CANCER GENOMICS

PTEN Loss and *BRCA1* Promoter Hypermethylation Negatively Predict for Immunogenicity in BRCA-Deficient Ovarian Cancer

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abstract

PURPOSE Ovarian cancers can exhibit a prominent immune infiltrate, but clinical trials have not demonstrated substantive response rates to immune checkpoint blockade monotherapy. We aimed to understand genomic features associated with immunogenicity in *BRCA1/2* mutation–associated cancers.

MATERIALS AND METHODS Using the Cancer Genome Atlas whole-exome sequencing, methylation, and expression data, we analyzed 66 ovarian cancers with either germline or somatic loss of *BRCA1/2* and whole-exome sequencing, immunohistochemistry, and CyTOF in 20 ovarian cancers with germline *BRCA1/2* pathogenic variants from Penn.

RESULTS We found two groups of *BRCA1/2* ovarian cancers differing in their immunogenicity: (1) 37 tumors significantly enriched for *PTEN* loss (11, 30%) and *BRCA1* promoter–hypermethylated (10, 27%; $P = .0016$) and (2) *PTEN* wild-type (28 of 29 tumors) cancers, with the latter group having longer overall survival (OS; $P = .0186$, median OS not reached *v* median OS = 66.1 months). *BRCA1/2*-mutant *PTEN* loss and *BRCA1* promoter–hypermethylated cancers were characterized by the decreased composition of lymphocytes estimated by gene expression ($P = .0030$), cytolytic index ($P = .034$), and cytokine expression but higher homologous recombination deficiency scores ($P = .00013$). Large-scale state transitions were the primary discriminating feature ($P = .001$); neither mutational burden nor neoantigen burden could explain differences in immunogenicity. In Penn tumors, *PTEN* loss and high homologous recombination deficiency cancers exhibited fewer CD3+ ($P = .05$), CD8+ ($P = .012$), and FOXP3+ ($P = .0087$) T cells; decreased PRF1 expression ($P = .041$); and lower immune costimulatory and inhibitory molecule expression.

CONCLUSION Our study suggests that within ovarian cancers with genetic loss of *BRCA1/2* are two subsets exhibiting differential immunogenicity, with lower levels associated with *PTEN* loss and BRCA hypermethylation. These genomic features of *BRCA1/2*-associated ovarian cancers may inform considerations around how to optimally deploy immune checkpoint inhibitors in the clinic.

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ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

BRCA1/2 are essential proteins involved in homologous recombination (HR)–based DNA repair.[1] Ovarian cancers with germline and somatic alterations in *BRCA1/2* share many phenotypic characteristics including defects in double-strand break repair, replication fork stalling, and mutational signatures reflective of underlying HR deficiency (HRD).[2,3] Consequently, they exhibit similar therapeutic vulnerabilities, namely, sensitivity to inhibitors of poly(ADP-ribose) polymerase (PARP) and platinum-based chemotherapy.[4-12] However, resistance to DNA-damaging therapy, whether intrinsic in the form of the absence of *BRCA1/2* allele–specific loss of heterozygosity[13] or acquired via secondary genetic events,[9,14-18] has necessitated the investigation of orthogonal treatment strategies.

One treatment strategy that has been hypothesized to impart clinical benefits in *BRCA1/2*-deficient ovarian cancer is immune checkpoint blockade (ICB).[19-25] *BRCA1/2*-deficient ovarian cancers tend to have a relatively increased neoantigen load, because of a reliance on error-prone double-strand break repair,[22] which can be predictive in some tumor types of ICB response.[20,22,23] *BRCA1/2*-deficient ovarian tumors are also characterized by a high presence of tumor-infiltrating lymphocytes (TILs),[20,22,26,27] which is a positive prognostic factor for survival.[20,22,24] PARP1

CONTEXT

Key Objective

To understand genomic features associated with immunogenicity in BRCA1/2 mutation–associated ovarian cancers.

Knowledge Generated

We found that BRCA1/2 mutation–associated ovarian groups clustered into two groups: Immune-High, associated with PTEN-loss and BRCA1 promoter–methylated tumors, and Immune-Low, with a significantly lower overall survival. BRCA1/2 mutation–associated ovarian cancers with PTEN loss had significantly higher homologous recombination deficiency scores, but exhibited significantly fewer CD3+, CD8+, and FOXP3+ T cells.

Relevance

Guided by molecular features, BRCA1/2 mutation–associated ovarian cancers can be divided into two groups with differing levels of immunogenicity, which may inform the use of immune checkpoint inhibitors in this patient group.

inhibition (PARPi), to which *BRCA1/2*-deficient ovarian cancers can respond,[5,16,28] has also been shown to increase TILs and synergistically combine with inhibitors of the immune checkpoint protein CTLA4 in mouse models.[29,30]

