

Preexisting and Post-COVID-19 Immune Responses to SARS-CoV-2 in Patients with Cancer



Tatjana Bilich^{1,2,3}, Malte Roerden^{2,3,4}, Yacine Maringer^{1,2,3}, Annika Nelde^{1,2,3}, Jonas S. Heitmann¹, Marissa L. Dubbelaar^{1,2,3,5}, Andreas Peter⁶, Sebastian Hörber⁶, Jens Bauer^{1,2,3}, Jonas Rieth^{1,2}, Marcel Wacker^{1,2,3}, Fiamma Berner⁷, Lukas Flatz^{7,8}, Stefanie Held⁹, Peter Brossart⁹, Melanie Märklin^{1,3}, Philipp Wagner¹⁰, Eva Erne¹¹, Reinhild Klein⁴, Hans-Georg Rammensee^{2,3,12}, Helmut R. Salih^{1,3}, and Julianne S. Walz^{1,2,3,13}



ABSTRACT

Patients with cancer, in particular patients with hematologic malignancies, are at increased risk for critical illness upon COVID-19. We here assessed antibody as well as CD4⁺ and CD8⁺ T-cell responses in unexposed and SARS-CoV-2-infected patients with cancer to characterize SARS-CoV-2 immunity and to identify immunologic parameters contributing to COVID-19 outcome. Unexposed patients with hematologic malignancies presented with reduced prevalence of preexisting SARS-CoV-2 cross-reactive CD4⁺ T-cell responses and signs of T-cell exhaustion compared with patients with solid tumors and healthy volunteers. Whereas SARS-CoV-2 antibody responses did not differ between patients with COVID-19 and cancer and healthy volunteers, intensity, expandability, and diversity of SARS-CoV-2 T-cell responses were profoundly reduced in patients with cancer, and the latter associated with a severe course of COVID-19. This identifies impaired SARS-CoV-2 T-cell immunity as a potential determinant for dismal outcome of COVID-19 in patients with cancer.

SIGNIFICANCE: This first comprehensive analysis of SARS-CoV-2 immune responses in patients with cancer reports on the potential implications of impaired SARS-CoV-2 T-cell responses for understanding pathophysiology and predicting severity of COVID-19, which in turn might allow for the development of therapeutic measures and vaccines for this vulnerable patient population.

See related commentary by Salomé and Horowitz, p. 1877.

¹Clinical Collaboration Unit Translational Immunology, German Cancer Consortium (DKTK), Department of Internal Medicine, University Hospital Tübingen, Tübingen, Germany. ²Institute for Cell Biology, Department of Immunology, University of Tübingen, Tübingen, Germany. ³Cluster of Excellence iFIT (EXC2180) "Image-Guided and Functionally Instructed Tumor Therapies," University of Tübingen, Tübingen, Germany. ⁴Department of Hematology, Oncology, Clinical Immunology and Rheumatology, University Hospital Tübingen, Tübingen, Germany. ⁵Quantitative Biology Center (QBiC), University of Tübingen, Tübingen, Germany. ⁶Institute for Clinical Chemistry and Pathobiochemistry, Department for Diagnostic Laboratory Medicine, University Hospital Tübingen, Tübingen, Germany. ⁷Institute of Immunobiology, Kantonsspital St. Gallen, St. Gallen, Switzerland. ⁸Department of Dermatology, University Hospital Tübingen, Tübingen, Germany. ⁹Department for Hematology and Oncology, University Hospital Bonn, Bonn, Germany. ¹⁰Department of Obstetrics and Gynecology, University Hospital of Tübingen, Tübingen, Germany. ¹¹Department of Urology, Medical

Faculty and University Hospital, Eberhard-Karls-University Tübingen, Tübingen, Germany. ¹²German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), partner site Tübingen, Tübingen, Germany.

¹³Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology and Robert Bosch Center for Tumor Diseases (RBCT), Stuttgart, Germany.

Note: Supplementary data for this article are available at Cancer Discovery Online (<http://cancerdiscovery.aacrjournals.org/>).

T. Bilich and M. Roerden contributed equally to this work.

Corresponding Author: Julianne S. Walz, Otfried-Müller-Str. 10, 72076 Tübingen, Germany. Phone: 0049-7071-2968746; Fax: 0049-7071-2925108; E-mail: juliane.walz@med.uni-tuebingen.de

Cancer Discov 2021;11:1982–95

doi: 10.1158/2159-8290.CD-21-0191

©2021 American Association for Cancer Research



INTRODUCTION

COVID-19 caused by SARS-CoV-2 has become a worldwide pandemic with dramatic socioeconomic consequences (1). The clinical course of SARS-CoV-2 infection is very heterogeneous, ranging from completely asymptomatic cases to severe COVID-19 lung disease with high mortality (2, 3). Critical illness of COVID-19 predominantly occurs in elderly individuals with medical comorbidities (2, 4, 5). Several recent studies reported on the increased risk of patients with cancer for a more severe course of COVID-19 and examined clinical predictors for mortality (6–8). Patients with hematologic malignancies (HM) were identified as one of the groups with poorest outcomes (6, 9). Several large-cohort studies are ongoing to better define risk groups such as patients undergoing specific cancer therapies (7, 10). The reasons for the overall increased SARS-CoV-2 mortality in patients with cancer so far remain ill-defined but mirror experiences with other viral pathogens (11–14). In addition to higher susceptibility to infection due to their overall poor health status and coexisting chronic diseases, patients with cancer have dysfunctional humoral and cellular immunity due to both the disease

itself and its treatment (15, 16). On the other hand, some authors have suggested that patients with cancer might be “protected” from severe COVID-19 morbidity due to their impaired ability to mount inflammatory immune responses (17, 18). As of now, data on immune responses and immunity to SARS-CoV-2 in patients with cancer are very limited. Two recent studies reported IgG antibody responses in 88% and 67%, respectively, of patients with acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL) who had COVID-19 (19, 20). To date, no data are available on SARS-CoV-2-directed T-cell responses in patients with cancer. In the meantime, multiple studies have identified the central role of SARS-CoV-2-specific T-cell responses for the clinical course of COVID-19 as well as for the development of long-term immunity (21–28). This comprises evidence for potential preexisting immunity mediated by T cells cross-reactive to human common cold coronaviruses (HCoV), which may provide a certain degree of protection against severe illness upon COVID-19 (21, 26, 27). We here conducted, to our knowledge, the first characterization of SARS-CoV-2-specific and cross-reactive T-cell and antibody responses in unexposed and SARS-CoV-2-infected patients with cancer. We report a reduced prevalence of preexisting,

cross-reactive T-cell responses, particularly in unexposed patients with hematologic malignancies. In addition, and in contrast to antibody responses, a reduced intensity, expandability, and diversity of T-cell responses in patients with cancer infected with SARS-CoV-2 was observed, with the latter being associated with a severe course of COVID-19.

RESULTS

Cohort of Patients with Cancer

SARS-CoV-2 immune responses were characterized in patients with cancer never exposed to SARS-CoV-2 (cancer-PRE group, $n = 199$, samples collected prior to SARS-CoV-2 pandemic, Table 1) and in patients with cancer with proven SARS-CoV-2 infection (cancer-COVID-19 group, $n = 17$, Table 2). PRE and COVID-19 groups comprised patients with various hematologic (HM-PRE, $n = 101$; HM-COVID-19, $n = 8$) and solid tumor (solid-PRE, $n = 98$; solid-COVID-19, $n = 9$) entities at different stages and time points during disease undergoing/after diverse anticancer treatments (Supplementary Tables S1 and S2). Patients with COVID-19 presented with a range of asymptomatic or mild (nonhospitalized, $n = 10$) to moderate and severe (hospitalized, $n = 7$) disease (Supplementary Table S2). To delineate particularities in SARS-CoV-2 immune responses in patients with cancer, previously described reference groups of noncancer SARS-CoV-2 convalescent healthy volunteers (HV-COVID-19, $n = 193$) and unexposed healthy individuals (HV-PRE, $n = 94$) were used for comparison (24).

Cross-Reactive SARS-CoV-2 T-cell Responses in Unexposed Patients with Cancer

To allow for standardized evaluation and determination of preexisting SARS-CoV-2 T-cell responses in unexposed patients with cancer (cancer-PRE), we employed broadly applicable human leukocyte antigen (HLA) class I and HLA-DR SARS-CoV-2 epitope compositions (EC). These comprised SARS-CoV-2 cross-reactive CD4⁺ and CD8⁺ T-cell epitopes recognized by both convalescents and individuals never exposed to SARS-CoV-2 (Supplementary Table S3), as described previously (24). Of the unexposed patients with cancer, 11.0% and 55.6% showed preexisting, cross-reactive T-cell responses to SARS-CoV-2 HLA class I and HLA-DR cross-reactive ECs, respectively, as assessed by IFN γ enzyme-linked immunospot (ELISPOT) assays after 12-day *in vitro* expansion (Fig. 1A–D). The recognition frequency of the HLA class I cross-reactive EC in unexposed patients with cancer was comparable to the HV-PRE group (11.0% vs. 16.0%; Fig. 1C). All unexposed patients with cancer with available HLA class I allotyping data (81%) showed at least one matching HLA allotype or HLA supertype (29) with the allotypes of the applied cross-reactive T-cell epitopes (Supplementary Data 1).

In contrast to HLA class I, the frequency of preexisting T-cell responses to the HLA-DR cross-reactive EC was significantly reduced in patients with cancer (55.6% vs. 77.7%, Fig. 1D). Subgroup analyses of cases with solid and hematologic malignancies revealed a markedly reduced frequency of cross-reactive CD4⁺ T-cell responses in patients with HMs compared with patients with solid tumors and healthy

Table 1. Characteristics of SARS-CoV-2 unexposed patients with cancer (cancer-PRE group)

Characteristic	Demographics			Clinical setting				
	Patients, n (%)	Female, n (%)	Median age (range), y	Diagnosis, n (%)	Watch and wait, n (%)	First-line therapy, n (%)	>First-line therapy, n (%)	Follow-up care, n (%)
Total	199	122 (61)	64 (22–89)	102 (51)	26 (13)	24 (12)	37 (19)	10 (5)
Hematologic malignancies								
AML/ALL	21 (11)	6 (29)	55 (22–78)	16 (76)	0 (0)	1 (5)	1 (5)	3 (14)
MDS	1 (1)	0 (0)	63 (63)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
CLL	30 (15)	11 (37)	58 (46–69)	1 (3)	19 (63)	7 (23)	1 (3)	2 (7)
MM	19 (10)	10 (53)	70 (40–89)	6 (32)	0 (0)	0 (0)	13 (68)	0 (0)
MGUS	4 (2)	3 (75)	72 (44–82)	0 (0)	4 (100)	0 (0)	0 (0)	0 (0)
CML	9 (5)	3 (33)	71 (59–80)	6 (67)	0 (0)	3 (33)	0 (0)	0 (0)
MPN	12 (6)	6 (50)	65 (39–85)	1 (8)	2 (17)	4 (33)	1 (8)	4 (33)
Others	5 (3)	2 (40)	60 (39–78)	4 (80)	0 (0)	1 (20)	0 (0)	0 (0)
Solid malignancies								
OvCa	67 (34)	67 (100)	61 (24–84)	62 (93)	0 (0)	0 (0)	5 (8)	0 (0)
NSCLC	20 (10)	6 (30)	68 (53–85)	0 (0)	0 (0)	7 (35)	13 (65)	0 (0)
Others	11 (6)	8 (73)	62 (23–80)	6 (55)	0 (0)	2 (18)	3 (27)	0 (0)

Abbreviations: ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; MM, multiple myeloma; MGUS, monoclonal gammopathy of undetermined significance; MPN, myeloproliferative neoplasms; NSCLC, non-small cell lung cancer; OvCa, ovarian carcinoma.

