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ANNALS OF ONCOLOGY driving innovation in oncology

ORIGINAL ARTICLE

Comprehensive cancer predisposition testing within the prospective MASTER trial identifies hereditary cancer patients and supports treatment decisions for rare cancers

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Background: Germline variant evaluation in precision oncology opens new paths toward the identification of patients with genetic tumor risk syndromes and the exploration of therapeutic relevance. Here, we present the results of germline variant analysis and their clinical implications in a precision oncology study for patients with predominantly rare cancers.

Patients and methods: Matched tumor and control genome/exome and RNA sequencing was carried out for 1485 patients with rare cancers (79%) and/or young adults (77% younger than 51 years) in the National Center for Tumor Diseases/German Cancer Consortium (NCT/DKTK) Molecularly Aided Stratification for Tumor Eradication Research (MASTER) trial, a German multicenter, prospective, observational precision oncology study. Clinical and therapeutic relevance of prospective pathogenic germline variant (PGV) evaluation was analyzed and compared to other precision oncology studies.

Results: Ten percent of patients ($n = 157$) harbored PGVs in 35 genes associated with autosomal dominant cancer predisposition, whereof up to 75% were unknown before study participation. Another 5% of patients ($n = 75$) were heterozygous carriers for recessive genetic tumor risk syndromes. Particularly, high PGV yields were found in patients with gastrointestinal stromal tumors (GISTs) (28%, $n = 11/40$), and more specifically in wild-type GISTs (50%, $n = 10/20$), leiomyosarcomas (21%, $n = 19/89$), and hepatopancreaticobiliary cancers (16%, $n = 16/97$). Forty-five percent of PGVs ($n = 100/221$) supported treatment recommendations, and its implementation led to a clinical benefit in 40% of patients ($n = 10/25$). A comparison of different precision oncology studies revealed variable PGV yields and considerable differences in germline variant analysis workflows. We therefore propose a detailed workflow for germline variant evaluation.

Conclusions: Genetic germline testing in patients with rare cancers can identify the very first patient in a hereditary cancer family and can lead to clinical benefit in a broad range of entities. Its routine implementation in precision oncology accompanied by the harmonization of germline variant evaluation workflows will increase clinical benefit and boost research.

Key words: precision medicine, rare cancer, hereditary cancer, biomarker, targeted therapy, prevention

INTRODUCTION

Hereditary cancer predisposition is gaining considerable attention, as it accounts for a substantial number of tumor cases[1,2] and is increasingly relevant for targeted treatment in patients with common cancers.[3] Precision oncology studies carried out mainly on patients with common cancers have shown that the current criteria for germline testing are too restrictive and about half of the patients with (likely) pathogenic germline variants (PGVs) remain undiagnosed.[4-6] Often, inclusion criteria do not apply for patients with rare cancers,[7,8] as PGV yields and their clinical relevance for this cohort remain largely under-studied.

The multicenter National Center for Tumor Diseases/German Cancer Consortium (NCT/DKTK) Molecularly Aided Stratification for Tumor Eradication Research (MASTER) trial was established in 2012 and aims to investigate the clinical value of exome/genome and transcriptome sequencing in advanced cases of either patients with rare cancers across all age groups or younger adults across different entities.[9,10] An important aim of the MASTER trial is the prospective evaluation of germline variants in genes associated with genetic tumor risk syndromes and their clinical translation.

The general clinical utility of precision oncology for patients with rare cancers in MASTER was recently reported.[11] Here, we analyzed the impact of germline variant evaluation for the identification of patients with genetic tumor risk syndromes and for treatment recommendations, as well as associations between germline variants and clinical phenotypes within an extended cohort of 1485 patients. Additionally, we compared our findings to other studies and proposed a workflow for germline variant evaluation in precision oncology.

PATIENTS AND METHODS

Patient cohort and study design of the NCT/DKTK MASTER trial

For this study, all patients ($n = 1485$) included in the NCT/DKTK MASTER program between August 2015 and July 2019 were selected. The actionable genome and transcriptome have been recently addressed in Horak et al. 2021,[11] whereof 1097 patients overlap with this study. All patients consented to banking of tumor (mostly fresh frozen) and control tissue (mostly blood or buffy coat), molecular profiling of both samples, and clinical data collection (S-206/2011, Ethics Committee of the Medical Faculty of Heidelberg University).[9,11] The study was conducted in adherence to the Declaration of Helsinki. According to the inclusion criteria, patients had exhausted conventional treatment options and were either younger than 51 years and/or were diagnosed with a rare cancer or rare subtypes of more common cancer entities. Exome ($n = 794/1485$) or genome ($n = 691/1485$) sequencing of tumor and control as well as tumor transcriptome sequencing (RNA-seq, $n = 1218/1485$) were carried out on Illumina (Illumina Inc., San Diego, CA, USA) platforms generating short paired-end reads yielding a mean average coverage of $\geq 50\times$ of analyzed genes in the control sample (Supplementary Table S1, available at https://doi.

org/10.1016/j.annonc.2022.07.008).[11] Tumor and control sample-derived next-generation sequencing reads were aligned against Hg19/GRCh37. Tumor variant calling for single-nucleotide variants (SNVs), indels, structural variants (SVs), copy number alterations, quantification of genomic and microsatellite instability, as well as mutational signatures, gene and variant expression, and identification of fusion genes from RNA sequencing was carried out as described in Horak et al.[11] Called tumor variants were flagged as somatic or germline variants based on absence or presence