Despite the immunogenic properties of *BRCA1/2*-deficient ovarian cancers, the results of clinical trials evaluating ICB have been variable.[31] A single-agent phase Ib clinical trial of the programmed death-1 (PD1) inhibitor avelumab (n = 125) with epithelial ovarian cancer found an objective response rate (ORR) of 9.6% and a 1-year progression-free survival (PFS) of 10.2%.[32] Patients with *BRCA1/2* mutations did not selectively benefit over *BRCA1/2* wild-type (WT) patients in this trial.[32,33] A phase II ovarian cancer study of pembrolizumab (KEYNOTE-100, n = 367) also showed modest activity with an ORR of 8.0%; BRCA mutation status was not evaluated as a biomarker.[34] In a single-institution study, BRCA mutation status, tumor mutational burden (TMB), and HRD were not associated with response to ICB in patients with ovarian cancer although a high fraction of genome altered was associated with improvement in PFS and overall survival.[35] A trial combining PARP1 inhibitor niraparib with the PD1 inhibitor pembrolizumab (n = 62) showed an ORR of 18% (5% complete response and 13% partial response[36]), which did not differ by tumor BRCA deficiency (11 carriers, 18% response). The accompanying correlative study found that having a marker of HRD, mutational signature 3 (n = 11, 33%), a positive immune score (n = 3, 9%), or both (n = 6, 18%) was predictive of response.[37] Thus, it remains unclear whether responses were driven primarily by PARPi or checkpoint blockade or the treatment of two subgroups in the population.[36,38,39] Although larger phase II and III trials evaluating anti–CTLA-4 and anti-PD1/programmed cell death ligand-1 in combination with targeted therapy are underway,[21,40] these results suggest that a subset of *BRCA1/2* ovarian cancers may harbor immunosuppressive mechanisms that impede clinical benefits.

Herein, we investigated 86 *BRCA1/2*-deficient ovarian cancers using genomic data from the Cancer Genome Atlas (TCGA) and performed genomic and histopathologic analyses in ovarian cancers from our institute to determine the genetic and genomic properties associated with intrinsic immunogenicity. Our findings illustrate that transcriptional pathways, loss of *PTEN*, and genomic signatures of HRD collectively inform intrinsic immunogenicity in *BRCA1/2*-deficient ovarian cancers and may aid in the evaluation of ICB in clinical trials.

MATERIALS AND METHODS

Materials and methods describing the acquisition and sequencing of Penn tumors, the acquisition of TCGA data, immunohistochemistry, CyTOF analysis, and bioinformatics are described in the Data Supplement.

RESULTS

Genomic and Transcriptomic Properties of BRCA1/2-Deficient Ovarian Cancers

We investigated immunogenicity in ovarian cancers from TCGA, comparing *BRCA1/2*-deficient with HR-proficient cancers (HR-WT cancers, Data Supplement). Ovarian cancers with mutations in non-*BRCA1/2* genes involved in HR[6] were excluded from our analysis because of the insufficient sample size for immunogenetic analysis (n = 10 RNAseq). TMB (Methods) was significantly different across BRCA1 versus HR-WT (P = .00032, Kruskal-Wallis) and across BRCA2 versus HR-WT cancers (P = .00043, Kruskal-Wallis, Data Supplement). The number of single-nucleotide nonsynonymous variants and neoantigen load were similar across *BRCA1* versus *BRCA2* cancers, and *BRCA1/2*-deficient ovarian cancers had a significantly higher mean number of single-nucleotide nonsynonymous variants (Data Supplement; P = .0036 and P = .0025) and neoantigen loads (Data Supplement; P = .018 and P = .0066, respectively) than HR-WT cancers. *BRCA1/2*-deficient cancers collectively exhibited a higher HRD score, a metric of genomic instability associated with *BRCA1/2* dysfunction,[8,13] than HR-WT cancers (Data Supplement; P = 6.74e–05). Notably, neither HRD nor TMB were significantly correlated with sequencing coverage (Data Supplement).

We investigated biologic pathways that may differentiate *BRCA1/2*-deficient ovarian cancers (Methods). Clustering of gene set variation analysis scores across all cancers found that three recurring biologic functions inclusive of immune, hematologic, and tumor-suppressive (p53 and ATM) pathways were significantly upregulated in cluster c2 (Fig 1A and Data Supplement). We evaluated the relationship between *PTEN* status and cluster assignment, as *PTEN* loss is a frequent driving event in *BRCA1/2* high grade serous ovarian cancer[41] and has been shown to impede immune cell infiltration in other solid tumors.[42,43] We confirmed in our cohort that *BRCA1/2* mutations were associated with a higher rate of *PTEN* loss; 17.3% of *BRCA1/2* cancers had homozygous *PTEN* loss versus 7.7% of HR WT tumors (P = .019, Fisher's exact test). *PIK3CA* amplification was found in 20% of all samples with higher rates in the *BRCA1* hypermethylation group at 30% than those with *BRCA1*/2 somatic and germline mutations (14%) and non-*BRCA1/2* (17%; Data Supplement). Using the chi-square test of independence, we found that *BRCA1/2*-deficient *PTEN*-WT cancers were significantly over-represented in one cluster (28 of 29 tumors), that is, c2, whereas *BRCA1* promoter–hypermethylated and *BRCA1/2*-deficient cancers with homozygous loss of *PTEN* (Methods) were significantly over-represented in cluster c1 (14 of 37 tumors, Fig 1B, P = .0016), but not *PIK3CA* amplification. When comparing overall survival across cluster c1 versus cluster c2 by multivariate Cox analysis, we found significantly worse prognosis in cluster c1 relative to cluster c2 when adjusting for grade, HRD level, ploidy, *BRCA* mutation, and stage (Fig 1C, P = .0186, hazard ratio = 4.19 [CI, 1.27 to 13.83]). When comparing PFS across clusters, we found a borderline significantly worse prognosis in cluster c1 relative to cluster c2 when adjusting for the same set of covariates (Fig 1C, P = .062, hazard ratio = 3.26 [CI, 0.94 to 11.33]).

