Frequencies

Tumor Type	Testing Method	HR Mutations Evaluated	No.; Frequency, %
Pancreas[49]	Whole-exome sequencing, somatic	DNA repair genes including Fanconi anemia genes, *ATM*, *CHEK2*, *BRCA1/2*	109; > 35.0
Bladder[50]	Whole-exome and targeted sequencing, somatic	DNA repair genes including (in targeted sequencing): *ATM*, *FANCD2*, *PALB2*, *BRCA1/2*	81; 34.4
Prostate[35]	Whole-exome and targeted sequencing, somatic and germline	DNA repair genes including: *BRCA1/2*, *ATM*, *FANCA*, *CHEK2*, *PALB2*, *RAD51*, *MRE11*, *NBN*	49; 33.0
Ovarian[51]	Whole-exome sequencing, somatic	*BRCA1/2*, *CDK12*, *RAD51C*, Fanconi anemia genes, *RAD50*, *PTEN*, *ATM*, *ATR*, *CHEK1*, *CHEK2*	316; 23.5
Multiple cancer types[52]	NGS600, Hotspot Illumina MiSeq, somatic	*ARID1A*, *ATM*, *ATRX*, *BAP1*, *BARD1*, *BLM*, *BRCA1/2*, *BRIP1*, *FANCA/C/D2/E/F/G/L*, *MRE11A*, *NBN*, *PALB2*, *RAD50*, *RAD51*, *RAD51B*, *WRN*	52,426; 17.4
Breast (triple-negative breast cancer)[53]	NGS Illumina MiSeq/ NextSeq, germline and somatic	*BRCA1/2*, Fanconi anemia genes, *BML*, *BARD1*, *BRIP1*, *CHEK2*, *FAM175A*, *NBN*, *PALB2*, *PTEN*, *RAD51D*, *TP53*	32; 15.6
Pancreas[54]	HRD score (LOH, TAI, LST) and targeted NGS, somatic	*BRCA1/2*, *ATM*, *ATR*, *BRIP1*, Fanconi anemia genes	78; 15.4
Gastric[55]	IHC, somatic	*ATM* (expression)	123; 14.0
Ovarian[42]	Hotspot BROCA panel, somatic and germline	*BRCA1/2*, *ATM*, *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, *RAD51D*	367; 7.6 (somatic), 22.6 (germline), 1.1 (both)
Breast[56]	Hotspot panel, somatic	*PTEN*, *ATM*, *CDKN2A*, *NPM1*	400; 2.0

Abbreviations: HR, homologous recombination; IHC, immunohistochemistry; LOH, loss of heterozygosity; LST, large-scale state transitions; NGS, next-generation sequencing; TAI, telomeric allelic imbalance.

to identify a substantial subset of patients who might benefit from specific therapies, including PARP inhibitors and platinum chemotherapy, though it is not fully defined which HR-DDR pathway mutations confer the greatest therapeutic impact, akin to patients with *BRCA1/2* mutations. In a recent evaluation of *BRCA1/2* mutational patterns, for example, a model to detect *BRCA1/2* deficiency failed to correlate *BRCA1/2* mutational patterns with pathogenic mutations in several additional HR-DDR pathway genes, including *ATM*, *ATR*, *CHEK2*, the *FANC* group of genes, *PALB2*, *PTEN RAD50*, and *RAD51C*.[48] The unique genetic signatures across the breadth of HR-DDR pathway genes are not well characterized, and, as such, it is unknown if the lack of a *BRCA*-like mutational pattern is associated with altered responses to PARP inhibitors and DNA-damaging chemotherapy.

Our reported frequencies of HRD are similar to previously published work, though a range of frequencies is appreciated (Table 5). Because there is not yet an established way to measure HRD, and previous studies have measured HRD by an HRD assay assessing large-scale transition scores and telomere allelic imbalances, germline mutations, somatic mutations, *BRCA*-like genetic signatures, or a combination of these methods, the differences in HRD prevalence may be related to nonuniformity of assessment. In addition, significant variation will occur between whole-exome sequencing versus use of hotspot panels, with the identification of HRD more common among studies that used whole-exome sequencing. Within our study, 17,566 tumors (33.5% of the total 52,426 tumors tested) underwent sequencing with the targeted whole-exome sequencing platform NGS600, which evaluated tumors for 592 genes, assuming DNA was sufficient. We also excluded an evaluation for tumor expression of deleterious *PTEN* mutations; *PTEN* is commonly mutated in malignant tumors. Consequently, studies including an evaluation for *PTEN* mutations are expected to report higher frequencies of HRD.

It is also possible frequencies of HRD may differ across studies as a result of differences in patient population. In our study, of the 52,426 tumors evaluated, 25,102 were sequenced from the primary tumor and 21,956 were sequenced

from a metastatic site of disease (tumor site was unknown for 5,368 cases; Table 1). Patients with tumors harboring HR-DDR pathway mutations may be more sensitive to DNA-damaging chemotherapy and therefore less likely to recur after initial treatment of localized disease. Although the clinical stage of patients included in our analysis at the time of tissue acquisition (and if tissue was obtained at presentation) was unknown, it is possible our population was enriched with patients with metastatic disease without HRD who were less likely to respond favorably to frontline treatment and, therefore, recurred.

Looking forward, classifying tumors as HR-DDR deficient will likely become increasingly important, as we now appreciate that HRD is common and has been associated with improved outcomes in some patients treated with DNA-damaging therapies. Several clinical trials are assessing in part the role of HRD in response to anticancer therapies and outcomes, including treatment with PARP inhibitor therapy alone, but also in combinations with chemotherapy, radiation therapy, or immunotherapy to enhance antitumor efficacy. In tandem, it will be important to generate a consensus regarding uniform testing to identify appropriate patients for these tailored therapies.

There are some limitations of this study to note. Given the nature of the study, we evaluated patients' tumor tissue for the presence of HR-DDR mutations and thus are unable to distinguish whether a given mutation was a somatic or germline mutation. We were also unable to assess tumors for epigenetic modifications such as DNA methylation, which could also lead to significant and clinically relevant alterations in gene expression affecting functions of the HR pathway.[57,58] In addition, HRD is defined by currently available literature regarding the suspected pathogenicity of each mutation. It is certainly possible that mutations labeled as a variant of unknown significance may prove important in the future, although the majority are reclassified as benign polymorphisms.[59-61] We also recognize our results are influenced by the overrepresentation of certain cancer lineages, including ovarian (n = 9,630), NSCLC (n = 8,119), CRC (n = 6,650), breast (n = 5,910), and endometrial (n = 5,540) cancers in the Caris database. And finally, although additional genes were of interest to evaluate (including *EMSY* and *RAD51C*), they either were not included in the Caris targeted NGS600 platform or variants have not been interpreted by a Caris geneticist and, as such, were excluded from this analysis.

Nevertheless, this study reveals that HRD is common in solid tumors and as our understanding of homologous recombination evolves and we further define the scope of clinical impact of mutations beyond *BRCA*, we may see an increase in the benefits gained from a wider range of HRD-directed therapies.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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