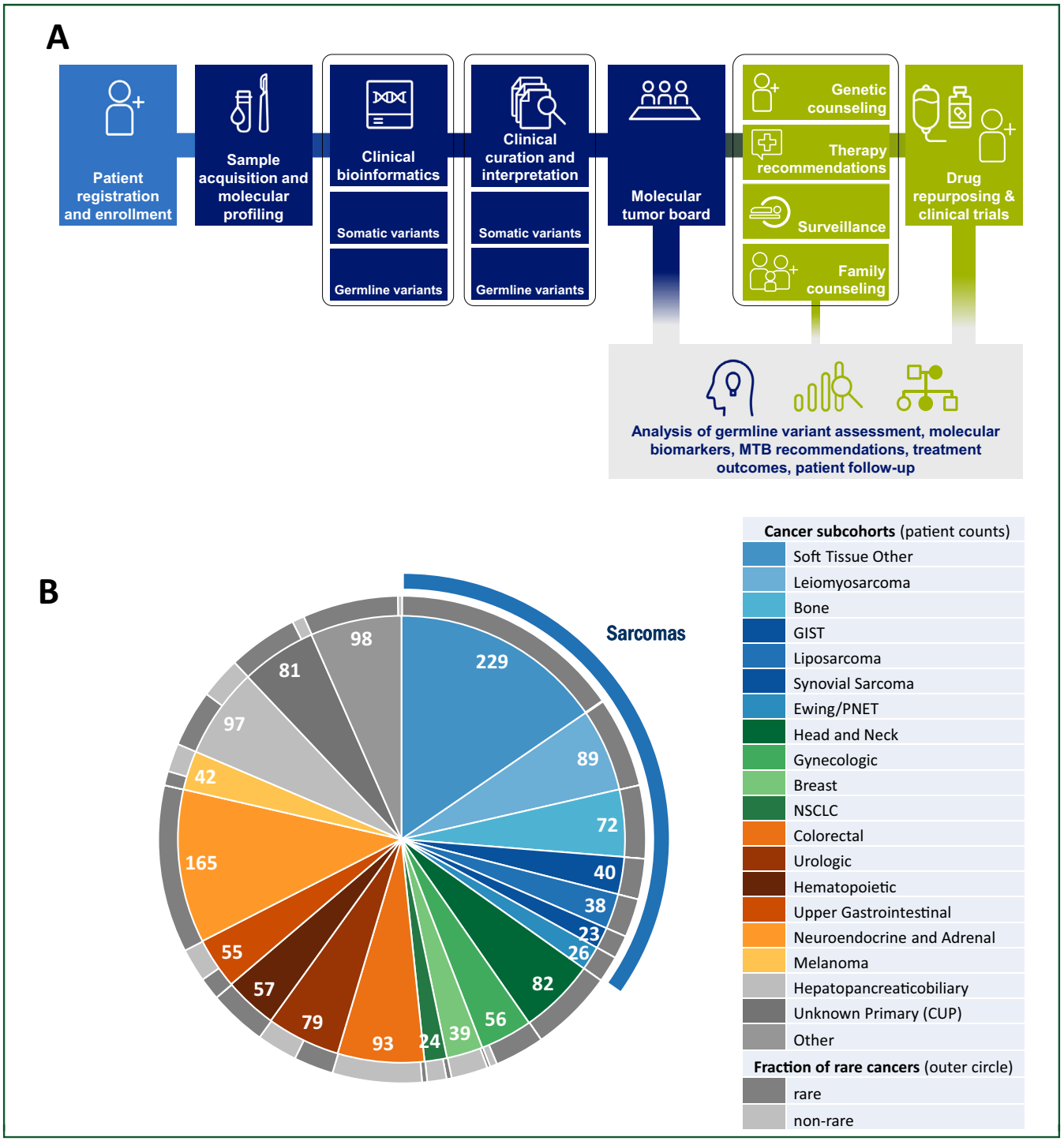


Figure 1. Workflow and patient cohort of the NCT/DKTK MASTER precision oncology study.
(A) Standardized MASTER workflow including interpretation of somatic and germline variants and analysis of clinical recommendations and implementations. (B) Distribution of patient subcohorts in the MASTER trial ($n = 1485$). Counts of patients in subcohorts; fractions of rare cancers (outer circle) and sarcomas (in blue) are highlighted. (C) Age of onset and sex distribution of the MASTER subcohorts. Violin plots are separated into females (left half) and males (right half, dashed) (white dot, median age of onset). (D) Classification of the MASTER germline genes. Center of Sankey plot, genes ($n = 142$) subgrouped by their clinical evidence for cancer predisposition; left, inheritance patterns ($n = 101$ genes); right, assignment of genes ($n = 142$) to biomarker baskets.
AD, autosomal dominant; AR, autosomal recessive; CC, cell cycle; DDR, DNA damage repair; DEV, developmental regulation; FR, female ratio (%); GIST, gastrointestinal stromal tumor; GUS, gene(s) of unknown significance in the context of cancer predisposition; *n*, number of patients in each subcohort; NSCLC, non-small-cell lung cancer; OTH, other; PAM, PI3K—AKT—mTOR; PNET, primitive neuroectodermal tumor; RME, RAF—MEK—ERK; som. mosaic, somatic mosaicism; TK, tyrosine kinases; XLR, X-linked recessive.

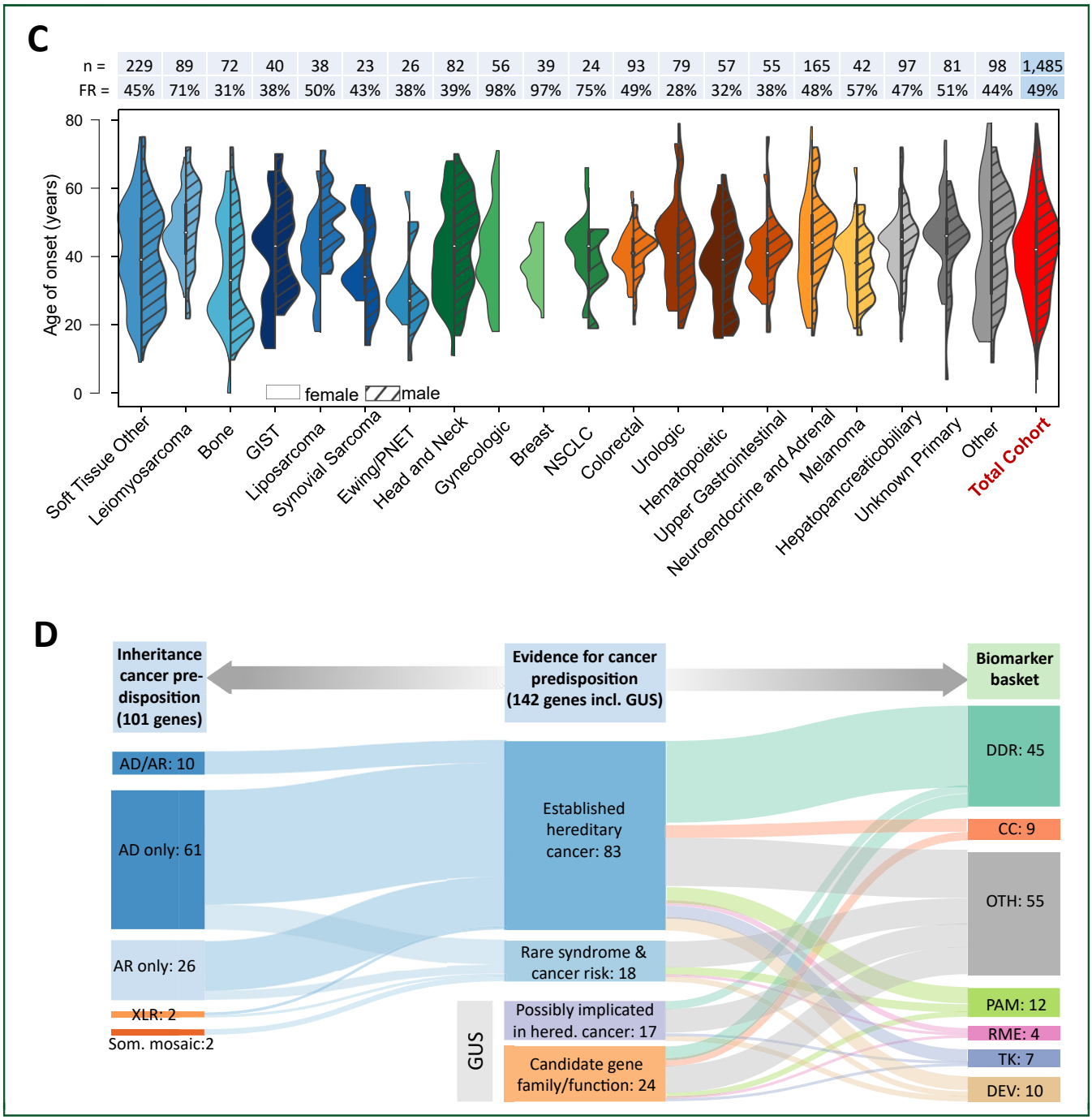
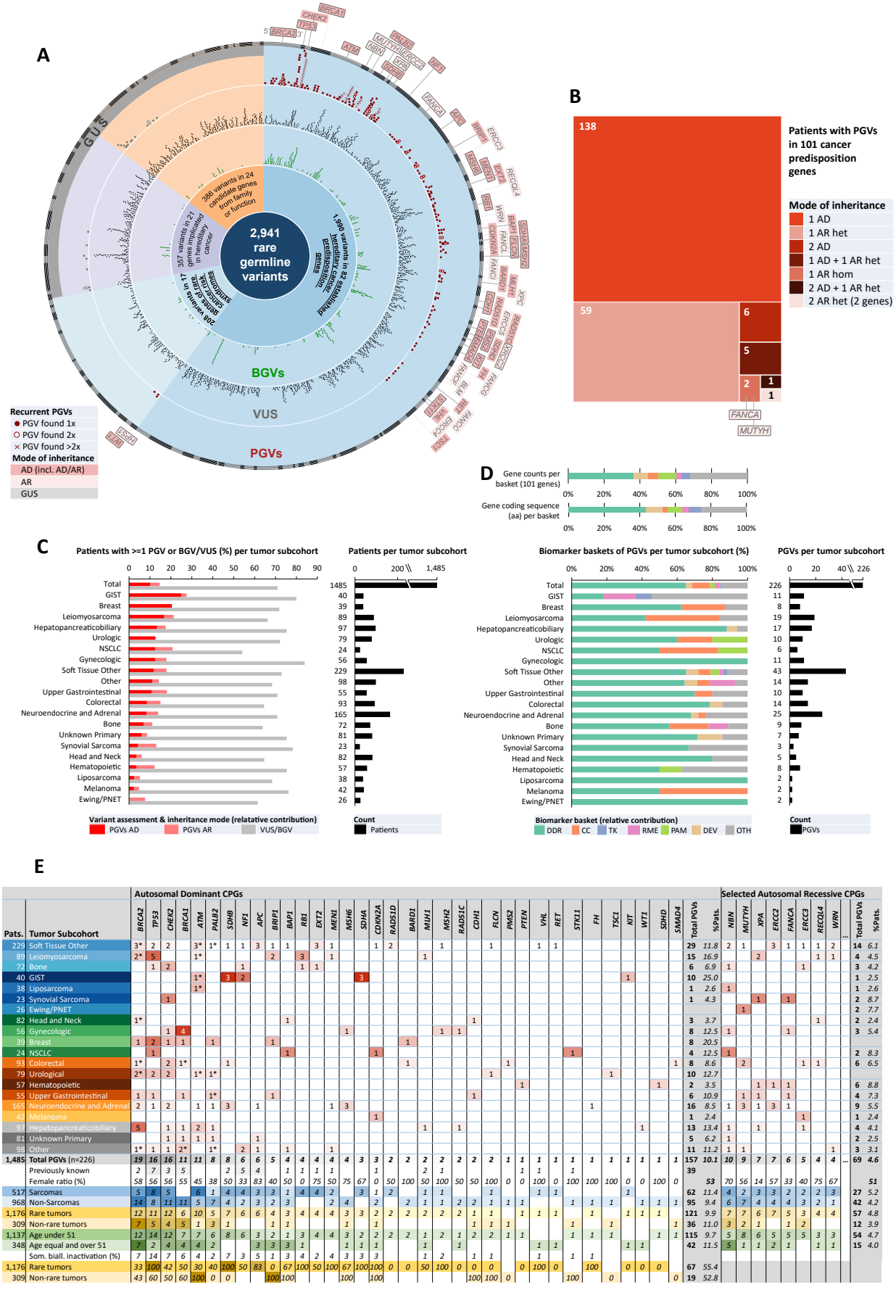


Figure 1. **Continued.**

in mappings from the matched control sample. For germline SNVs, a strict ExAc filter[12] (version r0.3.nonTCGA.sites, <3 homozygous or <40 heterozygous individuals) and gradually extended inclusion lists were applied (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2022.07.008). For manually curated analysis of biallelic inactivation, retrospective loss-of-heterozygosity annotation of the wild-type allele involving the position of the PGV [CNVkit for exome (version 2.1.0) and ACEseq for genome sequencing (version 5.1.0)], somatic SNV/indels/SVs,[11] and gene expression ≤0.3-fold change in comparison to a control cohort[11] were counted.