Immunogenicity of BRCA1/2-Deficient Ovarian Cancers by PTEN Status and Mechanism of BRCA1/2 Loss of Function

Given the transcriptional clusters observed in Figure 1A, we categorized *BRCA1/2*-deficient cancers on the basis of their *PTEN* status and mechanism of *BRCA1/2* loss.[44] We found no significant differences in TMB or neoantigen load when comparing on the basis of *PTEN* loss status within either germline or somatic *BRCA1/2*-deficient ovarian cancers or HR-WT cancers (Data Supplement). However, HRD scores were significantly higher in *BRCA1/2*-deficient *PTEN*-loss and *BRCA1* promoter–hypermethylated cancers as compared with *BRCA1/2*-deficient *PTEN*-WT and HR-WT cancers (Fig 2A; P = .024 and P = .024, respectively). Among the three components of the HRD score (Methods), large scale state transitions (LST) were significantly lower in *BRCA1/2*-deficient *PTEN*-WT ovarian cancers when compared with *BRCA1/2*-deficient *PTEN*-loss (P = .012) and *BRCA1* promoter–hypermethylated (P = .010 for LST) cancers (Fig 2B). The LST scores of *BRCA1/2*-deficient *PTEN*-WT cancers were similar to those of HR-WT cancers. Notably, non-telomeric allelic imbalance (NtAI) scores were significantly higher in *BRCA1/2*-deficient *PTEN* WT and HR-WT cancers versus *BRCA1/2*-deficient *PTEN*-loss cancers (Fig 2B).

We determined tumor immunogenicity by comparing expression-based immune indices, including cytolytic index (Clind)[45] and immune ESTIMATE (iEst) score.[46] Neither index differed significantly when comparing across cancers with *BRCA1* versus *BRCA2* alterations or germline versus somatic *BRCA1/2* alterations, or *PTEN*-loss versus *PTEN*-WT among HR-WT cancers (Data Supplement). Both indices correlated negatively with tumor purity estimated by copy number variation (CNV; see the Methods; Data Supplement; Clind: R = –0.25, P = .038; iEst: R = –0.38, P = .0013). The LST score correlated negatively with iEst scores and not with Clind (Data Supplement; Clind: R = –0.079, P = .52; iEst: R = –0.29, P = .019), whereas loss of heterozygosity score and NtAi scores did not exhibit any significant associations (data not shown). The HRD score showed a borderline significantly negative association with the iEst score, but not Clind (Data Supplement; Clind: R = –0.077, P = .53; iEst: R = –0.23, P = .067). When stratifying further, we found that *BRCA1/2*-deficient *PTEN*-loss and *BRCA1* promoter–hypermethylated cancers had lower Clind (P = .034 and P = .042, respectively) and, for the latter group, decreased iEst versus *BRCA1/2*-deficient *PTEN*-WT cancers (P = .003; Figs 2C and 2D). Tumor purity was also lower in *BRCA1/2*-deficient *PTEN*-WT versus *BRCA1/2*-deficient *PTEN*-loss tumors (Data Supplement, P = 9.34e–05). We analyzed the expression of multiple immune-regulatory genes that we curated from the literature[26,29,45,47,48] as having important roles with respect to response to ICB. We found significantly higher expression of *ADORA2A* (P = .033), *DOK3* (P = .0026), *HAVCR2* (P = .00055), *CD28* (P = .0037), *CD86* (P = .00091), *ICOS* (P = .05), and *TNFRSF17* (P = .033) in *BRCA1/2*-deficient *PTEN*-WT tumors versus *BRCA1* promoter–hypermethylated and *BRCA1/2 PTEN*-loss tumors (Fig 2E), consistent with the hypothesis that elevated expression of immune inhibitors (ADORA2A, DOK3, and HAVCR2) serves to counter-regulate heightened immune activity.[25,26] T-cell chemoattractants (CCL5, CXCL9, CXCL10, and CXCL11) coordinately trended toward higher expression in *BRCA1/2*-deficient *PTEN*-WT tumors, but not in *BRCA1* promoter–hypermethylated or *BRCA1/2*-deficient *PTEN*-loss tumors. HLA expression did not have any discernable pattern across the three groups.

We aimed to identify CNVs affecting gene regions involved in immune system function (Reactome, n = 946 immune-related genes) that may distinguish immunologically cold (*BRCA1/2*-deficient *PTEN*-loss + *BRCA1*-hypermethylated) cancers versus hot cancers (*BRCA1/2*-deficient, *PTEN*-WT), as focal CNVs affecting genes involved in immune