Table 2. Characteristics of convalescent patients with COVID-19 and cancer (cancer–COVID-19 group)

Characteristic	Demographics			Clinical setting				Follow-up care after allo-HSCT, n (%)
	Patients, n (%)	Female, n (%)	Median age (range), y	Diagnosis, n (%)	First-line therapy, n (%)	>First-line therapy, n (%)	Follow-up care, n (%)	
Total	17	9 (53)	58 (25–77)	0 (0)	2 (12)	2 (12)	9 (53)	4 (24)
Hematologic malignancies	8 (47)	4 (50)	60 (32–68)	0 (0)	1 (13)	1 (13)	2 (25)	4 (50)
AML	3 (18)	1 (33)	55 (32–66)	0 (0)	1 (33)	0 (0)	0 (0)	2 (67)
B-NHL	2 (12)	2 (100)	61 (53–68)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)
Other	3 (18)	1 (33)	65 (58–77)	0 (0)	0 (0)	1 (33)	0 (0)	2 (67)
Solid malignancies	9 (53)	6 (67)	56 (25–66)	0 (0)	1 (11)	1 (11)	7 (78)	0 (0)
Breast cancer	4 (24)	4 (100)	55 (46–58)	0 (0)	0 (0)	0 (0)	4 (100)	0 (0)
Urologic malignancy	2 (12)	2 (100)	56 (46–66)	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)
Other	3 (18)	2 (67)	62 (25–65)	0 (0)	0 (0)	1 (33)	2 (67)	0 (0)

Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplantation; B-NHL, B-cell non-Hodgkin lymphoma.

volunteers (HV; 34.3% vs. 77.3% and 77.7%, respectively; Fig. 1D). The entities with the lowest detection frequency of preexisting T-cell responses were myelodysplastic syndromes (MDS; 0.0%), myeloproliferative neoplasms (MPN; 0.0%), CLL (0.0%), and acute leukemias (AML and acute lymphoblastic leukemia, 6.3%; Fig. 1E) for HLA class I, as well as MDS (0.0%), chronic myeloid leukemia (CML; 14.3%), MPN (16.7%), and CLL (23.3%) in the case of HLA-DR (Fig. 1F). Univariate regression analysis revealed diagnosis of multiple myeloma, CLL, MPN, and CML as negative predictors for preexisting, cross-reactive T-cell responses to HLA-DR EC within HM (Supplementary Fig. S1A–S1D). Demographics (age and sex) and other clinical data of unexposed patients with cancer were not identified as predictors of cross-reactive SARS-CoV-2 T-cell responses (Supplementary Fig. S1A–S1D).

In contrast to the decreased frequency of cross-reactive CD4⁺ T-cell responses in patients with cancer, the intensity (spot counts per 5×10^5 cells, ELISPOT assays after 12-day *in vitro* expansion) of preexisting T-cell responses even showed a trend to increased intensities for HLA class I responses (Fig. 1G) in unexposed patients with cancer compared with HVs but was not significantly reduced for HLA-DR-directed responses (Fig. 1H).

Phenotyping of Cross-Reactive SARS-CoV-2 T Cells and Overall T-cell Function in Patients with Cancer

Characterization of cross-reactive T cells in unexposed patients with cancer using *ex vivo* flow cytometry-based assessment of surface markers and intracellular cytokine staining revealed that T-cell responses to the HLA class I cross-reactive EC were mediated by CD8⁺ T cells, with 1 of 3 patients showing an additional CD4⁺ T-cell response. T-cell responses to HLA-DR cross-reactive EC were predominantly mediated by CD4⁺ T cells, with 4 of 40 (10%) patients displaying an additional CD8⁺ T-cell response (Fig. 2A). Most cross-reactive CD4⁺

and CD8⁺ T cells were multifunctional, with positivity for several of the markers IL2, TNF, IFN γ , and CD107a (Fig. 2B; Supplementary Fig. S2A and S2B). In the patient showing additional CD4⁺ T-cell responses against HLA class I peptides, the frequency of CD8⁺ T cells dominated for all cytokines (Supplementary Fig. S2C). In patients showing additional CD8⁺ T-cell responses against HLA-DR-derived epitopes, multifunctional CD4⁺ T cells either dominated or were comparable to the frequency of CD8⁺ T cells (Supplementary Fig. S2D).

To put the frequency and intensity of cross-reactive SARS-CoV-2 T-cell responses in the broader context of antiviral T-cell immunity in patients with cancer, we compared preexisting SARS-CoV-2 T-cell responses with immune responses to chronic and past viral infections using HLA class I (matching the HLA allotypes of the cross-reactive ECs) and HLA-DR epitope panels, including Epstein-Barr virus (EBV), cytomegalovirus (CMV), adenovirus (ADV), and influenza virus (INF) peptides (Supplementary Table S3). Compared with preexisting, cross-reactive SARS-CoV-2 recognition, T-cell responses to other chronic and past viral infections were more frequent in patients with cancer (72.9% vs. 11.0% and 64.3% vs. 55.6% for HLA class I and HLA-DR, respectively). Concordance of T-cell responses to HLA class I and HLA-DR cross-reactive SARS-CoV-2 T-cell epitopes with recognition of other viral peptide panels was shown in 12 of 64 (18.8%) and 59 of 99 (59.6%) patients with HMs and 10 of 81 (12.3%) and 63 of 97 (64.9%) patients with solid tumors, respectively (Fig. 2C). A correlation between T-cell responses to HLA-DR SARS-CoV-2 cross-reactive EC and the HLA-DR viral peptide panel was observed (Fig. 2C; Pearson $p = 0.22$, $P = 0.002$). In line with the observations for cross-reactive SARS-CoV-2 T-cell responses, the frequency of T-cell responses to chronic and past viral infections was reduced in hematologic malignancies compared with solid tumors and HVs (61.4% vs. 84.7% and 95.2% for HLA class I; 54.5% vs. 74.5% and 97.6% for HLA-DR, respectively,

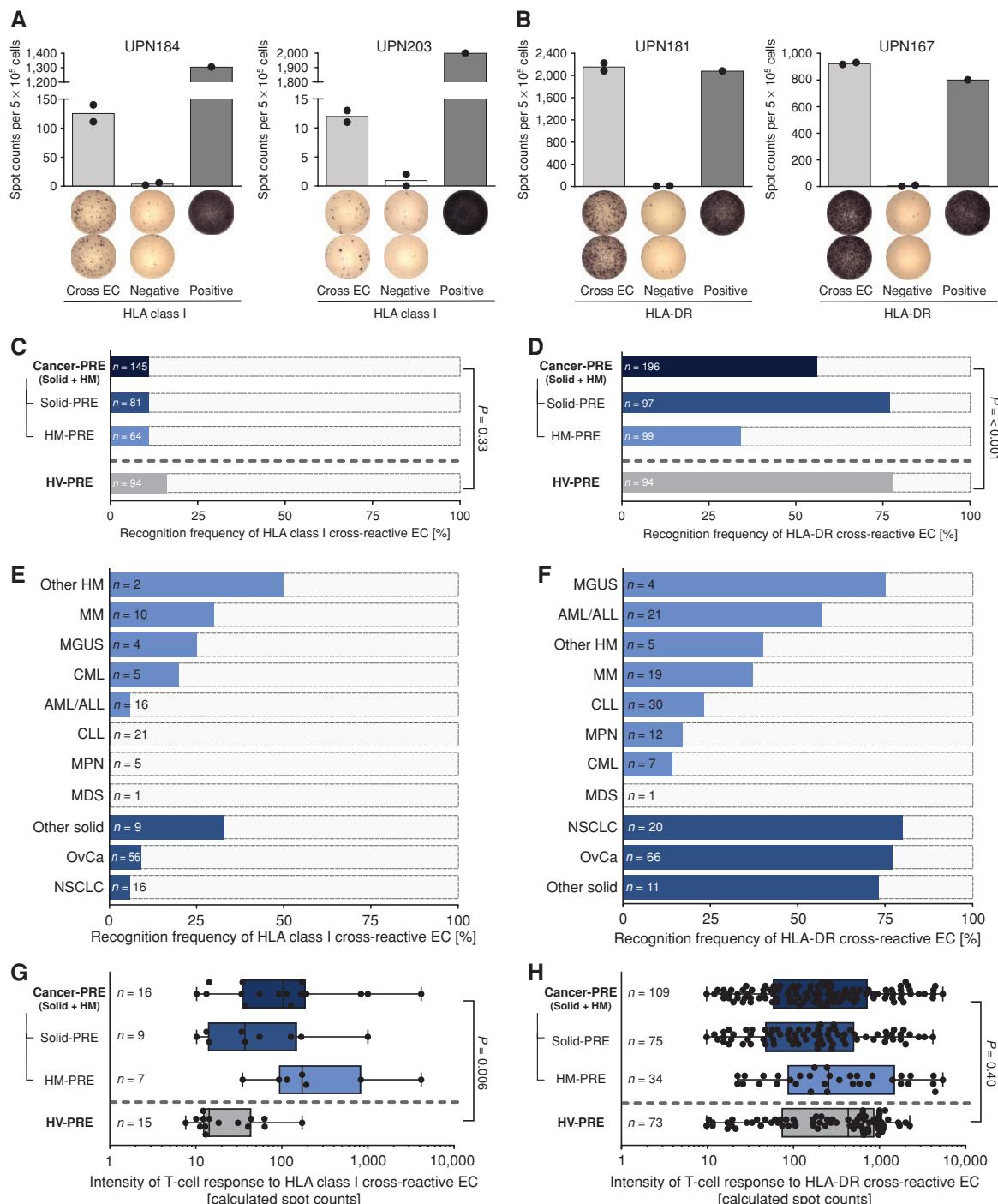


Figure 1. Cross-reactive SARS-CoV-2 T-cell responses in unexposed patients with cancer (cancer-PRE). **A** and **B**, Exemplary IFN γ ELISPOT assays of peripheral blood mononuclear cells from prepanemic patients with cancer after 12-day *in vitro* expansion with cross-reactive (**A**) HLA class I and (**B**) HLA-DR ECs. T-cell responses were considered positive when the mean spot count was ≥ 3 -fold higher than the respective negative control. Data are presented as scatter dot plot with mean. **C–F**, Recognition frequencies of cross-reactive (**C**, **E**) HLA class I and (**D**, **F**) HLA-DR ECs in unexposed patients with solid (solid-PRE) and hematologic malignancies (HM-PRE) compared with healthy volunteers (HV-PRE; two-sided Fisher exact test). Solid-PRE and HM-PRE represent subgroup analyses of the cancer-PRE cohort. Recognition frequencies are shown as absolute frequency revealing the proportion of patients with detected SARS-CoV-2 T-cell responses within the complete indicated cohorts. **G** and **H**, Intensities (calculated spot counts) of T-cell responses to cross-reactive (**G**) HLA class I and (**H**) HLA-DR EC in unexposed cancer-PRE patients and HV-PRE. Solid-PRE and HM-PRE represent subgroup analyses of the cancer-PRE cohort. Data are shown for donors with detectable T-cell responses; boxes represent median and 25th to 75th percentiles; whiskers are minimum to maximum, two-sided Mann-Whitney U test. ALL, acute lymphoblastic leukemia; cross EC, cross-reactive epitope composition; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; MPN, myeloproliferative neoplasm; NSCLC, non-small cell lung cancer; OvCa, ovarian carcinoma; UPN, uniform patient number.