Evaluation of germline variants and assessment of clinical actionability

Genes associated or potentially associated with cancer predisposition based on expert opinion, in-house lists, and peer-reviewed literature were nominated for germline variant extraction at the beginning of the study (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.07.008) and assigned to one of eight biomarker baskets.[9] Rare germline variants in the preselected gene list were classified by a team of fellows and board-certified specialists in clinical genetics according to the American



Pats.	Tumor Subcohort	BRCA2	TP53	CHEK2	BRCA1	ATM	PALB2	SDHB	NF1	APC	BRIP1	BAP1	RB1	EXT2	MEN1	MSH6	SDHA	CDKN2A	RAD51D	BARD1	MUH1	MSH2	RAD51C	CDH1	FLCN	PMS2	PTEN	VHL	RET	STK11	FH	TSC1	KIT	WT1	SDHD	SMAD4	Total PGVs	%Pats.	NBN	MUTYH	XPA	ERCC2	FANCA	ERCC3	RECQL4	WRN	...	Total PGVs	%Pats.
229	Soft Tissue Other	3*	2	2		3*	1*	1	1	3	1	1		3	1			1	2			1			1			1	1								29	11.8	2	1		3	1	1	1	2		14	6.1
89	Leiomyosarcoma	2*	5			1*					2		3		1						1																15	16.9			2				1	1		4	4.5
72	Bone		1	2					1			1	1																								6	6.9	1					1				3	4.2
40	GIST					1*		3	2								3															1					10	25.0										1	2.5
38	Liposarcoma					1*																														1	2.6	1									1	2.6
23	Synovial Sarcoma			1																																1	4.3			1		1					2	8.7
26	Ewing/PNET																																									1						2	7.7
82	Head and Neck	1*										1												1												3	3.7							1				2	2.4
56	Gynecologic			1	4										1						1	1														8	12.5	1					1				3	5.4
39	Breast	1	2	1	1		1				1									1																8	20.5											
24	NSCLC		1									1						1											1							4	12.5	1									2	8.3
93	Colorectal	1*		2	1*				1											1					1										1	8	8.6		2				1	1			6	6.5
79	Urological	2*	2	2		1*	1*																	1							1					10	12.7											
57	Hematopoietic																									1							1			2	3.5			1	1	1					6	8.8
55	Upper Gastrointestinal	1	1		1		1*				1													1												6	10.9		1	1		1					4	7.3
165	Neuroendocrine and Adrenal	2	1	2		1	1	3		1					1	3															1					16	8.5	1	3	1	3	1					9	5.5
42	Melanoma																	1																		1	2.4							1			1	2.4
97	Hepatopancreaticobiliary	5		1	1	2	1														1		1										1			13	13.4	1		1			1				4	4.1
81	Unknown Primary			1	1	1	1			1																										5	6.2	1									2	2.5
98	Other	1*	1	1	2*		1*		2	1		1			1																					11	11.2	1	1						1		3	3.1
1,485	**Total PGVs** (n=226)	19	16	16	11	11	8	8	6	6	5	4	4	4	4	4	3	3	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	157	10.1	10	9	7	7	6	5	4	4	...	69	4.6
	Previously known	2	7	3	5			2	5	4		1	1	1	1			1		2	1					1	1		1							39												
	Female ratio (%)	58	56	56	55	45	38	50	33	83	40	50	0	75	50	75	67	0	50	100	50	100	50	50	100	0	0	100	0	100	100	100	0	0	100	0		53		70	56	14	57	33	40	75	67			51
517	Sarcomas	5	8	5		6	1	4	4	3	3	1	4	4	2		3	1	2		1	1			1			1	1				1				62	11.4	4	2	3	3	2	2	2	3		27	5.2
968	Non-Sarcomas	14	8	11	11	5	7	4	2	3	2	3			2	4		2		2	1	1	2	2	1	1	1			1	1	1		1	1	1		95	9.4	6	7	4	4	4	3	2	1		42	4.2
1,176	Rare tumors	12	11	12	6	10	5	7	6	6	4	3	4	4	4	3	3	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	121	9.9	7	7	6	7	5	3	4	4		57	4.8
309	Non-rare tumors	7	5	4	5	1	3	1			1	1			1		1		1					1	1	1				1		1				36	11.0	3	2	1		1	2				12	3.9
1,137	Age under 51	12	14	12	7	7	6	8	6	3	2	1	3	4	4	3	2	2	2	2	1	2	1	2	2	1	1			1	1	1		1	1	115	9.7	5	8	6	5	5	5	3	3		54	4.7
348	Age equal and over 51	7	2	4	4	4	2		3	3	3	3	1			1	1	1			1		1					1	1				1	1		42	11.5	5	1	1	2	1		1	1		15	4.0
	Som. biall. inactivation (%)	7	14	7	6	4	2	7	3	5	1	3	4	2	4	3	3	3			1	2		1	1			1		1	1																	
1,176	Rare tumors	33	100	42	50	30	40	100	50	83	0	67	100	50	100	67	100	100	0	0	50	100	0	0	0	0	0	100	0		100		0	0	0	67	55.4											
309	Non-rare tumors	43	60	50	60	100	0	0			100	100			100		100							100	100	0			100		0		0		0	19	52.8											

College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) criteria[13] and further specifications[14,15]; potentially actionable variants were discussed in the multidisciplinary molecular tumor board (MTB) and integrated in the MTB report. Variants in genes of unknown significance (GUS) were assigned as variants of unknown significance (VUS) with respect to cancer predisposition. However, when applicable, additional assessment regarding non-cancer predisposition disease phenotypes was carried out and could lead to PGV classification. Curated pathogenic and likely pathogenic variants (PGVs) as well as benign and likely benign variants (BGVs) were combined leading to a three-tier variant assessment. Therapeutic recommendations supported by PGVs, other supporting biomarkers based on genome and transcriptome sequencing, and outcome parameters for patients were re-evaluated and the clinical benefit of implemented molecularly informed therapies was assessed, as described in Horak et al.[11] (data cut-off October 2020).

For the comparison of the studies, we extracted PGV yields[16,17] or germline variants, when precise annotations were available.[2,5,18-22] Automated variant assessment of germline variants across studies was carried out by CharGer (version 0.5.4) with a few modifications (forked version modifications available at https://github.com/NagaComBio/CharGer).

Kruskal–Wallis rank sum tests were used for multi-group comparisons and if $P < 0.05$, pairwise Wilcoxon rank sum tests with Bonferroni correction were carried out. Unless stated otherwise, Fisher's exact test (two-sided) was used for paired statistical data analyses.

RESULTS

Prospective identification of germline variants in tumor patients enrolled in NCT/DKTK MASTER trial

Between August 2015 and July 2019, 1485 patients were enrolled in the MASTER trial and prospective evaluation of germline variants was carried out by clinical geneticists. Relevant results for treatment recommendations, genetic counseling, and predictive testing in relatives were discussed in a multidisciplinary MTB and integrated in the MTB report (Figure 1A). Cancer entities were grouped in 20 subcohorts based on their histological and clinical characteristics (Supplementary Table S3 and S4, available at https://doi.org/10.1016/j.annonc.2022.07.008). The median age of onset was 42 years (range 0-79 years) (76.6% younger than 51 years, Figure 1C), and 79.2% of the patients had a rare cancer. Seven of these subcohorts represented sarcomas, accounting for 34.8% of the patients, followed by neuroendocrine and adrenal tumors (11.1%), and hepatopancreaticobiliary tumors (6.5%) (Figure 1B).

To identify PGVs associated with cancer predisposition, we filtered for rare germline variants within a preselected list of 142 genes (Figure 1D, Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.07.008). These genes included 101 established disease genes associated with cancer predisposition (CPGs) with autosomal dominant [AD, $n = 71$ and 10 of these with an additional autosomal recessive (AR) inheritance], AR ($n = 26$), and X-linked recessive inheritance ($n = 2$), as well as somatic mosaicism ($n = 2$). Another 41 candidate genes (GUS) were included without sufficient evidence for an association with a genetic tumor risk syndrome (Figure 1D). Variants in CPGs were evaluated according to ACMG/AMP criteria[13] with respect to cancer predisposition and reported as PGVs, VUS, and BGVs. All 142 genes were allocated to one of eight molecular biomarker baskets to facilitate therapeutic decisions (Figure 1D, Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.07.008). While the largest group, consisting of 55 genes, was assigned to the basket 'other' (38.7%) (OTH), 45 genes (31.7%) were assigned to the basket DNA damage repair (DDR).