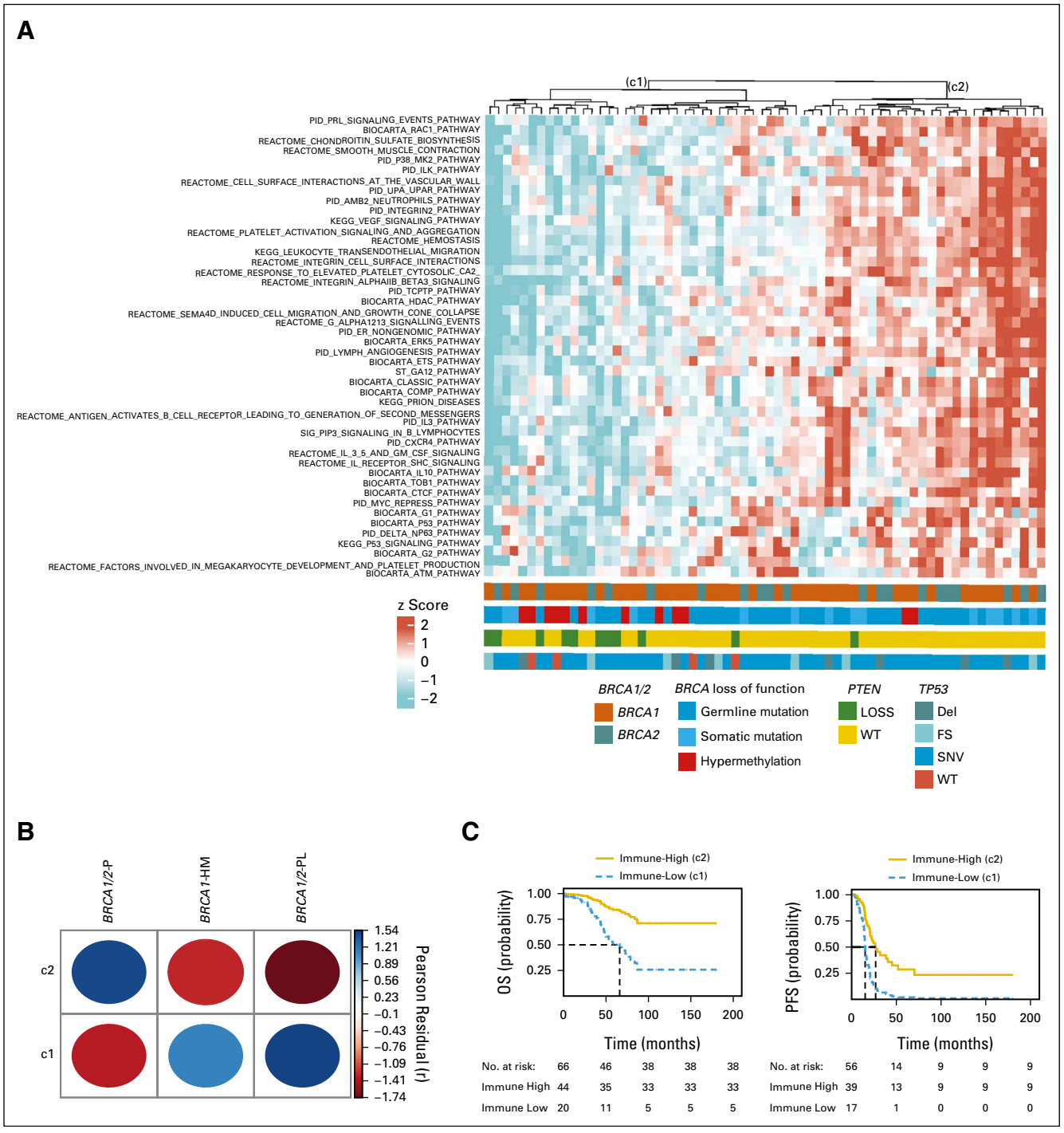

FIG 1. Mutational and transcriptomic features of the Cancer Genome Atlas ovarian cancers with somatic or germline *BRCA1/2* alterations: (A) GSVA illustrating significantly different canonical pathways (MSigDb, adjusted *P* value < .05) across c1 versus c2 GSVA signaling clusters in *BRCA1/2* ovarian cancers; (B) chi-square test of independence illustrating frequency of mutational subtypes within hierarchical clusters in *BRCA1/2* cancers (*P* = .0016); and (C) covariate-adjusted (grade, homologous recombination deficiency level, ploidy, stage, and *BRCA1/2* mutation) OS (hazard ratio = 4.19 [CI, 1.27 to 13.83]) and PFS (hazard ratio = 3.26 [CI, 0.94 to 11.33]) curves across c1 versus c2 GSVA signaling clusters in *BRCA1/2* ovarian cancers. **P* < .05, ***P* < .01, Student's *t*-test, Cox Proportional Hazards. *BRCA1*-HM, *BRCA1*-hypermethylated; *BRCA1/2*-P, *BRCA1/2 PTEN* wild-type; *BRCA1/2*-PL, *BRCA1/2 PTEN* loss; FS, Frameshift indel; GSVA, gene set variation analysis; OS, overall survival; PFS, progression-free survival; SNV, single-nucleotide variant; WT, wild-type.

function influence tumor immunophenotypes.[45] By an L1-regularized support vector machine,[49] we identified 42 genes distinguishing the two groups with an area under the receiver operating characteristic curve of 0.96 (Data Supplement, hg38 genomic locations). We observed deletions in *EIF2AK2*, *NCF2*, and *KPNA3*, among *BRCA1/2*-deficient