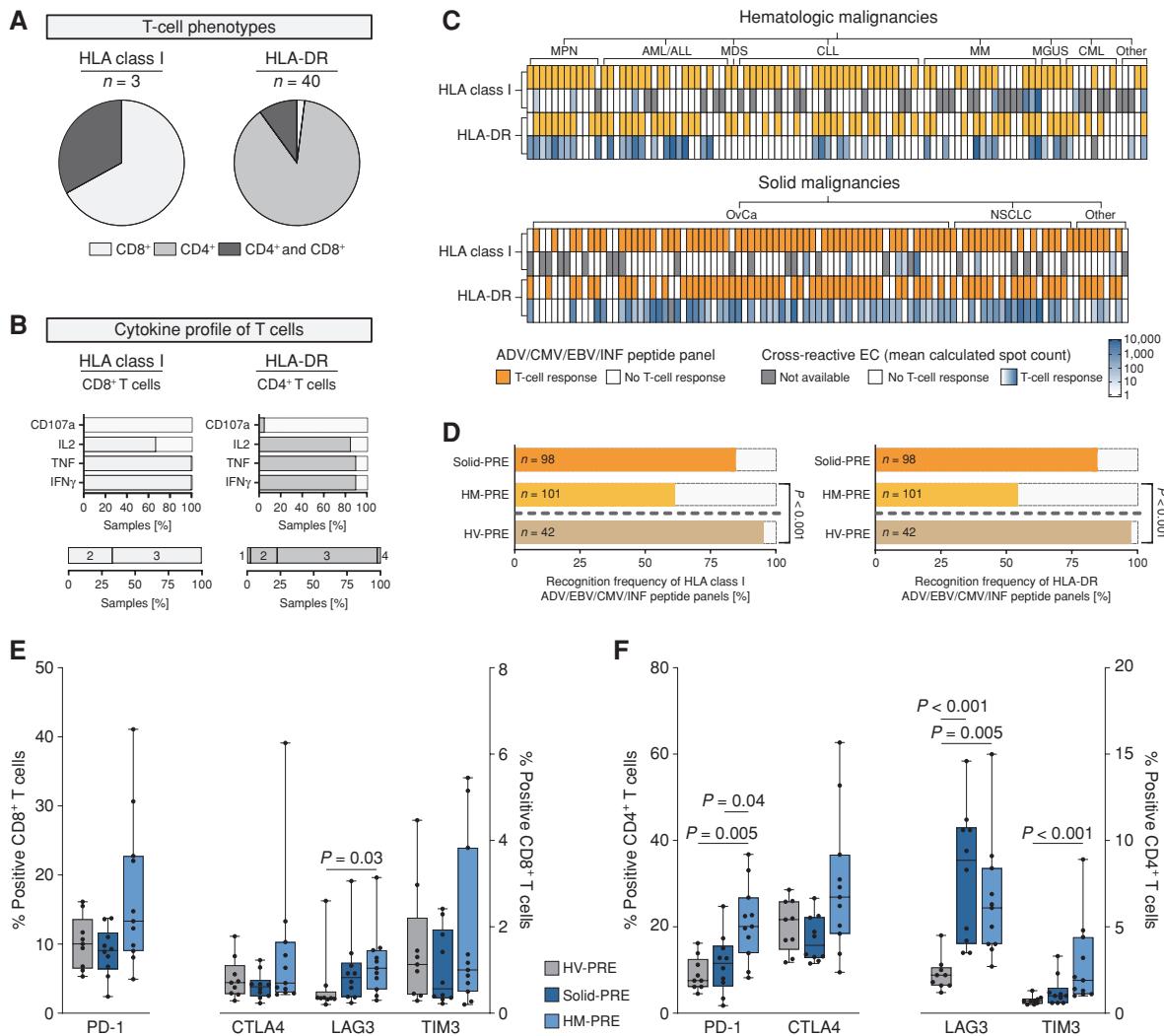


Figure 2. Characterization of SARS-CoV-2 T-cell phenotypes and general T-cell functionality in unexposed patients with cancer-PRE. **A** and **B**, Flow cytometry-based characterization of **(A)** phenotypes and **(B)** cytokine profiles of cross-reactive T-cell responses to HLA class I (left) and HLA-DR (right) using T cells from unexposed patients with cancer after 12-day *in vitro* expansion. **B**, Cytokine profiles (IFN γ , TNF, IL2) and degranulation marker (CD107a) expression of CD8 $^{+}$ (left) and CD4 $^{+}$ T (right) cells. Percentages of samples with CD107a $^{+}$, IL2 $^{+}$, TNF $^{+}$, and IFN γ $^{+}$ T-cell responses are shown in the top rows. Data are shown as absolute frequency revealing the proportion of samples within the total cohort with a detected cytokine production and degranulation in response to SARS-CoV-2 T-cell epitopes. The bottom rows display the proportion of samples revealing monofunctional (1), difunctional (2), trifunctional (3), or tetrafunctional (4) T-cell responses. **C**, Heat maps of recognition frequency and intensity (color gradient green) of T-cell responses to cross-reactive SARS-CoV-2 HLA class I and HLA-DR ECs in comparison to the recognition frequency of HLA class I and HLA-DR viral peptide panels (orange) comprising, for example, EBV, CMV, ADV, and INF peptides in unexposed patients with cancer (total n = 199; top, hematologic malignancies n = 101; bottom, solid malignancies n = 98). **D**, Recognition frequencies of HLA class I (left) and HLA-DR (right) ADV/CMV/EBV/INF peptide panels in unexposed patients with solid (solid-PRE) and hematologic malignancies (HM-PRE) compared with healthy volunteers (HV-PRE; Fisher exact test). Recognition frequencies are shown as absolute frequency revealing the proportion of patients with detected T-cell responses to ADV/CMV/EBV/INF viral peptide panels within the complete indicated cohorts. **E** and **F**, Flow cytometry-based analysis of T-cell exhaustion marker expression (PD-1, CTLA4, LAG3, TIM3) in **(D)** CD8 $^{+}$ and **(E)** CD4 $^{+}$ T cells of unexposed patients with SARS-CoV-2 and cancer (solid-PRE, n = 10; HM-PRE, n = 11) and healthy volunteers (HV-PRE, n = 9). HV-PRE, solid-PRE, and HM-PRE are depicted in gray, dark blue, and light blue, respectively. Boxes represent median and 25th to 75th percentiles; whiskers are minimum to maximum, Kruskal-Wallis test, P adjusted with Dunn multiple comparisons test.

Downloaded from http://aacrjournals.org/cancerdiscovery/article-pdf/11/8/1982/3102557/1982.pdf by ULB Bonn user on 30 May 2022

Fig. 2D). This suggests a generally reduced ability of patients with HMs to mount antiviral T-cell responses, rather than a SARS-CoV-2-specific effect.

CD4 $^{+}$ T Cells of Patients with HMs Show Patterns of Exhaustion

To uncover the reasons underlying the reduced frequency of preexisting SARS-CoV-2 cross-reactive CD4 $^{+}$ T-cell

responses in patients with HMs, we comparatively analyzed a panel of exhaustion markers (PD-1, CTLA4, LAG3, TIM3) in unstimulated CD8 $^{+}$ and CD4 $^{+}$ T cells in unexposed patients with HMs (n = 11), those with solid tumors (n = 10), and HVs (n = 9, Fig. 2E and F). Interestingly, and in contrast to CD8 $^{+}$ T cells from solid tumors and HMs as well as CD4 $^{+}$ T cells from solid tumors, a clear pattern of exhaustion was observed for CD4 $^{+}$ T cells of patients with HMs, with a profoundly higher

proportion of T cells expressing PD-1, LAG3, and TIM3 when compared with HVs (Fig. 2F). Exhaustion of CD4⁺ T cells may thus explain the observed reduction of preexisting cross-reactive HLA-DR SARS-CoV-2 T-cell responses in HMs.

Antibody and T-cell Responses to SARS-CoV-2 in Patients with Cancer and COVID-19

Two independent assays were employed to assess SARS-CoV-2 antibody responses in patients with cancer with proven SARS-CoV-2 infection (cancer-COVID-19; $n = 16$; Table 2) and in noncancer SARS-CoV-2 convalescents (HV-COVID-19; $n = 193$) to determine (i) ratios of IgG and IgA antibodies targeting the S1 domain of the spike protein, including the immunologically relevant receptor binding domain (RBD; EUROIMMUN; Fig. 3A and B), as well as (ii) antinucleocapsid antibody titers (Elecsys immunoassay including IgG; Fig. 3C). None of the patients received B cell-depleting therapy at or within 6 months prior to antibody analysis. Excluding borderline responses, 10 of 14 (71.4%), 11 of 16 (68.8%), and 14 of 16 (87.5%) patients with cancer and COVID-19 showed positive anti-S1 IgG and IgA and antinucleocapsid antibody responses, respectively. Neither antibody positivity rate nor antibody ratio or titer differed between cancer and HV convalescents, nor between patients with solid tumors and HMs (Fig. 3A–C). In line with previous reports (24, 30, 31), increased anti-S1 IgG ratios were observed in patients with cancer with a more severe course of COVID-19 requiring hospitalization and/or SARS-CoV-2 treatment but did not reach the level of statistical significance due to the small sample size (Fig. 3D).

Next, we aimed to enable standardized analyses of SARS-CoV-2 T-cell responses in patients with COVID-19 and cancer. To this end, we applied a SARS-CoV-2-specific EC recognized exclusively in COVID-19 convalescents in addition to the above-described HLA class I and HLA-DR SARS-CoV-2 cross-reactive ECs (Supplementary Table S3), as described previously (24). All analyzed patients with cancer had at least one HLA class I allotype matching the allotypes of the SARS-CoV-2-specific EC (Supplementary Data 1). Out of 17 patients with cancer and COVID-19, 14 (82.4%) showed T-cell responses to at least one of the HLA class I and HLA-DR specific or cross-reactive ECs, as assessed by *ex vivo* IFN γ ELISPOT assays (Fig. 3E–G; Supplementary Fig. S3A and S3B; Supplementary Table S2). Whereas no significant difference in the recognition frequency of T-cell responses to the SARS-CoV-2-specific HLA class I and HLA-DR ECs was observed, the frequency of T-cell responses to the HLA-DR cross-reactive EC was significantly reduced in patients with cancer compared with HVs (58.8% vs. 86.6%; Fig. 3F and G; Supplementary Fig. S3A and S3B). Alike in unexposed patients with cancer (cancer-PRE), a separate analysis for patients with COVID-19 and solid and hematologic neoplasms (cancer-COVID-19) revealed that this was due to the markedly reduced frequency of cross-reactive CD4⁺ T-cell responses in patients with HMs compared with patients with solid tumors and HVs (Supplementary Fig. S3B).