Correlation between phenotype and genotype in hereditary cancer risk syndrome-associated genes

In total, 2941 rare germline variants were identified in 84.6% of the patients (median 2, range 0-9) (Figure 2A, Supplementary Figure S1A and B, available at https://doi.org/10.1016/j.annonc.2022.07.008), of which 2198 were found in 101 CPGs and were evaluated as PGVs, VUS, and BGVs in 10.3%, 72.0%, and 17.7%, respectively. Overall, we identified 226 PGVs in 54 different CPGs in 212 patients: 150 patients (10.1%) harbored 157 PGVs in 35 AD CPGs and 68 patients (4.6%) harbored 69 PGVs in 19 AR CPGs (Figures 2B and 3A, Supplementary Table S5 and Figure S1C, available at https://doi.org/10.1016/j.annonc.2022.07.008). Only two patients had biallelic PGVs in AR CPGs (*MUTYH*, *FANCA*) and 13 patients showed PGVs in more than one gene (Figure 2B). PGV yield in AD CPGs was significantly higher among 105 patients with one or more

Figure 2. Germline variant distribution across genes and tumor subcohorts.
(A) Distribution and classification of rare germline variants across 142 genes. Genes classified in gene groups are sorted by PGV variant yield. Variants are depicted according to their position in the coding sequence and recurrence. (B) Patients with PGVs in 101 CPGs ($n = 212$ patients), subgrouped by the mode of inheritance. (C) Variant yields per subcohort. Left: distribution according to variant assessment and mode of inheritance of cancer predisposition [at least one PGV; or at least one BGV/VUS or both in 101 CPGs (some patients may be counted twice); subcohorts are ordered according to their PGV yield in AD CPGs]; right: counts of patients per subcohort. (D) Biomarker baskets of genes and PGVs. Left top: distribution of 101 CPGs according to biomarker basket; left bottom: distribution of biomarker baskets of PGVs in 101 CPGs according to subcohorts; right: counts of PGVs per subcohort. (E) Distribution of PGVs across 101 CPGs and subcohorts. Counts of PGVs in all affected genes with autosomal dominant (left, including autosomal dominant and autosomal recessive) and most affected genes with autosomal recessive (right) cancer predisposition as well as fraction of patients with PGVs [absolute and relative (%)]. Somatic events indicating somatic biallelic inactivation or loss of function are depicted [loss of heterozygosity (LOH), somatic variants, low expression]. Color grading represents relative PGV yield in a subcohort.
aa, amino acids; AD, autosomal dominant; AR, autosomal recessive; BGV, (likely) benign germline variant; CC, cell cycle; CPGs, cancer predisposition genes; DDR, DNA damage repair; DEV, developmental regulation; GIST, gastrointestinal stromal tumor; GUS, gene(s) of unknown significance in the context of cancer predisposition; het, heterozygous; hom, homozygous; NSCLC, non-small-cell lung cancer; OTH, other; PAM, PI3K–AKT–mTOR; PGV, (likely) pathogenic germline variant; PNET, primitive neuroectodermal tumor; RME, RAF–MEK–ERK; TK, tyrosine kinases; VUS, germline variant of unknown significance.
[a]Patients with cancers not commonly associated with PGVs in *ATM*, *BRCA1*, *BRCA2*, and *PALB2*.[24,36,37,55]

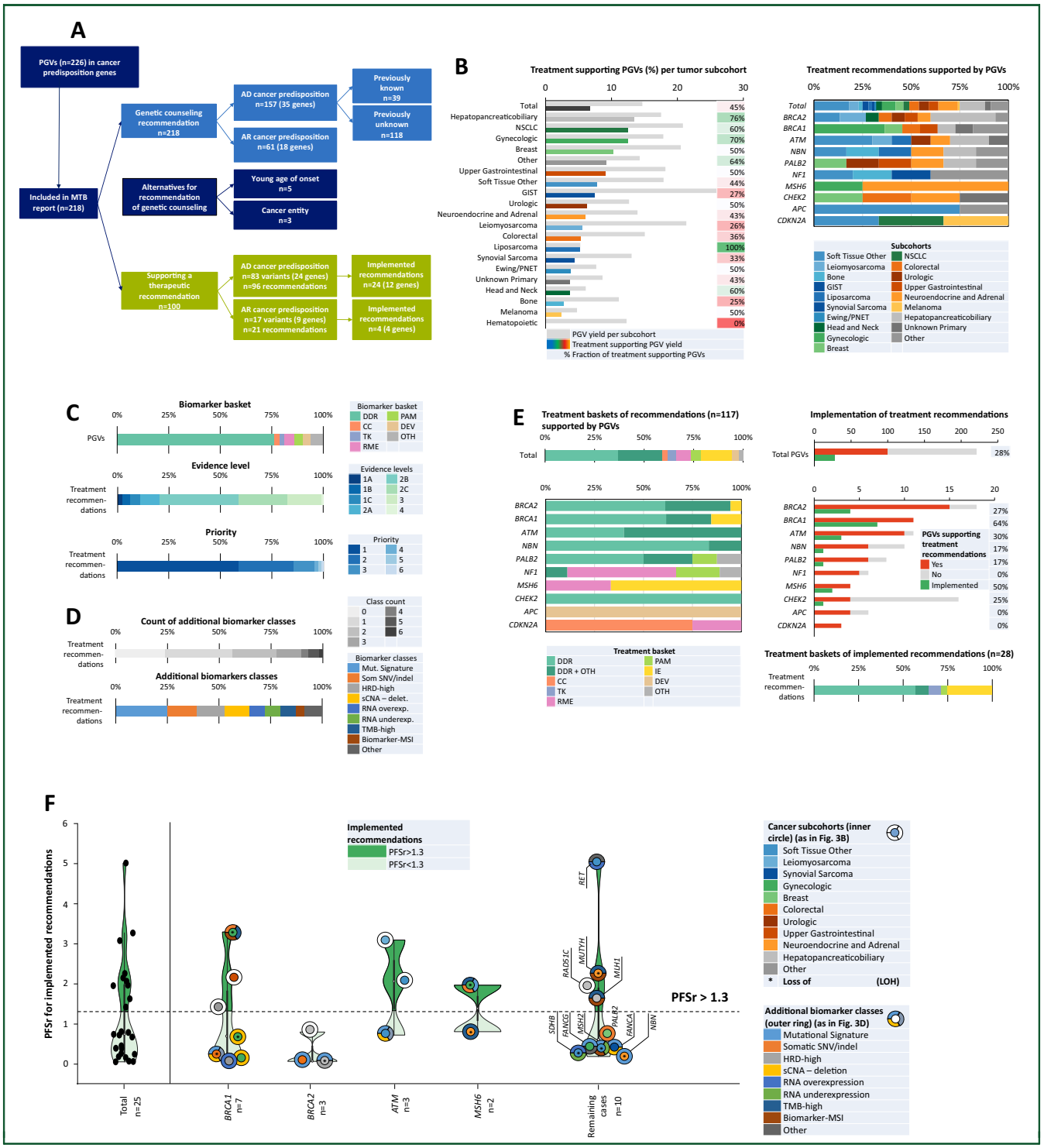


Figure 3. Recommendations based on germline variants and therapy outcome.

(A) Recommendations for genetic counseling and therapeutic recommendations supported by PGVs. (B) Treatment recommendations supported by PGVs. Left: PGV yield and fraction supporting therapeutic decisions in subcohorts; right: distribution of tumor subcohorts of treatment supporting PGVs for selected genes. (C) Characteristics of treatment recommendations supported by PGVs. Top: distribution of biomarker baskets of affected genes ($n = 100$ PGVs); middle: NCT/DKTK evidence level; bottom: priority (middle and bottom, $n = 117$ treatment recommendations). (D) Additional biomarker classes used for treatment recommendations supported by PGVs ($n = 89$ recommendations). Top: distribution of counts of additional biomarker classes; bottom: additional biomarker classes. (E) Treatment recommendations supported by PGVs (left: according to biomarker baskets for all recommendations and selected genes) and their implementation (top right: counts of therapy recommendation supporting PGVs and fractions of implemented recommendations for all PGVs and selected genes; bottom right: biomarker baskets of implemented recommendations). (F) Outcome of implemented therapies supported by PGVs. Distribution of progression-free survival ratio (PFSr) values for recommendations supported by PGVs [violin plots depict PFSr; white dot: median PFSr; darker areas: PFSr >1.3; each dot represents one treatment recommendation; inner circle: cancer subcohort; asterisk: loss of heterozygosity (LOH) of wild-type allele; outer circle: additional supporting biomarkers].