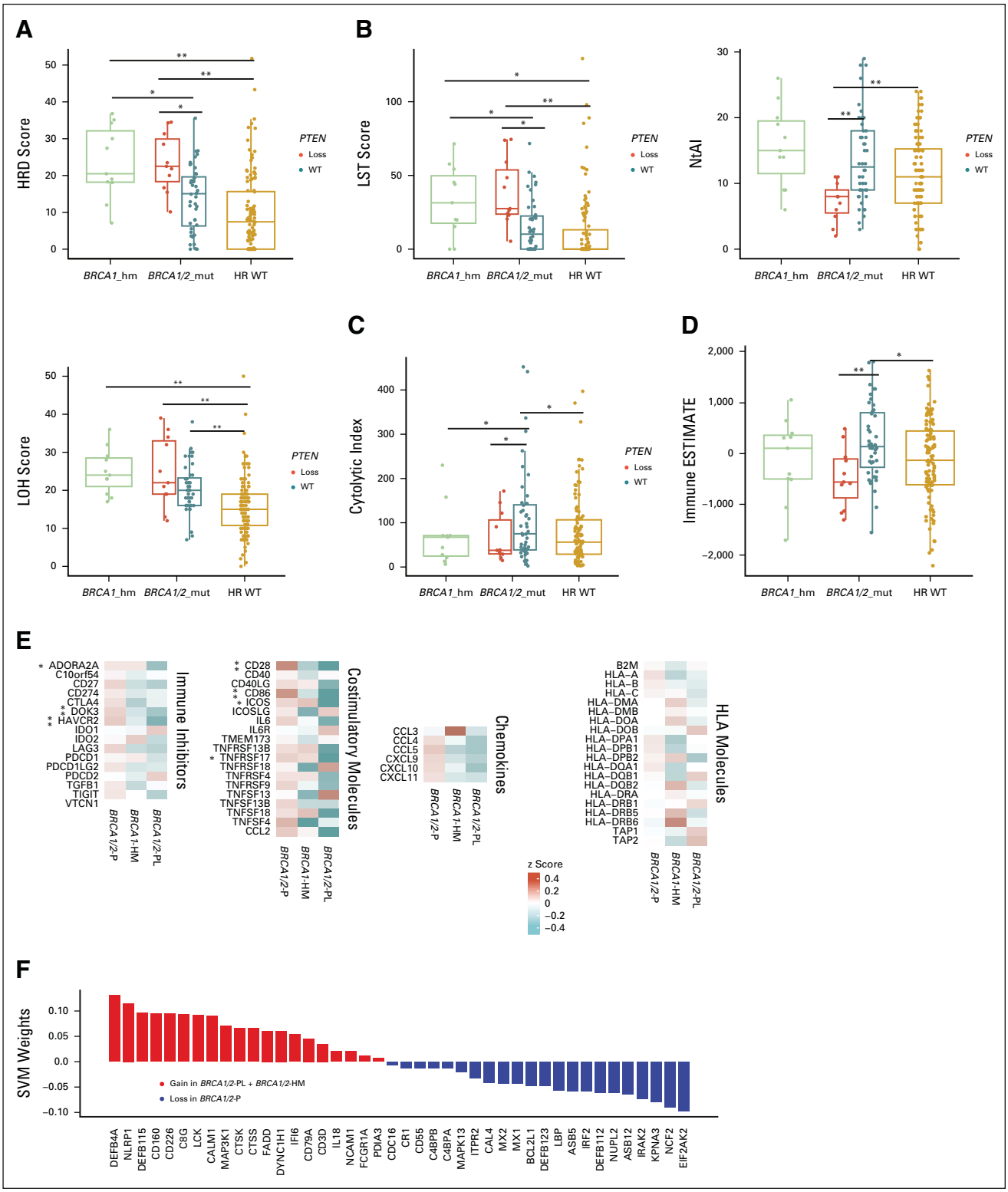

FIG 2. *BRCA1/2* ovarian cancer immunogenicity in relation to the mechanism of *BRCA1/2* loss of function and *PTEN* status: (A) HRD score in Penn + TCGA *BRCA1/2* and TCGA HR WT (N = 108) ovarian cancers by *PTEN* status (*PTEN* loss, n = 17; *PTEN* WT, n = 69) versus TCGA *BRCA1*-hm ovarian cancers (n = 11); (B) LST, NtAI, and LOH scores in Penn + TCGA *BRCA1/2* and TCGA HR WT (N = 108) ovarian cancers by *PTEN* status (*PTEN* loss, n = 17; *PTEN* WT, n = 69) versus TCGA *BRCA1*-hm ovarian cancers (n = 11); (C) in TCGA, CD8 T-cell Clind in *BRCA1/2* ovarian cancers by *PTEN* status (*PTEN* loss, n = 12; *PTEN* WT, n = 43) versus TCGA *BRCA1*-hm ovarian cancers (n = 11) and TCGA HR WT (N = 108); (D) immune infiltration inferred by ESTIMATE in *BRCA1/2* ovarian cancers by *PTEN* status (*PTEN* loss, n = 12; *PTEN* WT, n = 43) versus TCGA *BRCA1*-hm ovarian cancers (n = 11) and TCGA HR WT (N = 108); (E) expression of immune-regulatory molecules in *BRCA1/2* ovarian cancers (continued on following page)

FIG 2. (continued) by *PTEN* status (*PTEN* loss, n = 12; *PTEN* WT, n = 43) versus TCGA *BRCA1*-HM ovarian cancers (n = 11); and (F) coefficient weights from SVM analysis of copy number variations illustrating gene importance across TCGA *BRCA1/2*-P (n = 43) versus TCGA *BRCA1/2*-PL (n = 12) and *BRCA1*-HM (n = 11) ovarian cancers. $*P < .05$, $**P < .01$, Student's *t*-test, analysis of variance. *BRCA1/2*-P, *BRCA1/2 PTEN* wild-type; *BRCA1/2*-PL, *BRCA1/2 PTEN* loss; CIind, cytolytic index; HR, homologous recombination; HRD, homologous recombination deficiency; iEst, immune ESTIMATE; LOH, loss of heterozygosity; LST, large scale state transitions; NtAI, non-telomeric allelic imbalance; SVM, support vector machine; TCGA, the Cancer Genome Atlas; WT, wild-type.

PTEN-WT ovarian cancers. Gains in *DEFB4A* and *NLRP1* were more frequently observed in *BRCA1/2*-deficient *PTEN*-loss or *BRCA1* promoter–hypermethylated ovarian cancers.