No statistically significant differences were observed with regard to the intensities of HV and cancer patient T-cell responses to HLA class I cross-reactive and SARS-CoV-2-specific ECs (Fig. 3H; Supplementary Fig. S3C). In contrast to the unexposed donors (PRE group), the intensity (spot

counts per 5×10^5 cells) of T-cell responses to HLA-DR SARS-CoV-2-specific EC was significantly lower in patients with cancer and COVID-19 compared with HVs (Fig. 3I). A similar trend was observed for T-cell responses to the HLA-DR cross-reactive EC (Supplementary Fig. S3D). No correlation of T-cell or antibody responses with patients' lymphocyte counts was observed in patients with cancer after COVID-19 (Supplementary Fig. S3E and S3F).

Reduced Expandability and Diversity of SARS-CoV-2 CD4⁺ T-cell Responses in Patients with Cancer and COVID-19

To better characterize SARS-CoV-2 T-cell responses in patients with cancer, we investigated preexisting and postinfectious SARS-CoV-2 T-cell responses in a patient with squamous cell laryngeal carcinoma (UPN317, aged 62 years, Supplementary Fig. S4A–S4C). Preexisting SARS-CoV-2 T-cell responses to HLA class I and HLA-DR ECs were detected neither directly *ex vivo* (Supplementary Fig. S4A) nor after 12-day *in vitro* expansion (Supplementary Fig. S4B). Post-COVID-19 CD4⁺ and CD8⁺ T cells of the patient showed high expression of the exhaustion marker CTLA4 (Supplementary Fig. S4C). *De novo* SARS-CoV-2 CD4⁺ T-cell responses to HLA-DR specific and cross-reactive ECs were detected 18 days after confirmation of infection (Supplementary Fig. S4B). Single-epitope mapping using 20 validated HLA-DR T-cell epitopes (binding to several HLA-DR allotypes, derived from multiple open reading frames, Supplementary Table S3; as described previously, ref. 24) after 12-day *in vitro* expansion revealed recognition of only one HLA-DR SARS-CoV-2 T-cell epitope, indicative of reduced expandability and diversity of the patient's T cells upon infection (Supplementary Fig. S4A–S4C). To expand on this observation, we analyzed recognition frequencies and intensities of SARS-CoV-2 T-cell responses to our 20 HLA-DR T-cell epitopes in the cancer-COVID-19 cohort ($n = 17$, Fig. 4A). These SARS-CoV-2-derived HLA-DR peptides show promiscuous binding to multiple HLA-DR allelic variants and thus could be used for the assessment of SARS-CoV-2 T-cell responses independent of the HLA-DR allotype (24, 32). We observed T-cell responses against 15 of 20 (75%) of these HLA-DR single T-cell epitopes in the cancer-COVID-19 cohort. T-cell response intensity after *in vitro* 12-day expansion, as a measure of expandability of SARS-CoV-2 T cells, showed high interindividual and interpeptide heterogeneity (Fig. 4B). Of note, for 73.3% (11/15) of SARS-CoV-2 HLA-DR peptides, reduced intensity of SARS-CoV-2 T-cell responses after *in vitro* expansion was observed in patients with cancer and COVID-19 compared with COVID-19 convalescent HVs, reaching the level of significance for 5 of 15 peptides (Fig. 4B). Patterns of T-cell phenotypes and functionality of SARS-CoV-2 T-cell responses were comparable between patients with cancer and COVID-19 and unexposed patients with cancer (Supplementary Fig. S5A–S5C).

Most important, the diversity of SARS-CoV-2 CD4⁺ T-cell responses—that is, the recognition of multiple different T-cell epitopes implicated as prerequisite for effective immunity (24, 33)—was significantly reduced in patients with cancer compared with HVs, with the most pronounced impairment observed in patients with HMs (median percentage of recognized peptides in cancer-COVID-19 and HM-COVID-19 vs. HV-COVID-19,

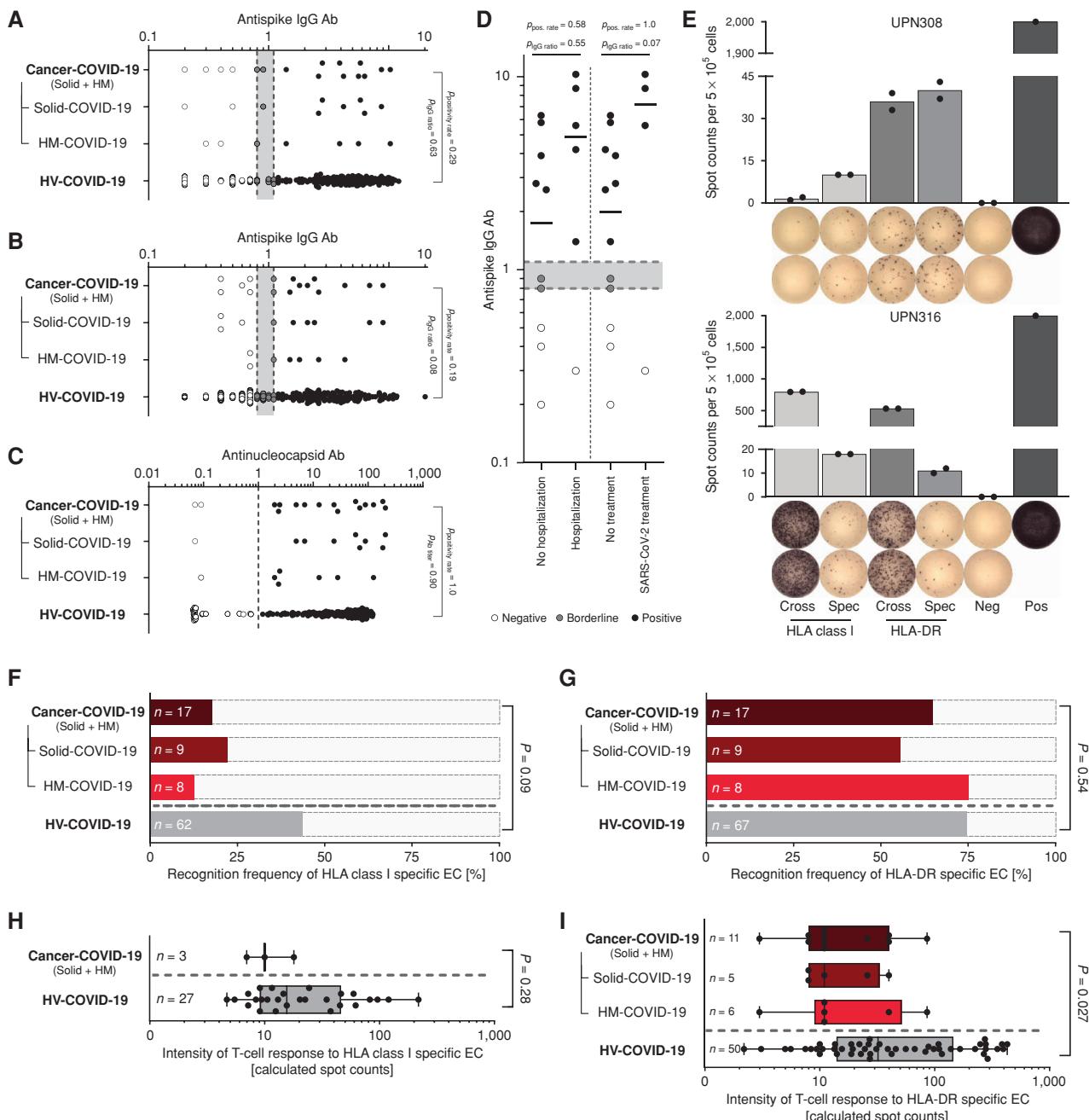


Figure 3. Antibody and T-cell responses to SARS-CoV-2 in patients with COVID-19 and cancer (cancer-COVID-19). **A–D**, SARS-CoV-2-directed antibody responses in cancer-COVID-19 and healthy volunteers (HV-COVID-19) after SARS-CoV-2 infection. SARS-CoV-2 serum antispike (**A**) IgG and (**B**) IgG S1 ratios (EUROIMMUN) as well as (**C**) antinucleocapsid antibody titers (Roche Elecsys). Solid-COVID-19 and HM-COVID-19 represent subgroup analyses of the cancer-COVID-19 cohort. **D**, Antispike IgG ratios (EUROIMMUN) of cancer-COVID-19 grouped by severity of COVID-19 (requiring hospitalization or SARS-CoV-2-specific treatment; Fisher exact test for positivity rate, Mann-Whitney U test for antibody ratios/titers of positive patients). Donors with negative and borderline responses are marked in white and gray, respectively. **E**, Exemplary ex vivo IFN γ ELISPOT assays using T cells from two cancer-COVID-19 patients stimulated with cross-reactive or SARS-CoV-2-specific HLA class I and HLA-DR epitope compositions. T-cell responses were considered positive when the mean spot count was ≥ 3 -fold higher than the negative control. **F** and **G**, Recognition frequencies of SARS-CoV-2-specific (**F**) HLA class I and (**G**) HLA-DR EC in cancer-COVID-19 patients and healthy volunteers (HV-COVID-19; Fisher exact test). Recognition frequencies are shown as absolute frequency revealing the proportion of patients with detected SARS-CoV-2 T-cell responses within the complete indicated cohorts. **H** and **I**, Intensities (calculated spot counts) of T-cell responses to SARS-CoV-2-specific (**H**) HLA class I and (**I**) HLA-DR EC in cancer-COVID-19 and HV-COVID-19. Data shown for donors with T-cell responses; boxes represent median and 25th to 75th percentiles; whiskers are minimum to maximum, two-sided Mann-Whitney U test. Solid-COVID-19 and HM-COVID-19 represent subgroup analyses of the cancer-COVID-19 cohort. cross, cross-reactive EC; pos, positive control; pos. rate, positivity rate; spec, SARS-CoV-2-specific EC; neg, negative control; UPN, uniform patient number.