CC, cell cycle; DDR, DNA damage repair; DEV, developmental regulation; GIST, gastrointestinal stromal tumor; HRD, homologous recombination deficiency; IE, immune evasion; MSI, microsatellite instability; Mut. signature, mutational signature; NSCLC, non-small-cell lung cancer; OTH, other; PAM, PI3K—AKT—mTOR; PNET, primitive neuroectodermal tumor; RME, RAF—MEK—ERK; sCNA-delet., somatic copy number deletion; TK, tyrosine kinases; TMB, tumor mutational burden.

previous cancer diagnoses compared to patients without previous diagnosis (24.8%, $n = 26/105$ versus 9.0%, $n = 124/1380$, $P = 0.0001$) (Supplementary Figure S1E, available at https://doi.org/10.1016/j.annonc.2022.07.008). Contrarily, there was no association between PGV yields in AD CPGs and age of onset, neither for the overall cohort nor for the common and rare cancer or sarcoma subgroups (Supplementary Figure S1F, available at https://doi.org/10.1016/j.annonc.2022.07.008). Analyzing known genotype—phenotype associations within specific entity subgroups revealed a younger age at cancer diagnosis associated with PGVs in patients with leiomyosarcomas, but not in patients with gastrointestinal stromal tumors (GISTs) or breast cancer.[2,23] However, this might be masked in our cohort due to the age inclusion criteria.

The fraction of patients with PGVs in CPGs differed between subcohorts and ranged from 4.8% to 27.5% in patients with melanoma ($n = 2/42$) and GISTs ($n = 11/40$), respectively (Figure 2C). Considering only PGVs in AD CPGs, subcohorts with the highest PGV yields were GIST (25%, $n = 10/40$), followed by breast cancer (20.5%, $n = 8/39$), leiomyosarcoma (16.9%, $n = 15/89$), hepatopancreaticobiliary cancer (13.4%, $n = 13/97$), and urological cancer (12.7%, $n = 10/79$) (Figure 2C). In the GIST group ($n = 40$), 90.9% of PGVs ($n = 10/11$) were detected in 20 cancers without *KIT* or *PDGFRA* mutation (wild type). Overall, 65% of all PGVs were detected in genes assigned to the DDR biomarker basket, 15% in OTH, and 10.2% in cell cycle with considerable differences between subcohorts (Figure 2D).

Most PGVs in AD CPGs were found in *BRCA2* (8.4%, $n = 19/226$), followed by *TP53* and *CHEK2* (both 7.1%, $n = 16$), *BRCA1* and *ATM* (both 4.9%, $n = 11$), and *PALB2* as well as *SDHB* (both 3.5%, $n = 8$) (Figure 2E). The most frequently affected AR CPGs were *NBN* (4.4%, $n = 10$) and *MUTYH* (4.0%, $n = 9$). While the frequency of all the observed rare variants in a gene was associated with the coding size of the gene, the frequency of PGVs could only be partially explained by this relation (Supplementary Figure S1D, available at https://doi.org/10.1016/j.annonc.2022.07.008). PGVs in *BRCA2* were detected in ten of the 20 subcohorts; interestingly, five were found in sarcomas, five in hepatopancreaticobiliary cancers, and one in breast cancer. Similarly, PGVs in *PALB2* ($n = 8$) were detected in eight different subcohorts, four of them not commonly associated with PGVs in *PALB2*[24] (Figure 2E). In contrast, no PGV in *BRCA1* was detected in sarcomas, while 45.5% ($n = 5/11$) were found in gynecologic and breast cancers, and overall PGVs in *BRCA1* were significantly more frequent in common compared to rare cancers (χ^2: $P = 0.045$). Somatic alterations indicating biallelic inactivation or biallelic loss of function (loss of heterozygosity, somatic variants, low expression) were found in 7/19 cases with PGVs in *BRCA2*, 6/11 cases with PGVs in *BRCA1*, and 2/8 cases with PGVs in *PALB2*, both in rare (range 33%-50%) and except for *PALB2* in common cancers (43%-60%, Figure 2E and Supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2022.07.008). For certain genes, PGVs were (nearly) exclusively found in patients with rare cancers, such as PGVs in *NF1* ($n = 6/6$), *APC* ($n = 6/6$, whereof 4 in aggressive fibromatosis), *ATM* ($n = 10/11$), and *SDHB* ($n = 7/8$, whereof 3 each in GIST and pheochromocytoma/paraganglioma). PGVs in *RB1* ($n = 4/4$, χ^2: $P = 0.006$) and *EXT2* ($n = 4/4$, χ^2: $P = 0.006$) were exclusively seen in sarcoma patients, and three of four *RB1* PGVs in leiomyosarcoma patients. A high proportion of patients with PGVs in *MSH6* ($n = 3/4$) and *MUTYH* ($n = 3/9$) had neuroendocrine and adrenal cancers. Notably, we found a high number of patients with PGVs in *TP53*, and half of them ($n = 8/16$) had sarcomas, and in particular leiomyosarcomas ($n = 5$). One-third ($n = 5/16$) of them had common cancers known to be associated with Li—Fraumeni syndrome, such as early-onset breast, prostate, and non-small-cell lung cancers (NSCLC).

Recommendations based on germline variants and therapy outcome

All 157 PGVs in AD CPGs and 61 of 69 (88.4%) PGVs in AR CPGs were included in the MTB report with recommendations for clinical genetics follow-up and management (Figure 3A, Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2022.07.008). Only 39 of 157 PGVs in AD CPGs had been previously known, meaning an AD genetic tumor risk syndrome was newly diagnosed in 118 patients within this study. Moreover, 46% of PGVs ($n = 100/218$) in 34 genes supported 117 therapeutic recommendations in 6.6% of the patients ($n = 98$) (Figure 3A, Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2022.07.008). The highest fraction of PGVs supporting treatment recommendations was found in hepatopancreaticobiliary cancer ($n = 13/17$), followed by gynecologic cancer (except breast) and NSCLC ($n = 7/11$ and $n = 3/6$, respectively) (Figure 3B). PGVs in AD CPGs led twice as frequently to a treatment recommendation compared to PGVs in AR CPGs (52.9%, $n = 83/157$ versus 24.5%, $n = 17/69$, $P = 0.0001$) (Supplementary Figure S2A and B, available at https://doi.org/10.1016/j.annonc.2022.07.008).

The majority of these PGVs ($n = 76/100$) were found in genes assigned to the DDR biomarker basket (Figure 3C). Eleven percent of recommendations supported by PGVs and possibly other biomarkers had NCT/DKTK molecular evidence levels 1A-C[25] ($n = 13/117$, same tumor type), 71.8% evidence levels 2A-C ($n = 84/117$, other tumor type) (Figure 3C, Supplementary Table S7, available at https://doi.org/10.1016/j.annonc.2022.07.008), and 59% of them ($n = 70/117$) received the highest priority (Figure 3C). In 28 patients, the PGV was the only biomarker supporting the treatment rationale. On average, 1.6 biomarker groups (range 0-6) supported recommendations in addition to PGVs (Figure 3D, Supplementary Figure S2C and Table S3A, available at https://doi.org/10.1016/j.annonc.2022.07.008), i.e. mutational signatures (25%), somatic SNVs/indels (14.4%), and high homologous recombination deficiency (HRD, 13.3%). In most cases, treatment recommendations targeted DDR and DDR + OTH (59.4%), followed by immune evasion (15.6%) (Figure 3E).

Overall, 23.9% of these treatment recommendations ($n = 28/117$) were implemented, 24 of these (85.7%) based on PGVs in AD CPGs (Figure 3A), such as *BRCA1* and *MSH6,* which had the highest fractions of implemented treatment recommendations (64% and 50%, respectively) (Figure 3E). In 18 cases (64.3%) a poly (ADP-ribose) polymerase (PARP) inhibitor (twice combined with a treatment classified as OTH), in seven cases (25.9%) an immune checkpoint inhibitor (ICI), and in two cases a tyrosine kinase inhibitor were administered, and once the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway was targeted (in combination with a tyrosine kinase inhibitor) (Figure 3F, Supplementary Table S8, available at https://doi.org/10.1016/j.annonc.2022.07.008). The progression-free survival ratio (PFSr) between the PFS of the molecularly guided therapy (PFS2) and the PFS of the last systemic therapy before MTB (PFS1) was calculated for 25 cases. In 10 cases (40%), a PFSr >1.3 was observed and the targeted therapy was considered to be clinically beneficial[26] (Figure 3F).

Comparison of germline variant evaluation between studies analyzing common and rare adult as well as pediatric cancers

Various studies assessing germline variants from genome, exome, and multi-gene-panel sequencing of matched tumor/control samples have been published (Supplementary Table S9, available at https://doi.org/10.1016/j.annonc.2022.07.008), focusing on adult patients with a broad range of mainly common cancers,[2,18,19,20] pediatric cancers,[16] and in our study, on rare cancers in adults. The fraction of patients with PGVs in these studies ranged between 7.6%[16] and 17.8%.[19] To better understand the reasons for the different variant yields, we analyzed the workflows and resulting variants. All six studies limited germline variant analysis on proprietary nominations of gene panels, selecting 142-187 genes per study (Figure 4A, Supplementary Table S10, available at https://doi.org/10.1016/j.annonc.2022.07.008). The set union of all the genes amounted to a total of 318 genes, with only 70 of these being commonly analyzed by all six studies and 124 genes nominated only once. The fractions of PGVs in the 70 commonly analyzed genes as compared to all PGVs of the respective study ranged between 63%[19] and 100%[20] (Figure 4B). The number of genes affected by PGVs in the common gene group ranged from 15[20] to 54 in the largest study,[2] affecting a total of 62 genes across studies (Figure 4C).