Association of PTEN Loss and HRD Score With Immune Infiltration and Cytotoxicity in *BRCA1/2*-Deficient Penn Ovarian Cancers

To validate the immunologic effects of *PTEN* loss, we evaluated Penn ovarian cancers associated with germline *BRCA1/2* mutations (n = 18, Data Supplement). Multiple markers corresponding to adaptive and innate immune cell function were compared by *PTEN* status (Methods). *BRCA1/2*-deficient *PTEN*-loss cancers (n = 5) were characterized by significantly lower intratumoral CD3+ cells ($P = .05$), intratumoral CD8+ ($P = .012$), and intratumoral and stromal FOXP3+ ($P = .0087$ and $P = .037$, respectively) immune cells (Figs 3A and 3B), revealing that *PTEN* loss is associated with T-cell exclusion in *BRCA1/2*-deficient ovarian cancer. Other immune cell types (CD4, CD20, and CD68) did not demonstrate a statistically significant difference by *PTEN* status (Data Supplement). Furthermore, *BRCA1/2*-deficient *PTEN*-loss cancers were characterized by lower numbers of PRF1 (perforin 1)-positive cells ($P = .041$, Figs 3C and 3D), suggesting lower antitumor cytolytic activity in this subset. To determine whether the difference in cytolytic activity was due to fewer CD8+ cells or due to lower immune activity on a per-cell basis, we calculated the ratio of PRF1+ to CD8+ cells and found no significant difference ($P = .71$) across *PTEN*-WT versus *PTEN*-loss *BRCA1/2*-deficient cancers. MATH score, a measure of intratumoral heterogeneity,[50] was significantly higher in *BRCA1/2*-deficient *PTEN*-loss cancers than *BRCA1/2*-deficient *PTEN*-WT cancers (Fig 3E, $P = .0042$), in agreement with previous work demonstrating that CD8+ T-cell infiltration is negatively associated with ovarian cancers exhibiting greater clonal diversity.[51]

CyTOF analysis was performed on 11 available Penn tumor digests (n = 8) or ascites samples (n = 3) with *BRCA1/2* mutations (2 of 11 with homozygous *PTEN* loss) to measure the expression of immune-regulatory molecules in CD3+ T-cell subsets in the tumor microenvironment (Methods, Data Supplement). viSNE plots mapped the location of immune subsets such as CD8+ and CD8– T cells (Data Supplement). viSNE analysis[52,53] identified dense clusters and higher frequencies of T cells in *BRCA1/2*-deficient *PTEN*-WT tumors expressing relatively high amounts of inhibitory immune checkpoints CTLA4, LAG3, and TIGIT, as well as FOXP3+ cells in a representative *BRCA1* ovarian cancer, in contrast to comparatively lower overall expression in a *BRCA1 PTEN*-loss cancer (Fig 4A). The proinflammatory and costimulatory molecules interleukin (IL)-2, interferon gamma, IL-6, and CD28 were also expressed by more T cells at high levels in the *PTEN*-WT cancer relative to the *PTEN*-loss cancer (Fig 4B), underscoring the association of *PTEN* loss with reduced T-cell activation in the tumor microenvironment.

We investigated the association of the HRD score with immune cell subsets and immunomodulatory molecules in *BRCA1/2*-deficient ovarian cancers (Fig 4C). We found negative correlations between the HRD score and the frequency of tumor-associated T cells expressing CTLA4, LAG3, and CD160 immune inhibitory molecules and FOXP3+ T cells, which have been shown to accumulate at sites of cytotoxic T cells to mitigate antitumor immune attack.[54,55] Furthermore, proinflammatory cytokines (interferon gamma, IL-2), cytolytic pore-forming molecules (PRF and GZMB), and markers of proliferating or activated T cells (pSTAT5, Ki67, CD25, and GITR) were all negatively correlated with the HRD score (Fig 4C). Despite the negative correlations, the *P* values are nonsignificant likely because of the sample size.

Notably, the *BRCA1/2*-deficient *PTEN*-loss tumors had a higher preponderance of CD103+CD69+CD127– resident memory T cells (Trm) expressing PD-1 relative to the *PTEN*-WT tumor (Data Supplement). Furthermore, the HRD score is positively associated with CD103+CD69+CD127– Trm cells expressing PD-1 (Data Supplement).[56,57] Taken together, these results further illustrate that *PTEN* loss or high HRD may inform immunologic states in *BRCA1/2*-deficient ovarian cancers.

DISCUSSION

Our work sheds light on the tumor-immune heterogeneity in ovarian cancers with *BRCA1/2* alterations and potentially gives novel insights into the treatment of these cancers with ICB. Similar to other studies of ovarian cancer, we found *PIK3CA* amplification in 20% of ovarian cancers but did not identify an association with immunogenicity.[58] Our results are similar to immunologic studies in other cancers[59-64] with *PTEN* loss and are also consistent with a study of 5,400 ovarian cancers, with 3,244 being high-grade serous, which demonstrated a correlation between cytoplasmic PTEN staining and low CD8+ T cells.[63] Notably, in *BRCA1/2* breast cancers, we also found that markers of immunogenicity were inversely correlated with the HRD score in both TGCA and local tumor analyses, although likely