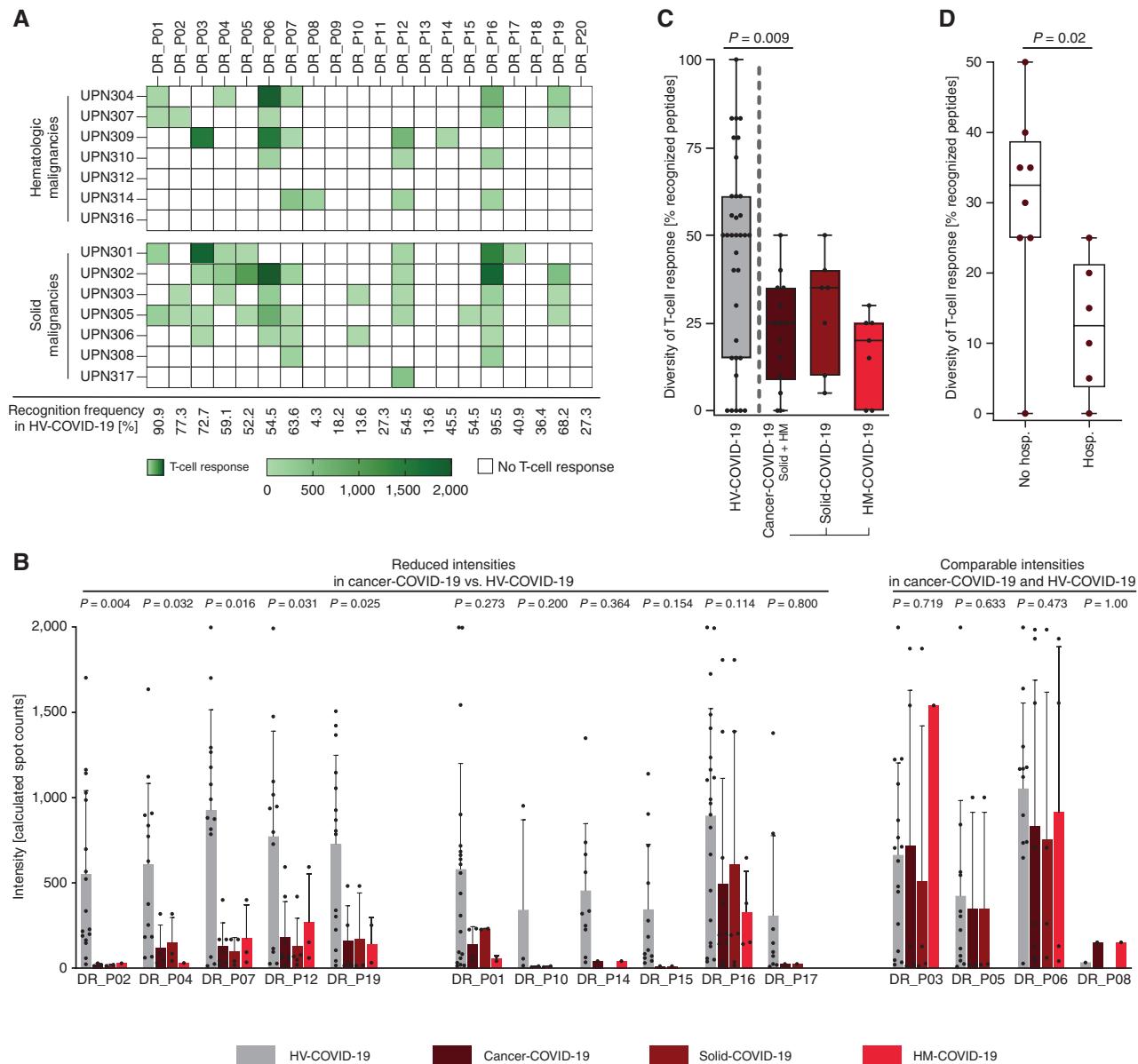


Figure 4. Diversity and intensity of SARS-CoV-2 T-cell responses to single HLA-DR epitopes in cancer-COVID-19 patients. **A**, Heat map of recognition frequencies and intensities (calculated spot count, color gradient green) of SARS-CoV-2 T cells to single SARS-CoV-2-derived HLA-DR T-cell epitopes in IFN γ ELISPOT assays after 12-day *in vitro* expansion using peripheral blood mononuclear cells from cancer-COVID-19 patients ($n = 14$) with hematologic (top panel, HM-COVID-19, $n = 7$) and solid tumors (bottom panel, solid-COVID-19, $n = 7$). Recognition frequencies of the respective HLA-DR T-cell epitopes in COVID-19 convalescent healthy volunteers (HV-COVID-19, $n = 34$) in IFN γ ELISPOT assays after 12-day *in vitro* expansion are listed below. **B**, Intensities of T-cell responses against individual HLA-DR SARS-CoV-2 T-cell epitopes in cancer-COVID-19 ($n = 14$) and HV-COVID-19 ($n = 34$) after SARS-CoV-2 infection. Data shown for donors with T-cell responses; mean with SD (error bars); indicated P values represent comparison of HV-COVID-19 and cancer-COVID-19. **C**, Diversity of SARS-CoV-2 HLA-DR T-cell responses (recognized peptides/tested peptides) in patients with cancer (cancer-COVID-19, $n = 14$; HM-COVID-19, $n = 7$; solid-COVID-19, $n = 7$) and HV-COVID-19 ($n = 34$) after SARS-CoV-2 infection as assessed in IFN γ ELISPOT assays after 12-day *in vitro* expansion; boxes represent median and 25th to 75th percentiles; whiskers are minimum to maximum, two-sided Mann-Whitney U test. **D**, Diversity of SARS-CoV-2 HLA-DR T-cell responses (recognized peptides/tested peptides) in hospitalized ($n = 8$) versus nonhospitalized patients with cancer ($n = 8$); boxes represent median and 25th to 75th percentiles; whiskers are minimum to maximum, two-sided Mann-Whitney U test. Solid-COVID-19 and HM-COVID-19 represent subgroup analyses of the cancer-COVID-19 cohort. hosp., hospitalization; no hosp., no hospitalization; UPN, uniform patient number.

25% and 20% vs. 50%, respectively; $P = 0.009$; Fig. 4C). Alike in convalescent donors without cancer (24), reduced T-cell response diversity in patients with cancer associated with a more severe course of COVID-19 (Fig. 4D), providing evidence that a broad SARS-CoV-2 T-cell response is lacking in patients with cancer and results in impairment of protective COVID-19 immunity.

DISCUSSION

Immune control of SARS-CoV-2 has been extensively studied during the COVID-19 pandemic, resulting in the delineation of distinct immune response patterns associated with severity of disease (24, 30, 31). We here report on SARS-CoV-2

T-cell and antibody responses in unexposed patients with cancer and patients with COVID-19 and cancer, providing insights into immune control of SARS-CoV-2 and unraveling factors that contribute to critical illness and high mortality of COVID-19 in this vulnerable patient cohort (2, 4–9, 34–36).

Cross-reactivity of T cells for different virus species or even among different pathogens is a well-known phenomenon postulated to enable heterologous immunity after exposure to a nonidentical pathogen (37–40). This heterologous immunity, facilitated by cross-reactive T-cell responses, can mediate both beneficial and adverse effects (39, 41, 42). Preexisting SARS-CoV-2 T-cell responses have been described by several groups in up to 81% of unexposed donors (24, 25, 27, 28). Sequence and physiochemical similarities as well as comparable affinity of SARS-CoV-2 cross-reactive T-cell epitopes to the known human common cold coronaviruses (HCoV-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKU1) provide a functional basis for preexisting SARS-CoV-2 T-cell responses (24, 26–28). Expandable, cross-reactive SARS-CoV-2 T cells are thought to provide beneficial heterologous immunity in COVID-19, which might contribute to the highly heterogeneous course of disease (43, 44). We here show that SARS-CoV-2 cross-reactive T-cell responses are detectable in unexposed patients with cancer. However, compared with HVs and patients with solid tumors, the detection frequency of cross-reactive CD4⁺ T cells was found to be significantly reduced in patients with HMs, who, among patients with cancer, are at increased risk for severe COVID-19 (6–9, 35, 36). This observation is critical, as previous data on acute and chronic viral infection (45–47) as well as on T-cell responses in COVID-19 convalescent and unexposed individuals have shown that CD4⁺ T cells play a central role in SARS-CoV-2 immunity. The pathophysiologic relevance is mirrored by a higher frequency of SARS-CoV-2 CD4⁺ T cells compared with CD8⁺ T cells detectable in convalescents and unexposed donors as well as an increased T-cell response intensity and a broader cytokine profile of CD4⁺ T cells (24, 48).

The recognition of SARS-CoV-2 HLA-DR-presented peptides not only by CD4⁺ but, to a lesser degree, also by CD8⁺ T cells is due to several embedded SARS-CoV-2 HLA class I peptides within the HLA-DR-binding sequences. HLA-DR epitopes with embedded HLA class I peptides, which induce both CD4⁺ and CD8⁺ T-cell responses, are widely used for anticancer and antiviral immunotherapy (49, 50). In addition, we were able to show that the SARS-CoV-2 HLA class I ECs could also be recognized by CD4⁺ T cells, which is a frequently described phenomenon especially in viral diseases (51, 52), as both HLA class I and HLA class II molecules could bind to primary and secondary peptide anchor motifs covering the central 9 to 10 amino acids. Moreover, we observed antiviral T-cell responses to HLA class I cross-reactive SARS-CoV-2 EC and ADV/CMV/EBV/INF-derived peptide pools not only in patients with cancer with an HLA allotype matching those for which the respective peptide pools were validated. This observation suggests that the used HLA class I T-cell epitopes are not restricted to a single HLA allotype and can be explained by HLA supertype clusters (29). This finding is in line with supertype-based promiscuously recognized HLA class I T-cell epitopes described for a variety of different viral diseases (53–58). Moreover, HLA supertypes have also been examined as a variable in studies of disease association, rates of

susceptibility, and outcome (58–61). However, as HLA class I cross-reactive SARS-CoV-2 T-cell responses are observed in only a very small proportion of HVs and patients with cancer, further large cohort studies with HLA-matched groups are required to delineate HLA allotype- or supertype-specific effects of cross-reactive T-cell responses potentially mediating heterologous SARS-CoV-2 immunity.

In the group of unexposed patients with HMs, we identified T-cell exhaustion as a potential reason for the reduced frequency of SARS-CoV-2 cross-reactive CD4⁺ T cells. T-cell exhaustion is a well-described phenomenon in patients with cancer, particularly in HMs (62–64). T-cell exhaustion in patients with HMs is accompanied by decreased T-cell counts, and hampered T-cell functionality is mediated by the disease itself as well as by immunosuppressive treatment regimens, resulting in reduced immune control and increased susceptibility to viral infections (65, 66).

Analysis of antibody responses in patients with cancer and in HVs with SARS-CoV-2 infection revealed comparable positivity rates as well as antibody ratios and titers. This is in line with recent findings in patients with CLL and AML (19, 20), indicative of functional humoral SARS-CoV-2 immunity in these patients. Similar to previous reports in patients with COVID-19 but without cancer (24, 30, 31), a trend to increased anti-S1 IgG ratios was observed in patients with cancer with a more severe course of COVID-19. Even if RBD antibody levels reportedly correspond to virus-neutralizing activity (67), the protective efficacy of the SARS-CoV-2 antibodies detected in patients with cancer remains unclear and needs to be validated in future studies employing neutralizing assays in larger cohorts.