In addition to various gene nominations, variant filtering across studies was heterogeneous (Supplementary Table S9, available at https://doi.org/10.1016/j.annonc.2022.07.008). As an example, variant extraction in AR CPGs was limited to biallelic germline variants for the pediatric cohort.[16] Within the 70 genes analyzed by all six studies, Huang et al.[2] reported less than half of the PGV yield (6% of the patients) compared to Schrader et al.[18] (12.1%) or our study (13.3%) (Figure 4C). On a single gene level, high variations in the PGV yield were detected, e.g. for *TP53*, *MUTYH*, *BRCA1*, *NF1*, and especially for *CHEK2.* The PGV yield in *CHEK2* ranged from 0.11%[16] to 2.82%[20] (Figure 4D, Supplementary Figure S3A, available at https://doi.org/10.1016/j.annonc.2022.07.008). Furthermore, we observed a variable discovery rate for the well-known Eastern European founder variant *CHEK2*: c.1100delC (p.Thr367Metfs*15),[27] which we consider as PGV, from 0%[2] to 2.69%.[20] While this difference can be partially explained by different ethnic backgrounds, it is also possible that this variant was removed bioinformatically due to a high prevalence in population databases.

To analyze heterogeneity in variant interpretation between studies, we applied CharGer[2] to automatically and consistently re-classify germline variants. The recall of unambiguous PGVs of five studies by CharGer was on average 84%, ranging from 66%[18] to 100%[20] (Supplementary Figure S3B and Table S11, available at https://doi.org/10.1016/j.annonc.2022.07.008). The inconsistencies included lower class assessment of PGVs from different studies as VUS (12.3%) or BGV (0.6%) by CharGer. The highest deviations between CharGer and study variant assessments were identified in *CHEK2,* including the variant c.470T>C (p.Ile157Thr) (Figure 4E). This variant should most likely be classified as BGV/risk factor consistent with recent literature.[28]

To further investigate the prevalence of cancer predisposition, we extended our analysis and included three additional studies analyzing mainly common adult cancer,[5] adult sarcoma,[21] and pediatric cancer patients[22] (Supplementary Table S9 and S10, available at https://doi.org/10.1016/j.annonc.2022.07.008). In all the nine studies, only 36 genes were commonly evaluated for germline variants (Figure 4F). Overall, 7.9% among 22 407 patients had PGVs in those 36 genes. PGVs were more frequent in rare adult cancers ($n = 1176$) and pediatric cancers ($n = 1665$; each 10.8% and $P < 0.0001$) compared to common adult cancers (7.1%, $n = 18\,095$). The occurrence of PGVs in *RB1*, *TP53*, *NF1*, and *APC* was significantly associated with rare and pediatric cancers compared to common adult cancers ($P < 0.001$ each), and *RB1* was also more frequently affected in pediatric compared to rare adult cancers ($P < 0.001$) (Figure 4G). Conversely, PGVs in *BRCA1* and *ATM* were detected significantly more frequently in adult common cancers compared to pediatric cancers ($P < 0.01$). In addition, PGVs in *ATM* were more frequent in rare adult compared to pediatric cancers ($P < 0.01$). *BRCA1/2* PGVs were also more frequent in common adult cancers compared to rare adult cancers, although this difference was not significant. In comparison to a general adult population screening (Healthy Nevada Project[17]), a significantly higher PGV yield in *BRCA1* was only found in adult patients with common cancers ($P < 0.001$), while the PGV yield in *BRCA2* was significantly higher in both adult common and rare cancers ($P < 0.001$ and $P = 0.05$, respectively).

DISCUSSION

Tumor-control sequencing in precision oncology studies allows for the identification of patients with previously unknown genetic tumor risk syndromes and contributes to therapeutic stratification. However, its clinical value for adult patients with rare cancers is not well established. The

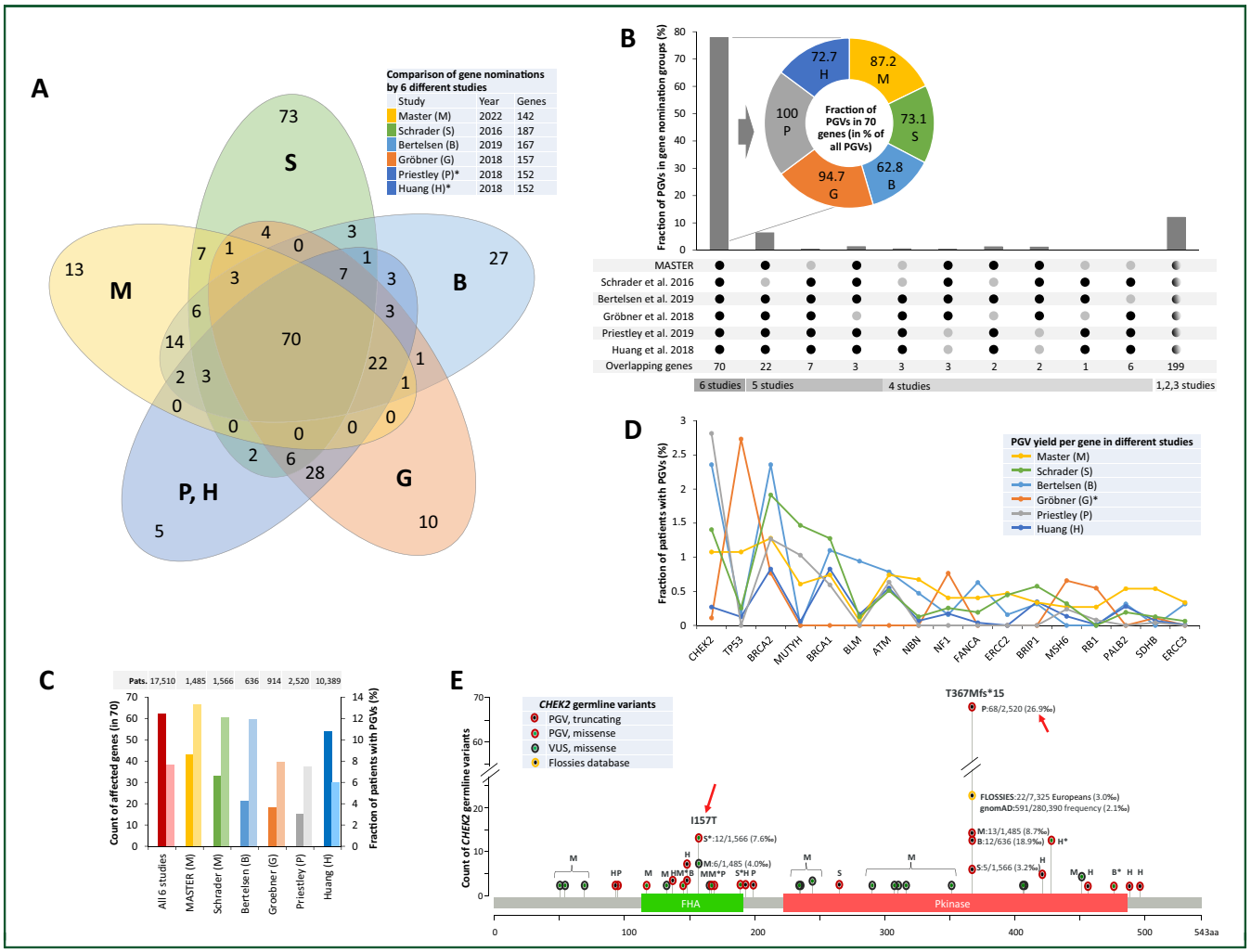


Figure 4. Genes and germline variant assessments in broad cancer sequencing studies. Proposal of a workflow.