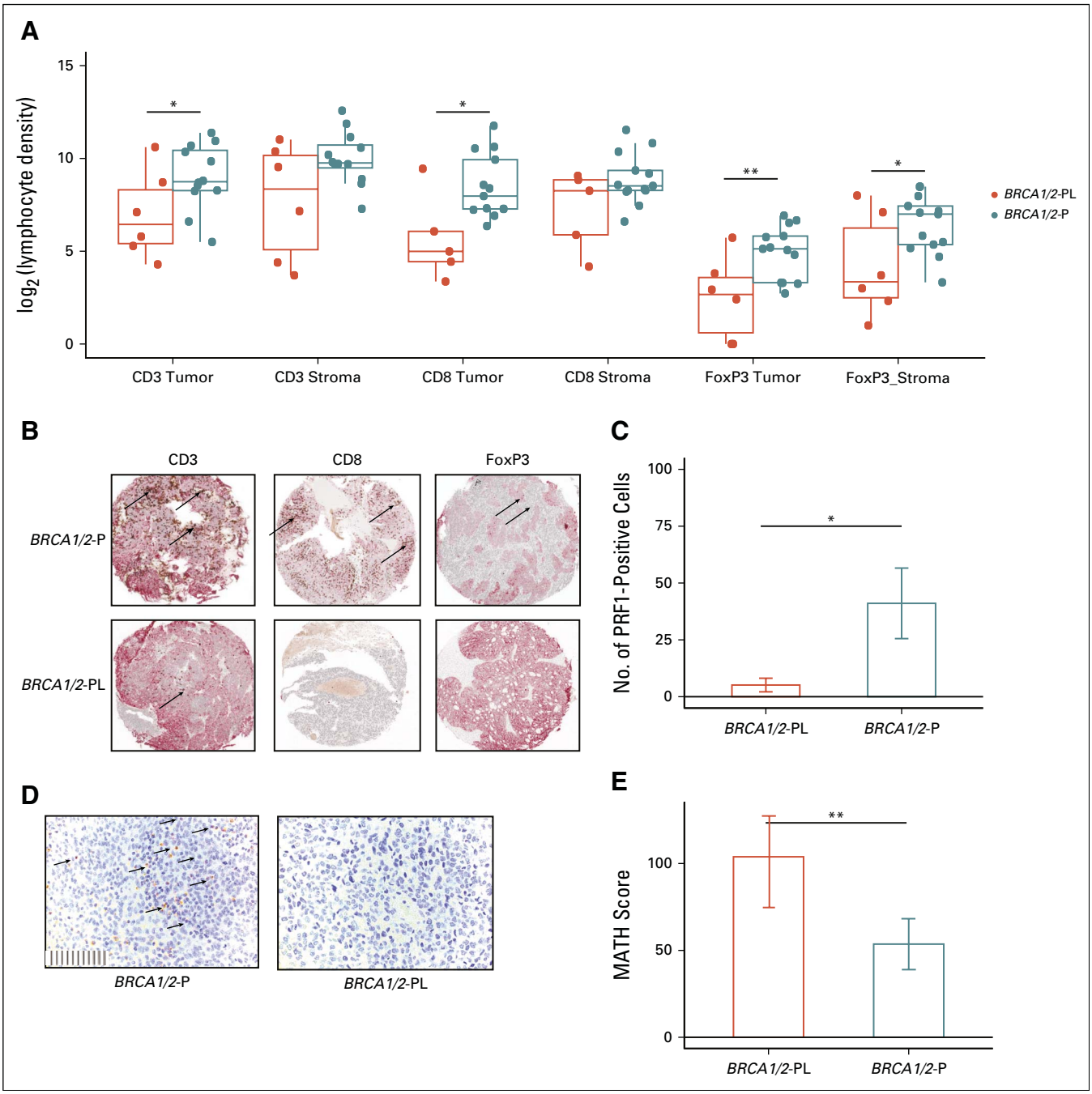

FIG 3. Evaluation of tumor infiltrates, antitumor immune activity, and somatic mutation clonality in Penn *BRCA1/2* ovarian cancers by *PTEN* status. Immune cell populations and immune effector molecules as a function of *PTEN* status in Penn ovarian cancers associated with germline *BRCA1/2* mutations. (A) Levels of intratumoral and stromal CD3+, CD8+, and FoxP3+ T cells in *BRCA1/2*-PL (n = 5) or *BRCA1/2*-P (n = 13) ovarian cancers. (B) Representative 10× immunohistochemical images of CD3+, CD8+, and FoxP3+ T cells in *BRCA1/2* ovarian cancers (the scale bar represents 0.1 mm at 0.01 mm increments). (C) Number of PRF1-positive cells in *BRCA1/2*-PL (n = 5) or *BRCA1/2*-P (n = 13) ovarian cancers. (D) Representative images of intratumoral PRF1 expression in *BRCA1/2*-PL or *BRCA1/2*-P ovarian cancers (the scale bar represents 0.1 mm at 0.01 mm increments). (E) MATH scores by *PTEN* status. Error bars, standard error, $*P < .05$, $**P < .01$, Student's *t*-test. *BRCA1/2*-P, *BRCA1/2 PTEN* wild-type; *BRCA1/2*-PL, *BRCA1/2 PTEN* loss; MATH, Mutant Allele Tumor Heterogeneity.

through distinct underlying genetic mechanisms.[65] Furthermore, Davoli et al and Thorrson et al found negative correlations between CNV burden and measures of immunogenicity,[51,64-69] consistent with our analysis.