The frequency of T-cell responses to HLA class I and HLA-DR SARS-CoV-2-specific ECs did also not differ between patients with cancer and HVs. In contrast, the frequency of T-cell responses to cross-reactive HLA-DR epitopes was significantly reduced in patients with HMs and COVID-19, which might reflect the lack of preexisting SARS-CoV-2 CD4⁺ T cells in unexposed patients with HMs. In contrast to unexposed patients with cancer, patients with cancer and COVID-19 presented with a lower intensity of SARS-CoV-2 T-cell responses compared with convalescent HVs. This might be explained by the observed impairment of expandability of SARS-CoV-2 T cells in the cancer–COVID-19 patient group and is in accordance with the reduced ability of patients with cancer to fight viral infections (65, 68, 69). T-cell exhaustion and reduced T-cell functionality were also reported for patients without cancer who had severe and critical illness upon COVID-19, and this impairment is observed even prior to the onset of acute respiratory distress syndrome (70). In line with these observations and based on the ability of immune checkpoint inhibition to restore functionality of exhausted T cells, allowing them to efficiently counteract viral infection (71, 72), clinical trials are currently examining the efficacy of anti-PD-1 antibody treatment to combat COVID-19 in both patients with and without cancer (NCT04333914, NCT04268537, NCT04356508, NCT04343144, and NCT04413838).

Previous work on viral diseases including SARS-CoV-2 implicates diversity of T-cell responses (i.e., recognition of multiple T-cell epitopes) as an important prerequisite for effective immunity (24, 33). We here show that diversity of

SARS-CoV-2 T-cell responses is decreased in patients with COVID-19 and cancer, particularly in HMs. The observed correlation of decreased T-cell response diversity with severity of COVID-19 delineates an immunologic cause for critical illness and high mortality of COVID-19 in patients with cancer.

Caveats of this study include the limited sample size of patients with cancer and COVID-19, the very heterogeneous patient cohort in terms of cancer diagnosis, disease status, cancer drug treatment, and the unequal coverage of ethnic backgrounds. Further large cohort studies, including entity- and cancer treatment-based subgroup analyses, are required to better define the role of SARS-CoV-2 T-cell immunity for susceptibility to SARS-CoV-2 infection and for the course of COVID-19. These future studies will further enable the delineation of the underlying mechanisms of impaired T-cell responses and T-cell exhaustion in patients with cancer in more detail, particularly in patients with HMs.

Nevertheless, this first characterization of SARS-CoV-2 immune responses in unexposed and SARS-CoV-2-infected patients with cancer has important implications for understanding the pathophysiology of COVID-19, as well as the selection and development of therapeutic measures and vaccines for these high-risk patient cohorts.

METHODS

Patients and Blood Samples

Peripheral blood mononuclear cells (PBMC) asserted from blood donations of patients with cancer were collected prior to the SARS-CoV-2 pandemic (April 2009 to November 2019) at three centers (University Hospital Tübingen, Germany; University Hospital Bonn, Germany; University Hospital St. Gallen, Switzerland) to assess the prevalence of preexisting cross-reactive SARS-CoV-2 T-cell responses (PRE group; $n = 199$).

Blood and serum samples from patients with cancer after SARS-CoV-2 infection (cancer–COVID-19 group; $n = 17$) were collected at the University Hospital Tübingen, Germany, from April 2020 to December 2020. SARS-CoV-2 infection was confirmed by PCR after nasopharyngeal swab. Sample collection for patients with COVID-19 and cancer was performed between 14 and 263 days (median, 47 days) after positive PCR. In nonhospitalized patients, donor characteristics and COVID-19 symptoms were assessed by questionnaire. For hospitalized patients, data were obtained from clinical data records.

Written informed consent was obtained in accordance with the Declaration of Helsinki protocol. The study was approved by and performed according to the guidelines of the local ethics committees (University of Tübingen: 454/2016/BO2, 406/2019/BO2, 179/2020/BO2; University of Bonn: 266/08; Kantonsspital St. Gallen: Ethikkommission Ostschweiz [EKOS] 16/079).

PBMCs were isolated by density gradient centrifugation. Serum was separated by centrifugation for 10 minutes and the supernatant was stored at -80°C . Detailed characteristics of patients with cancer are provided in Tables 1 and 2 and Supplementary Tables S1 and S2. HLA allotype data were obtained from clinical routine records of the respective study centers or performed from cell or DNA material of the study participants using single-molecule real-time sequencing (Histogenetics LLC; Supplementary Data 1). HLA class I allotyping data were available for 81% (117/145) of unexposed patients with cancer and for 100% (17/17) of patients with cancer and COVID-19. HLA allotype and supertype matching with the applied SARS-CoV-2 and ADV/CMV/EBV/INF-derived T-cell epitopes are indicated in Supplementary Data 1.

To delineate differences in SARS-CoV-2 immune responses in patients with cancer, a reference group of SARS-CoV-2 convalescent

and unexposed healthy individuals, described in a previous work, was applied (24). PBMCs of unexposed HVs (HV-PRE, $n = 94$) were collected prior to the SARS-CoV-2 pandemic (June 2007 to November 2019). Sample collection for COVID-19 convalescent HVs (HV-COVID-19, $n = 193$) was performed between 16 and 59 days (median, 41 days) after positive PCR.

Peptides

Synthetic peptides were provided by EMC Microcollections GmbH and INTAVIS Peptide Services GmbH & Co. KG. The SARS-CoV-2 HLA class I and HLA-DR T-cell epitopes as well as the applied ECs were characterized in detail in a previous work (24) analyzing T-cell responses in convalescents after COVID-19 and in healthy donors never exposed to the virus. To standardize analyses of SARS-CoV-2 T-cell responses, broadly applicable HLA class I and HLA-DR SARS-CoV-2-specific ECs (16 and 5 HLA class I and HLA-DR peptides, respectively) recognized exclusively in COVID-19 convalescents or cross-reactive ECs (9 and 10 HLA class I and HLA-DR peptides, respectively) recognized by both convalescents and individuals never exposed to SARS-CoV-2 (Supplementary Table S3) were used. HLA class I T-cell epitopes are derived from the nine most common HLA class I allotypes covering more than 69% (cross-reactive EC) and 90% (specific EC) of the European population with at least one allotype (<http://tools.iedb.org/population/>; ref. 73). For the analyses of T-cell response diversity, which requires the HLA-independent analysis of multiple peptides, promiscuous SARS-CoV-2 HLA-DR T-cell epitopes (20 peptides with multiple HLA-DR restrictions) were used. These promiscuous SARS-CoV-2-derived HLA-DR peptides were validated to bind multiple HLA-DR allelic variants and thus could be used for the assessment of SARS-CoV-2 T-cell responses independent of the HLA-DR allotype (24, 32).

HLA class I (matching the HLA allotypes of the cross-reactive EC and the corresponding HLA supertypes predicted by NetMHCpan-4.1) and HLA-DR viral peptide panels comprising peptides derived from EBV, CMV, ADV, and influenza (Supplementary Table S3) were used to assess the general T-cell functionality in patients with cancer.

IFN γ ELISPOT Assay Ex Vivo or Following 12-Day In Vitro Expansion

For 12-day *in vitro* expansion, PBMCs were pulsed with HLA class I or HLA-DR peptide pools (1 $\mu\text{g}/\text{mL}$ per peptide for HLA class I or 5 $\mu\text{g}/\text{mL}$ for HLA-DR) and cultured for 12 days adding 20 U/mL IL2 (Novartis) on days 3, 5, and 7. Peptide-stimulated (*in vitro* expanded) or freshly thawed (*ex vivo*) PBMCs were analyzed by IFN γ ELISPOT assay as described previously (24). Cell counts and viability assessed using the NucleoCounter NC-250 (chemometec) are listed in Supplementary Data 2. In brief, 2 to 8 $\times 10^5$ cells per well were incubated with 1 $\mu\text{g}/\text{mL}$ (HLA class I) or 2.5 $\mu\text{g}/\text{mL}$ (HLA-DR) ECs or single peptides in 96-well ELISPOT plates coated with anti-IFN γ antibody (clone 1-D1K, 2 $\mu\text{g}/\text{mL}$, MabTech, cat. 3420-3-250, RRID: AB_907283). PHA (Sigma-Aldrich) served as a positive control. An irrelevant HLA-matched control peptide (HLA-DR, ETVIT-VDTKAAGKGK, FLNA_HUMAN_{1669–1683}) or 10% dimethyl sulfoxide (DMSO) in double-distilled water (ddH₂O) for HLA class I served as a negative control. After 24 hours of incubation, spots were revealed with anti-IFN γ biotinylated detection antibody (clone 7-B6-1, 0.3 $\mu\text{g}/\text{mL}$; MabTech, cat. 3420-6-250, RRID: AB_907273), ExtrAvidin-Alkaline Phosphatase (1:1,000 dilution; Sigma-Aldrich), and BCIP/NBT (5-bromo-4-chloro-3-indolyl-phosphate/nitro-blue tetrazolium chloride; Sigma-Aldrich). Spots were counted using an ImmunoSpot S5 analyzer (CTL) and T-cell responses were considered positive when the mean spot count was ≥ 3 -fold higher than the mean spot count of the negative control. The intensity of T-cell responses is depicted as calculated spot counts, which represent the mean spot count of duplicates normalized to 5 $\times 10^5$ cells minus the normalized mean spot

count of the respective negative control. The recognition frequency of T-cell responses within groups indicates the percentage of donors recognizing the respective EC or peptide. The diversity of T-cell responses for single donors represents the number of recognized SARS-CoV-2-derived peptides (positive peptides/tested peptides).

Intracellular Cytokine and Cell Surface Marker Staining

Peptide-specific T cells were characterized by intracellular cytokine and cell surface marker staining as previously described (24). In brief, PBMCs were incubated with SARS-CoV-2 peptide/EC or negative control peptide Brefeldin A (Sigma-Aldrich), and GolgiStop (BD Biosciences). Staining was performed using Cytofix/Cytoperm solution (BD), Aqua live/dead (1:400 dilution; Invitrogen), APC/Cy7 antihuman CD4 (1:100 dilution; BioLegend, cat. 300518, RRID: AB_314086), PE/Cy7 antihuman CD8 (1:400 dilution; Beckman Coulter, cat. 737661, RRID: AB_1575980), Pacific Blue antihuman TNF (1:120 dilution; BioLegend, cat. 502920, RRID: AB_528965), FITC antihuman CD107a (1:100 dilution; BioLegend, cat. 328606, RRID: AB_1186036), and PE antihuman IFN γ monoclonal antibodies (1:200 dilution; BioLegend, cat. 506507, RRID: AB_315440). PMA and ionomycin (Sigma-Aldrich) served as positive control. All samples were analyzed on a FACS Canto II cytometer (BD).