(A) Comparison of gene nominations used for germline variant filtering by six studies sequencing matched tumor and control DNA. (B) Gene nominations and PGV yields across six different studies. Fractions of PGVs in common gene nominations by different studies as per all PGVs across all studies (upset blot depicting studies with common gene nominations; count of commonly nominated genes per group shown; all genes nominated only 1-3 times displayed together); circle: fraction of PGVs in 70 commonly analyzed genes per study. (C) PGV yields in 70 genes commonly analyzed by six studies. Left bars (darker): count of genes affected by PGVs; right bars (lighter): fractions of patients with PGVs (one PGV per patient assumed). (D) PGVs in a selection of commonly analyzed genes. Fractions of patients with PGVs in genes with highest variance between studies. (E) Germline variants and assessments in *CHEK2* across five studies. Variants showing discrepancies between studies and between study and CharGer assessment marked with red arrows and asterisks, respectively [*note: for the study of Gröbner et al.[16] detailed information about germline variants was not available; variant frequencies from the FLOSSIES database, found in >70-year-old healthy females[56]; and the gnomAD database (v2.1.1) (https://gnomad.broadinstitute.org/)[57] added]. (F) Comparison of PGV yields in broader cancer subgroups. Top: left bars (darker), count of genes affected by PGVs in 36 commonly analyzed genes; right bars (lighter): fractions of patients with PGVs of all patients in the respective group compiled from nine studies (bottom: counts and compilation of broad cancer subgroups; outer circles indicate patient selection from a specific study). Statistical comparison of PGV yields between common and rare adult as well as pediatric cancers was performed with Kruskal—Wallis rank sum and pairwise Wilcoxon rank sum tests (*$P < 0.05$). (G) PGV yields in broader cancer subgroups (e.g. common adult, rare adult, pediatric, sarcomas) in 36 genes commonly analyzed by nine studies. Fraction of patients with PGVs in selected genes (average of PGV yields of single-study cohorts compiling a broader cancer subgroup, sorted by descending yield of total PGVs per gene; note: Gröbner et al. 2018[16] did not include carrier status in AR CPGs and Fiala et al. 2021[22] counted the risk allele p.Ile1307Lys in *APC* as pathogenic). Box top right: distribution of biomarker baskets of PGVs across broader cancer subgroups. Statistical comparison of PGV yields per gene between common and rare adult as well as pediatric cancers was performed with Kruskal—Wallis rank sum and pairwise Wilcoxon rank sum test (*$P < 0.05$). (H) Workflow proposal for comprehensive germline variant evaluation in precision oncology programs and routine clinical genetic diagnostics.

aa, amino acids; ACMG, American College of Medical Genetics and Genomics; AD, autosomal dominant; AR, autosomal recessive; *CHEK2* transcript, NM_007194; CNA, copy number alteration; FHA, forkhead-associated domain; GUS, gene(s) of unknown significance in the context of cancer predisposition; HRD, homologous recombination deficiency; MSI, microsatellite instability; Pkinase, C-terminal serine/threonine kinase domain; som. mosaic, somatic mosaicism; SV, structural variants; TMB, tumor mutational burden; XLR, X-linked recessive.

[a]Note: Huang et al. 2018[2] and Priestley et al. 2019[20] used the same gene list.

[b]Note: Gröbner et al. 2018[16] did not include carrier status in AR CPGs.

prospective evaluation of germline variants within the NCT/DKTK MASTER trial revealed that a substantial proportion of patients with rare cancer entities or early-onset cancer had actionable PGVs (14.3%), and nearly half of them supported therapeutic recommendations. About 10% of patients were diagnosed with an AD cancer predisposition syndrome, of which 75% were newly diagnosed.

Comparing the MASTER cohort comprising predominantly rare cancers with other pan-cancer precision oncology studies indicated that rare adult cancers had

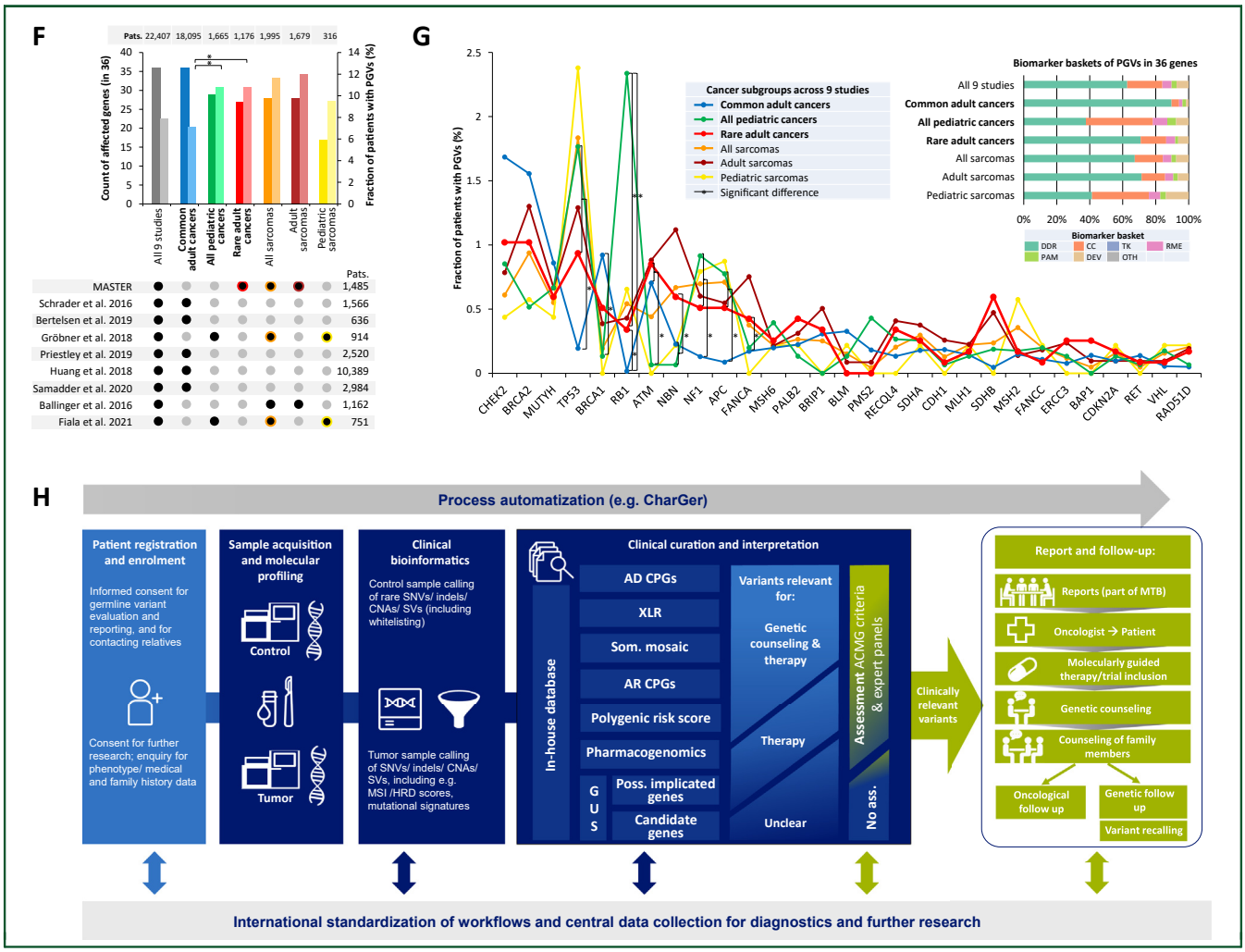


Figure 4. **Continued.**

significantly higher PGV yields than common adult cancers. This finding and the large proportion of underdiagnosed PGVs show that genetic tumor risk syndromes affect a substantial and underserved patient group. Patients with rare adult cancers and family members should be considered for genetic testing.[29,30] This could be accomplished by extending the inclusion criteria for genetic testing,[4,6] e.g. by including patients with specific entities or multiple tumors,[31] or by offering genetic testing to all cancer patients entering routine care, as done in an increasing number of precision oncology studies.[17,32-35]

Supporting known genotype—phenotype associations, patients with certain rare adult cancers harbored more PGVs in genes such as *RB1*, *TP53*, and *NF1*,[2,21] while an inverse correlation was observed for PGVs in *BRCA1*. Interestingly, PGVs in certain genes, primarily associated with breast cancer and other common cancers,[36] such as *BRCA2*, *ATM*, and *PALB2*, were found in a broad range of (rare) entities in our cohort. Indications for somatic biallelic inactivation in more than one-third of the cases support a potential relevance of these PGVs for tumorigenesis. Across >22 000 patients in nine precision oncology studies, PGVs in *ATM* were more frequent in adult patients with sarcomas and other rare cancers. In contrast, PGVs in *ATM* were hardly detected in pediatric cancer cohorts. This supports the association of PGVs in *ATM* with a broad range of common and also rare adult cancers and raises the question of appropriate surveillance and treatment.[37] However, the group sizes for individual rare cancer entities remain small and the relevance of PGVs in these genes in tumorigenesis needs further investigation in larger cohorts that can only be achieved by international collaborations.

Nearly half of the PGVs identified in the MASTER study had an impact on therapeutic recommendations. A similar yield (51%, $n = 1041/2037$) was reported for a large cohort of mainly common adult cancers analyzed with the MSK-IMPACT panel.[38] In the MSK study, higher levels of evidence for treatment recommendations (50% level 1 and level 1 microsatellite instable (MSI)-high versus 11% level 1 in MASTER) and higher implementation rates were reported (41% in MSK-IMPACT versus 24% in MASTER). This is likely due to the retrospective MSK study assignment, as it considered recent Food and Drug Administration approvals of, e.g. PARP inhibitors in pancreatic and prostate cancers,[39-41] and the high proportion of rare cancers in the MASTER study.