Flow Cytometry-Based Analysis of T-cell Exhaustion Marker Expression

T-cell exhaustion was assessed using PBMC samples of unexposed patients with cancer. Analysis was based on cell surface expression of CD279 (PD-1) and CD366 (TIM3) as well as intracellular expression of CD152 (CTLA4) and CD223 (LAG3). T cells analyzed for expression of exhaustion markers were not peptide-stimulated. Staining was performed using Cytofix/Cytoperm solution (BD), Pacific Blue antihuman CD4 (1:100 dilution; BioLegend, cat. 300524, RRID: AB_493099), FITC antihuman CD8 (1:100 dilution; BioLegend, cat. 300905, RRID: AB_314908), PE antihuman CD152 (1:50 dilution; BioLegend, cat. 349905, RRID: AB_10645522), PE/Cy7 antihuman CD223 (1:100 dilution; BioLegend, cat. 369309, RRID: AB_2629752), APC antihuman CD279 (1:100 dilution; BioLegend, cat. 621609, RRID: AB_2832829), and APC/Cy7 antihuman CD366 (1:100 dilution; BioLegend, cat. 345025, RRID: AB_2565716). Viable cells were determined using Aqua live/dead (1:400 dilution; Invitrogen). All samples were analyzed on a FACS Canto II cytometer (BD).

SARS-CoV-2 IgG and IgA ELISA (EUROIMMUN)

SARS-CoV-2 IgG and IgA ELISA (EUROIMMUN) assays were performed as previously described (24) on an automated BEP 2000 Advance system (Siemens Healthcare Diagnostics GmbH) according to the manufacturer's instructions. The assay detects anti-SARS-CoV-2 IgG and IgA directed against the S1 domain of the viral spike protein (including the immunologically relevant receptor binding domain) and relies on an assay-specific calibrator to report a ratio of specimen absorbance to calibrator absorbance. The final interpretation of positivity is determined by the ratio above a threshold value given by the manufacturer: positive (ratio ≥ 1.1), borderline (ratio 0.8–1.0), or negative (ratio < 0.8). Quality control was performed following the manufacturer's instructions on each day of testing.

Elecsys Anti-SARS-CoV-2 Immunoassay (Roche Diagnostics GmbH)

The Elecsys anti-SARS-CoV-2 electrogenerated chemiluminescence immunoassay was performed as previously described (24). The assay detects high-affinity antibodies (including IgG) directed against the nucleocapsid protein of SARS-CoV-2 in human serum. Readout was performed on a Cobas e411 analyzer. Negative results were defined by a cutoff index of < 1.0 . Quality control was performed following the manufacturer's instructions on each day of testing.

Software and Statistical Analysis

Data are displayed as mean with standard deviation (for $n \geq 3$), scatter dot plot with mean, box plot as median with 25th or 75th percentiles, and minimum/maximum whiskers. Description of the applied tests used for statistical analysis is provided within the respective figure legends. Continuous data were tested for distribution (Shapiro-Wilk test), and individual groups were tested by use of Wilcoxon, Mann-Whitney U, Kruskal-Wallis test, or Kruskal-Wallis with Dunn multiple comparisons test, where appropriate. Categorical data were tested by use of Fisher exact test or Pearson χ^2 test. Univariate logistic regression analysis was performed to assess the predictive value of patient demographics and clinical parameters for SARS-CoV-2 cross-reactive EC recognition. Flow cytometric data were analyzed using FlowJo 10.0.8 (BD). Graphs were plotted using RStudio and GraphPad Prism 9.0.0 (GraphPad Software). Statistical analyses were conducted using GraphPad Prism 9.0.0 and SPSS 26 (IBM) software. P values of < 0.05 were considered statistically significant.

Authors' Disclosures

T. Bilich reports a patent for PCT/EP 20 169 047.6 pending and a patent for PCT/EP 20 190 070.1 pending. A. Nelde reports a patent for PCT/EP 20 169 047.6 pending and a patent for PCT/EP 20 190 070.1 pending. L. Flatz reports grants from Swiss National Science Foundation and grants from Swiss Cancer League during the conduct of the study; grants from Hookipa Pharma, grants from Novartis Foundation, grants from Novartis, and grants from Bristol-Meyers Squibb outside the submitted work. H. Rammensee reports grants from DFG, grants from Ernst-Jung-Stiftung, and grants from Landesforschungspreis Baden-Württemberg during the conduct of the study; has a patent for COVID peptides (PCT/EP 20 169 047.6 and PCT/EP 20 190 070.1) pending and a patent for a new adjuvant, XS15, pending; and is shareholder of CureVac, immatics, and Synimmune. J.S. Walz reports a patent for CoVac-1 peptide cocktail, application number: PCT/EP 20 190 070.1 pending to University Hospital Tübingen and a patent for SARS-CoV-2 CD8 $^{+}$ and CD4 $^{+}$ T-cell epitopes, application number: PCT/EP 20 169 047.6 pending to University Hospital Tübingen. No disclosures were reported by the other authors.

Authors' Contributions

T. Bilich: Conceptualization, data curation, formal analysis, investigation, visualization, methodology, writing-original draft, writing-review and editing. **M. Roerden:** Conceptualization, data curation, formal analysis, investigation, visualization, methodology, writing-original draft, writing-review and editing. **Y. Maringer:** Investigation, writing-review and editing. **A. Nelde:** Investigation, methodology, writing-review and editing. **J.S. Heitmann:** Data curation, formal analysis, investigation, methodology, writing-review and editing. **M.L. Dubbelaar:** Visualization, writing-review and editing. **A. Peter:** Resources, investigation, writing-review and editing. **S. Horber:** Investigation, writing-review and editing. **J. Bauer:** Investigation, writing-review and editing. **J. Rieth:** Investigation, writing-review and editing. **M. Wacker:** Investigation, writing-review and editing. **F. Berner:** Investigation, writing-review and editing. **L. Flatz:** Resources, investigation, writing-review and editing. **S. Held:** Investigation, writing-review and editing. **P. Brossart:** Resources, investigation, writing-review and editing. **M. Marklin:** Investigation, writing-review and editing. **P. Wagner:** Resources, supervision, investigation, methodology, writing-review and editing. **E. Erne:** Resources, supervision, investigation, writing-review and editing. **R. Klein:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, writing-original draft, project administration, writing-review and editing. **H. Rammensee:** Resources, supervision, investigation, methodology, writing-review and editing. **H.R. Salih:** Resources, supervision, investigation, writing-review and

editing. **J.S. Walz:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, writing—original draft, project administration, writing—review and editing.

Acknowledgments

We thank Ulrike Schmidt, Sabrina Sauter, Hannah Zug, Sabrina Augstein, Celine Reiß, Valentina Agrusa, Michael Beller, Richard Schaad, and Claudia Falkenburger for technical support and project coordination. This work was supported by the Bundesministerium für Bildung und Forschung (BMBF, FKZ:01KI20130, Juliane S. Walz), the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, Grant WA 4608/1-2, Juliane S. Walz), the Deutsche Forschungsgemeinschaft under Germany's Excellence Strategy (Grant EXC2180-390900677, Juliane S. Walz, Helmut R. Salih, Hans-Georg Rammensee), the German Cancer Consortium (DKTK; Juliane S. Walz, Helmut R. Salih, Hans-Georg Rammensee), the Wilhelm Sander Stiftung (Grant 2016.177.2, Juliane S. Walz), the José Carreras Leukämie-Stiftung (Grant DJCLS 05 R/2017, Juliane S. Walz), and the Fortüne Program of the University of Tübingen (Fortüne number 2451-0-0 and 2581-0-0, Juliane S. Walz and Malte Roerden).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 12, 2021; revised April 15, 2021; accepted May 12, 2021; published first May 19, 2021.

REFERENCES

- Khan S, Siddique R, Shereen MA, Ali A, Liu J, Bai Q, et al. Emergence of a novel coronavirus, severe acute respiratory syndrome coronavirus 2: biology and therapeutic options. *J Clin Microbiol* 2020;58:e00187–20.
- Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;395:1054–62.
- Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *JAMA* 2020;323:1488–94.
- Myers LC, Parodi SM, Escobar GJ, Liu VX. Characteristics of hospitalized adults with COVID-19 in an integrated health care system in California. *JAMA* 2020;323:2195–8.
- Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York city area. *JAMA* 2020;323:2052–9.
- Yang K, Sheng Y, Huang C, Jin Y, Xiong N, Jiang K, et al. Clinical characteristics, outcomes, and risk factors for mortality in patients with cancer and COVID-19 in Hubei, China: a multicentre, retrospective, cohort study. *Lancet Oncol* 2020;21:904–13.
- Rivera DR, Peters S, Panagiotou OA, Shah DP, Kuderer NM, Hsu C-Y, et al. Utilization of COVID-19 treatments and clinical outcomes among patients with cancer: a COVID-19 and Cancer Consortium (CCC19) cohort study. *Cancer Discov* 2020;10:1514–27.
- Robilotti EV, Babady NE, Mead PA, Rolling T, Perez-Johnston R, Bernardes M, et al. Determinants of COVID-19 disease severity in patients with cancer. *Nat Med* 2020;26:1218–23.
- Dai M, Liu D, Liu M, Zhou F, Li G, Chen Z, et al. Patients with cancer appear more vulnerable to SARS-CoV-2: a multicenter study during the COVID-19 outbreak. *Cancer Discov* 2020;10:783–91.
- Lee LY, Cazier JB, Starkey T, Turnbull C, Team UCCMP, Kerr R, et al. COVID-19 mortality in patients with cancer on chemotherapy or other anticancer treatments: a prospective cohort study. *Lancet* 2020;395:1919–26.
- Chemaly RF, Vigil KJ, Saad M, Vilar-Compte D, Cornejo-Juarez P, Perez-Jimenez C, et al. A multicenter study of pandemic influenza A (H1N1) infection in patients with solid tumors in 3 countries: early therapy improves outcomes. *Cancer* 2012;118:4627–33.
- Curigliano G. Cancer patients and risk of mortality for COVID-19. *Cancer Cell* 2020;38:161–3.
- Kim Y-J, Lee ES, Lee Y-S. High mortality from viral pneumonia in patients with cancer. *Infect Dis* 2019;51:502–9.
- Schluck K, Grundbichler M, Auburger J, Kern JM, Hell M, Hohla F, et al. Cytomegalovirus reactivation and its clinical impact in patients with solid tumors. *Infect Agent Cancer* 2015;10:45.
- Raab MS, Podar K, Breitkreutz I, Richardson PG, Anderson KC. Multiple myeloma. *Lancet* 2009;374:324–39.
- Thommen DS, Schumacher TN. T cell dysfunction in cancer. *Cancer Cell* 2018;33:547–62.
- Reda G, Noto A, Cassin R, Zamprogna G, Borella C, Scarfo L, et al. Reply to “CLL and COVID-19 at the Hospital Clinic of Barcelona: an interim report” Analysis of six hematological centers in Lombardy. *Leukemia* 2020;34:2531–2.
- Treon SP, Castillo JJ, Skarbnik AP, Soumerai JD, Ghobrial IM, Guerera ML, et al. The BTK inhibitor ibrutinib may protect against pulmonary injury in COVID-19-infected patients. *Blood* 2020;135:1912–5.
- Roeker LE, Knorr DA, Pessin MS, Ramanathan LV, Thompson MC, Leslie LA, et al. Anti-SARS-CoV-2 antibody response in patients with chronic lymphocytic leukemia. *Leukemia* 2020;34:3047–9.
- O'Nions J, Muir L, Zheng J, Rees-Spear C, Rosa A, Roustan C, et al. SARS-CoV-2 antibody responses in patients with acute leukaemia. *Leukemia* 2021;35:289–92.
- Weiskopf D, Schmitz KS, Raadsen MP, Grifoni A, Okba NMA, Endemann H, et al. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. *Sci Immunol* 2020;5:eabd2071.
- Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* 2021;371:eabf4063.
- Bilich T, Nelde A, Heitmann JS, Maringer Y, Roerden M, Bauer J, et al. T cell and antibody kinetics delineate SARS-CoV-2 peptides mediating long-term immune responses in COVID-19 convalescent individuals. *Sci Transl Med* 2021;13:eabf7517.
- Nelde A, Bilich T, Heitmann JS, Maringer Y, Salih HR, Roerden M, et al. SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. *Nat Immunol* 2021;22:74–85.
- Le Bert N, Tan AT, Kunasegaran K, Tham CY, Hafezi M, Chia A, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature* 2020;584:457–62.
- Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* 2020;181:1489–501.
- Braun J, Loyal L, Frentsch M, Wendisch D, Georg P, Kurth F, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature* 2020;587:270–4.
- Mateus J, Grifoni A, Tarke A, Sidney J, Ramirez SI, Dan JM, et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science* 2020;370:89–94.
- Sidney J, Peters B, Frahm N, Brander C, Sette A. HLA class I supertypes: a revised and updated classification. *BMC Immunol* 2008;9:1–15.
- Laing AG, Lorenc A, Del Barrio IDM, Das A, Fish M, Monin L, et al. A dynamic COVID-19 immune signature includes associations with poor prognosis. *Nat Med* 2020;26:1623–35.
- Chen X, Pan Z, Yue S, Yu F, Zhang J, Yang Y, et al. Disease severity dictates SARS-CoV-2-specific neutralizing antibody responses in COVID-19. *Signal Transduct Target Ther* 2020;5:180.
- Tarke A, Sidney J, Kidd CK, Dan JM, Ramirez SI, Yu ED, et al. Comprehensive analysis of T cell immunodominance and immuno-prevalence of SARS-CoV-2 epitopes in COVID-19 cases. *Cell Rep Med* 2021;2:100204.
- Yager EJ, Ahmed M, Lanzer K, Randall TD, Woodland DL, Blackman MA. Age-associated decline in T cell repertoire diversity leads to holes

- in the repertoire and impaired immunity to influenza virus. *J Exp Med* 2008;205:711–23.
34. Passamonti F, Cattaneo C, Arcaini L, Bruna R, Cavo M, Merli F, et al. Clinical characteristics and risk factors associated with COVID-19 severity in patients with haematological malignancies in Italy: a retrospective, multicentre, cohort study. *Lancet Haematol* 2020;7:e737–e45.
 35. Yu J, Ouyang W, Chua MLK, Xie C. SARS-CoV-2 transmission in patients with cancer at a tertiary care hospital in Wuhan, China. *JAMA Oncol* 2020;6:1108–10.
 36. Kuderer NM, Choueiri TK, Shah DP, Shyr Y, Rubinstein SM, Rivera DR, et al. Clinical impact of COVID-19 on patients with cancer (CCC19): a cohort study. *Lancet* 2020;395:1907–18.
 37. Friberg H, Burns L, Woda M, Kalayanarooj S, Endy TP, Stephens HA, et al. Memory CD8+ T cells from naturally acquired primary dengue virus infection are highly cross-reactive. *Immunol Cell Biol* 2011;89:122–9.
 38. Su LF, Kidd BA, Han A, Kotzin JJ, Davis MM. Virus-specific CD4(+) memory-phenotype T cells are abundant in unexposed adults. *Immunity* 2013;38:373–83.
 39. Petrova G, Ferrante A, Gorski J. Cross-reactivity of T cells and its role in the immune system. *Crit Rev Immunol* 2012;32:349–72.
 40. Vali B, Tohn R, Cohen MJ, Sakhdari A, Sheth PM, Yue FY, et al. Characterization of cross-reactive CD8+ T-cell recognition of HLA-A2-restricted HIV-Gag (SLYNTVATL) and HCV-NS5b (ALYDVVSKL) epitopes in individuals infected with human immunodeficiency and hepatitis C viruses. *J Virol* 2011;85:254–63.
 41. Welsh RM, Che JW, Brehm MA, Selin LK. Heterologous immunity between viruses. *Immunol Rev* 2010;235:244–66.
 42. Watkin LB, Mishra R, Gil A, Aslan N, Ghersi D, Luzuriaga K, et al. Unique influenza A cross-reactive memory CD8 T-cell receptor repertoire has a potential to protect against EBV seroconversion. *J Allergy Clin Immunol* 2017;140:1206–10.
 43. Lipsitch M, Grad YH, Sette A, Crotty S. Cross-reactive memory T cells and herd immunity to SARS-CoV-2. *Nat Rev Immunol* 2020;20:709–13.
 44. Saletti G, Gerlach T, Jansen JM, Molle A, Elbahesh H, Ludlow M, et al. Older adults lack SARS CoV-2 cross-reactive T lymphocytes directed to human coronaviruses OC43 and NL63. *Sci Rep* 2020;10:1–10.
 45. Wilkinson TM, Li CK, Chui CS, Huang AK, Perkins M, Liebner JC, et al. Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. *Nat Med* 2012;18:274–80.
 46. Zhou M, Xu D, Li X, Li H, Shan M, Tang J, et al. Screening and identification of severe acute respiratory syndrome-associated coronavirus-specific CTL epitopes. *J Immunol* 2006;177:2138–45.
 47. Sogholian DZ, Jessen H, Flanders M, Sierra-Davidson K, Cutler S, Pertel T, et al. HIV-specific cytolytic CD4 T cell responses during acute HIV infection predict disease outcome. *Sci Transl Med* 2012;4:123ra25.
 48. Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* 2021;184:861–80.
 49. May RJ, Dao T, Pinilla-Ibarz J, Korontsvit T, Zakhaleva V, Zhang RH, et al. Peptide epitopes from the Wilms' tumor 1 oncoprotein stimulate CD4+ and CD8+ T cells that recognize and kill human malignant mesothelioma tumor cells. *Clin Cancer Res* 2007;13:4547–55.
 50. Melief CJ. License to kill? reflects joint action of CD4 and CD8 T cells. *Clin Cancer Res* 2013;19:4295–6.
 51. Wang M, Tang ST, Lund O, Dziegieł MH, Buus S, Claesson MH. High-affinity human leucocyte antigen class I binding variola-derived peptides induce CD4+ T cell responses more than 30 years post-vaccinia virus vaccination. *Clin Exp Immunol* 2009;155:441–6.
 52. Boyle LH, Goodall JC, Gaston JS. Major histocompatibility complex class I-restricted alloreactive CD4+ T cells. *Immunology* 2004;112:54–63.
 53. Bertoni R, Sidney J, Fowler P, Chesnut RW, Chisari FV, Sette A. Human histocompatibility leukocyte antigen-binding supermotifs predict broadly cross-reactive cytotoxic T lymphocyte responses in patients with acute hepatitis. *J Clin Invest* 1997;100:503–13.
 54. Burrows SR, Elkington RA, Miles JJ, Green KJ, Walker S, Haryana SM, et al. Promiscuous CTL recognition of viral epitopes on multiple human leukocyte antigens: biological validation of the proposed HLA A24 supertype. *J Immunol* 2003;171:1407–12.
 55. Oseroff C, Kos F, Bui H-H, Peters B, Pasquetto V, Glenn J, et al. HLA class I-restricted responses to vaccinia recognize a broad array of proteins mainly involved in virulence and viral gene regulation. *Proc Natl Acad Sci U S A* 2005;102:13980–5.
 56. Ovsyannikova IG, Jacobson RM, Vierkant RA, Pankratz VS, Poland GA. HLA supertypes and immune responses to measles-mumps-rubella viral vaccine: findings and implications for vaccine design. *Vaccine* 2007;25:3090–100.
 57. Sylvester-Hvid C, Nielsen M, Lamberth K, Røder G, Justesen S, Lundsgaard C, et al. SARS CTL vaccine candidates; HLA supertype, genome-wide scanning and biochemical validation. *Tissue Antigens* 2004;63:395–400.
 58. Trachtenberg E, Korber B, Sollars C, Kepler TB, Hraber PT, Hayes E, et al. Advantage of rare HLA supertype in HIV disease progression. *Nat Med* 2003;9:928–35.
 59. MacDonald KS, Fowke KR, Kimani J, Dunand VA, Nagelkerke NJ, Blake Ball T, et al. Influence of HLA supertypes on susceptibility and resistance to human immunodeficiency virus type 1 infection. *J Infect Dis* 2000;181:1581–9.
 60. Balamurugan A, Sharma SK, Mehra NK. Human leukocyte antigen class I supertypes influence susceptibility and severity of tuberculosis. *Nat Rev Immunol* 2004;189:805–11.
 61. Taylor GM, Hussain A, Verhage V, Thompson PD, Ferguson WD, Watkins G, et al. Strong association of the HLA-DP6 supertype with childhood leukaemia is due to a single allele, DPB1* 0601. *Leukemia* 2009;23:863–9.
 62. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol* 2015;15:486–99.
 63. Yang Z-Z, Grote DM, Ziesmer SC, Niki T, Hirashima M, Novak AJ, et al. IL-12 upregulates TIM-3 expression and induces T cell exhaustion in patients with follicular B cell non-Hodgkin lymphoma. *J Clin Invest* 2012;122:1271–82.
 64. Riches JC, Davies JK, McClanahan F, Fatah R, Iqbal S, Agrawal S, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. *Blood* 2013;121:1612–21.
 65. Wade JC. Viral infections in patients with hematological malignancies. *Hematology Am Soc Hematol Educ Program* 2006;2006:368–74.
 66. Atkins S, He F. Chemotherapy and beyond: infections in the era of old and new treatments for hematologic malignancies. *Infect Dis Clin North Am* 2019;33:289–309.
 67. Ju B, Zhang Q, Ge J, Wang R, Sun J, Ge X, et al. Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature* 2020;584:115–9.
 68. Falsey AR, Hennessy PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. *N Engl J Med* 2005;352:1749–59.
 69. Lehnert N, Tabatabai J, Prifert C, Wedde M, Putthenparambil J, Weissbrich B, et al. Long-term shedding of influenza virus, parainfluenza virus, respiratory syncytial virus and nosocomial epidemiology in patients with hematological disorders. *PLoS One* 2016;11:e0148258.
 70. De Biasi S, Meschiari M, Gibellini L, Bellinazzi C, Borella R, Fidanza L, et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. *Nat Commun* 2020;11:1–17.
 71. Wykes MN, Lewin SR. Immune checkpoint blockade in infectious diseases. *Nat Rev Immunol* 2018;18:91.
 72. Gambichler T, Reuther J, Scheel CH, Becker JC. On the use of immune checkpoint inhibitors in patients with viral infections including COVID-19. *J Immunother Cancer* 2020;8:e001145.
 73. Bui H-H, Sidney J, Dinh K, Southwood S, Newman MJ, Sette A. Predicting population coverage of T-cell epitope-based diagnostics and vaccines. *BMC Bioinformatics* 2006;7:153.