In a few cases, PARP inhibition was recommended in common cancers in the MASTER study before regulatory approval, such as in one patient with a metastatic prostate cancer with a PGV in *PALB2* and somatic signs of HRD, who responded to the treatment,[42] as well as six patients with pancreatic cancer and PGVs in HR-related genes. Additionally, PARP inhibitor therapy based on PGVs in *BRCA1/2* and other HR-related genes was recommended and explored in rare cancer entities. This included six patients with cholangiocarcinoma. In two of them, PARP inhibitor recommendation was implemented and one of these patients, who had a PGV in *RAD51C*, showed a clinical benefit (PFSr = 2). The second most frequent recommendation supported by PGVs was ICIs which are established in the treatment of MSI tumors. ICIs were recommended to nine patients with PGVs in genes associated with mismatch repair, two of them with a rare MSI sarcoma.[43] As exemplified in these cases, the comprehensive molecular analysis of tumors and controls may substantially improve omics-based stratification for personalized treatment options. However, additional basket protocols will be required to further explore the predictive value of composite biomarkers.[44] The NCT initiated a histology-independent clinical trial (ClinicalTrials.gov NCT03127215) investigating the genomic imprints of HRD. Other examples of promising[3] yet sparse germline-guided clinical trials investigate targeted treatments in early-stage cancers (NCT03499353), combination therapies (NCT04548752), targeted treatments in rare genetic tumor risk syndromes (NCT03871257, NCT03190915), and prevention (NCT04094675, NCT04711434).

The comparison of several precision oncology programs[2,16,18-20] revealed considerable differences in the respective germline variant evaluation workflow, such as (i) the employed gene lists, (ii) bioinformatics pipelines, and (iii) variant interpretation and reporting, which affected PGV yields and limited the comparability of studies. Based on our experience from prospective germline variant evaluation in the MASTER trial and literature review, we propose here a streamlined workflow for germline variant evaluation in large-scale precision oncology programs (Figure 4H, Supplementary Table S12, available at https://doi.org/10.1016/j.annonc.2022.07.008). Involvement of clinical genetics should be started early on and in parallel to the oncological management. Clinical data collection and molecular profiling, preferably from parallel tumor and matched control sequencing, should be carried out to improve variant detection as well as evaluation and hence the identification of patients with tumor risk syndromes and treatment recommendation.[45,46] Results of germline variant assessment according to ACMG/AMP criteria[13] should be discussed in a multidisciplinary MTB. Clinically relevant germline variants should be reported to the treating oncologist to initiate therapeutic regimens and further genetic work-up for the patient and relatives at risk within the framework of genetic counseling.

Using a pre-defined gene list to allow for timely and efficient germline variant identification and reporting of clinically relevant germline variants is recommended and widely applied in precision oncology programs. Applying an updated list of 141 CPGs (Supplementary Table S13, available at https://doi.org/10.1016/j.annonc.2022.07.008) from nine different studies, 89% of all PGVs across the studies would have been detected. Bioinformatics pipelines, gene lists, and filter criteria should be regularly updated and harmonized between studies to enable comparability and to further enable the power of genome sequencing, as well as to improve variant detection in known disease genes, such as SVs and deep-intronic variants.[47] Integration of tools such as CharGer,[48] Varsome,[49] or Franklin (https://franklin.genoox.com) with automatized variant prioritization and subsequent manual assessment will allow to cater for the expected increase in the number of patients and genes in the future. We suggest a similar strategy, but with a limited gene list (60 actionable AD CPGs including *MUTYH*, Supplementary Table S13, available at https://doi.org/10.1016/j.annonc.2022.07.008) for routine clinical genetic diagnostics of cancer patients, independent of tumor entity and age. Using this approach, 72% of all PGVs in the nine studies reviewed, including all variants in AD CPGs and biallelic variants in *MUTYH* in the MASTER study, would have been detected.

Certain aspects of this endeavor, such as cancer predisposition-related findings, are being addressed by the ACMG,[50] the ClinGen-[15] and Variant Interpretation for Cancer Consortium (VICC, https://cancervariants.org/), the ERN GENTURIS network,[51] GENIE (Genomics Evidence Neoplasia Information Exchange Consortium[52]), OncoKB,[53] or national consortia such as the German Consortium for Hereditary Breast and Ovarian Cancer,[28] and the German Consortium for Hereditary Non-Polyposis Colorectal Cancer.[54] However, further multidisciplinary collaborations of experts and institutions are required to implement and maintain this framework. Public access and collection of clinical and genetic data including follow-up information on molecularly deeply characterized patients with rare genetic tumor risk syndromes is likely to foster further research and translation in the field of oncology and cancer predisposition.

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DISCLOSURE

AJ: Honoraria: AstraZeneca. CH: Honoraria: Roche, Novartis; research funding: Boehringer Ingelheim; consulting or advisory board: Boehringer Ingelheim. CVG: Employed by Illumina since August 2021. LM reports non-financial support from Celgene outside the submitted work (travel expenses). MAu: Consulting or advisory board membership: Bristol-Myers Squibb, Ipsen, Merck KGaA, MSD Sharp & Dohme, Novartis, Pfizer, Roche, PharmaMar; research funding: AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Exelixis, Ipsen, Merck KGaA, Novartis, Pfizer, PharmaMar, Roche; honoraria: Blueprint Medicines, Bristol-Myers Squibb, Ipsen, Janssen-Cilag, Merck KGaA, Pfizer, PharmaMar. PHoh: Advisory board membership: Roche, Deciphera, PharmaMar, Pfizer, BluMedicine, GSK; honoraria: Bristol-Meyers-Squibb, Astrazeneca; research funding: Novartis, Siemens. MK: Honoraria: Bayer, MSD, Eisai, Ipsen, Eli Lilly; research funding: Eli Lilly, Ipsen, Loxo Oncology. RFS: Consulting or advisory board membership: Astellas, Bristol-Myers Squibb, Celgene, Pfizer; research funding: AstraZeneca, Boehringer Ingelheim, Daiichi Sankyo, Pfizer, PharmaMar, Roche; honoraria: Daiichi Sankyo, Pfizer. GF: Honoraria: Amgen, Armo Biosciences, Bayer, Bristol-Myers Squibb, Eli Lilly, HRA Pharma, Merck KGaA, MSD Sharp & Dohme, Pierre Fabre, Roche, Sanofi, Servier, Shire; research funding: Merck KGaA. SB: Consulting or advisory board membership: ADC Therapeutics, Blueprint Medicines, Daichii-Sankyo, Deciphera, Eli Lilly, Exelixis, Janssen-Cilag, Mundipharma, Novartis, Plexxikon; honoraria: Bayer, Eli Lilly, GlaxoSmithKline, Novartis, Pfizer, PharmaMar; research funding: Blueprint Medicines, Incyte, Novartis. JTS: Consulting or advisory board membership: AstraZeneca, Bayer, Bristol Myers Squibb, Celgene, Immunocore, Novartis, Roche, Shire; honoraria: AstraZeneca, Aurikamed, Baxalta, Bristol Myers Squibb, Celgene, Falk Foundation, iomedico, Immunocore, Novartis, Roche, Shire; research funding: Bristol-Myers Squibb, Celgene, Roche; minor equity in iTheranostics and Pharma15; member of the Board of Directors for Pharma15, all outside the submitted work. ALI: Honoraria: Bristol-Myers Squibb, Janssen-Cilag, Takeda, Roche. NvB: Consulting or advisory board membership: Novartis; honoraria: Novartis, Takeda. PJJ: Consulting or advisory board membership, honoraria, research funding, travel or accommodation expenses: Abbvie, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, Novartis, Pfizer, Roche, Servier. KHM: Consulting: Celgene/BMS, Novartis, Jazz Pharmaceuticals, Pfizer; honoraria: Celgene/BMS, Daiichi Sankyo, Astellas, AbbVie, Novartis; research funding: Celgene. AS: Consulting or advisory board membership, honoraria: AGCT, AstraZeneca, Bayer, Bristol-Myers Squibb, Chugai, Eli Lilly, Illumina, Janssen, MSD Sharp & Dohme, Novartis, Pfizer, Roche, Seattle Genetics, Takeda, Thermo Fisher; research funding: Bayer, Bristol-Myers Squibb, Chugai. WW: Consulting or advisory board membership, honoraria: Agilent, Amgen, Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Illumina, Merck, MSD Sharp & Dohme, Pfizer, NewOncology, Novartis, Roche, Takeda; research funding: AstraZeneca, Bristol-Myers Squibb, MSD Sharp & Dohme, Roche. SF: Consulting or advisory board membership: Bayer, Illumina, Roche; honoraria: Amgen, Eli Lilly, PharmaMar, Roche; research funding: AstraZeneca, Pfizer, PharmaMar, Roche; travel or accommodation expenses: Amgen, Eli Lilly, Illumina, PharmaMar, Roche. ES: Honoraria: AstraZeneca, Illumina. All other authors have declared no conflicts of interest.

DATA SHARING

All evaluated germline variants can be found in Supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2022.07.008. Sequencing data have been deposited in the European Genome-phenome Archive (https://www.ebi.ac.uk/ega/datasets) under accession EGAS00001005537.

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