Our findings are also consistent with previous observations in melanoma in which T-cell exclusion is a mechanism of mitigating immune attack in *PTEN*-loss cancers.[69,70] Our data suggest that immune exclusion in *PTEN*-loss cancers

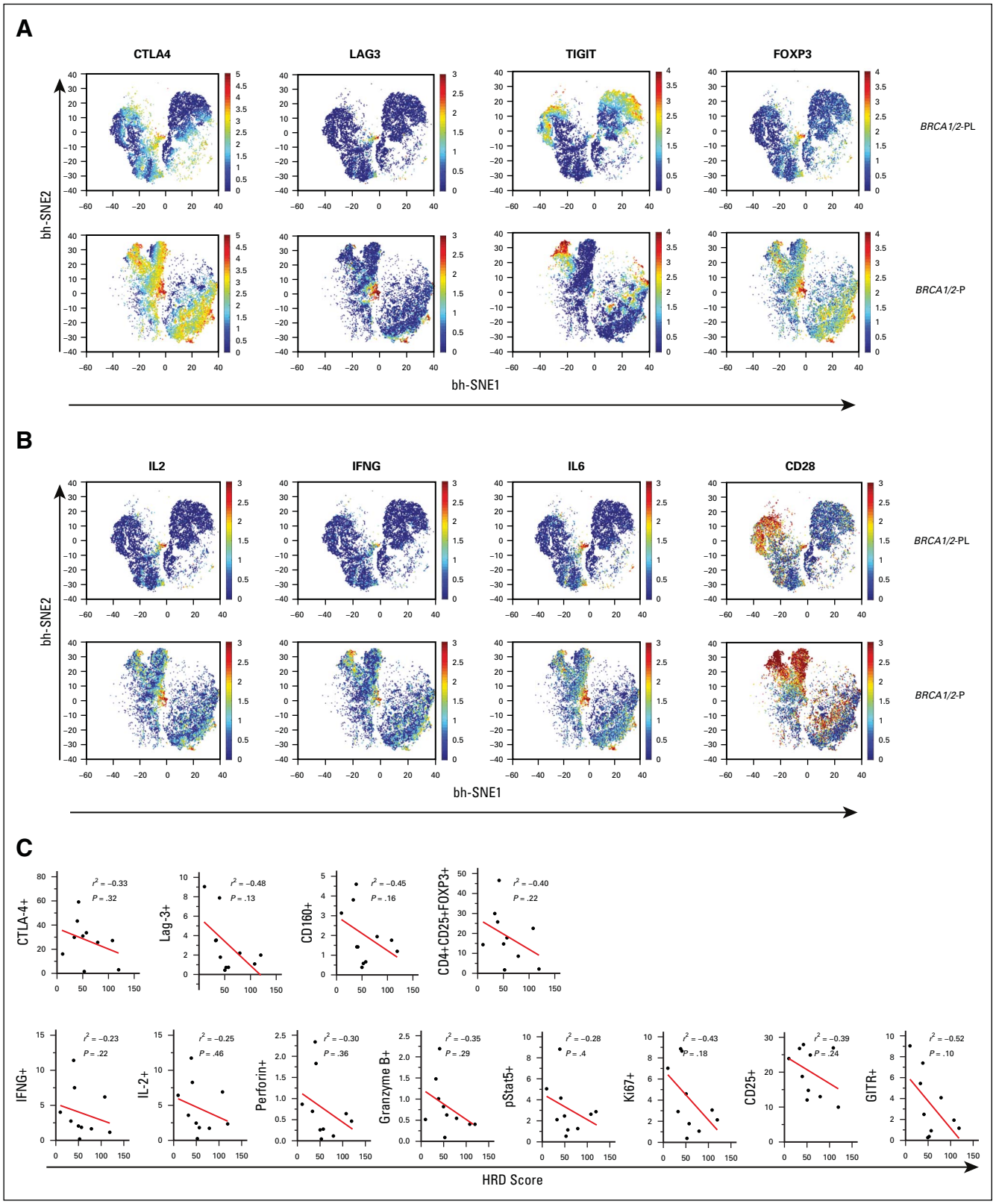

FIG 4. CyTOF analysis of immunoinhibitory and cytolytic activity in Penn *BRCA1/2* ovarian cancers by *PTEN* status and HRD score: (A) viSNE maps of CD4+ and CD8+ T cells illustrating differences in expression of inhibitory immune checkpoint molecules CTLA4, LAG3, TIGIT, and FOXP3 from a patient with a germline *BRCA1/2*-PL tumor versus a patient with a *BRCA1/2*-P; (B) viSNE maps of CD4+ and CD8+ T cells illustrating differences in expression of proinflammatory and costimulatory immune molecules IL2, IFNG, IL6, and CD28 from a patient with a germline *BRCA1/2*-PL versus a patient with a *BRCA1/2*-P; and (C) Scatterplots illustrating negative correlations between the HRD score and the expression of inhibitory (continued on following page)

FIG 4. (continued) immune molecules and immune cell subsets (top) and between the HRD score and proinflammatory and immune-activating molecules (bottom) by CyTOF in *BRCA1/2* ovarian cancers (n = 11). *BRCA1/2*-P, *BRCA1/2 PTEN* wild-type; *BRCA1/2*-PL, *BRCA1/2 PTEN* loss; HRD, homologous recombination deficiency; IFNG, interferon gamma; IL, interleukin; r^2, Pearson Correlation Coefficient.

may result from reduced expression of chemokines that play important roles in T-cell recruitment.[70] This finding is consistent with our recent finding that a reduction of both tumor intrinsic CCL5 expression and CCL5-driven CXCL9 expression by macrophages promotes TIL desertification and immune blunting, whereas CCL5 and CXCL9 overexpression in the ovarian cancer microenvironment is associated with CD8+ T-cell infiltration.[48]

Our observations with HRD and immunogenicity highlight that although HRD contributes to an increase in the number of neoantigens, chronic exposure of TILs to their cognate antigens may contribute to a quiescent phenotype in ovarian cancer.[71-73] CyTOF analysis showed a higher preponderance of PD1-expressing CD103+CD69+CD127– Trm cells in *PTEN*-loss or HRD-high cancers. Trm cells have been shown in ovarian cancer to be quiescent,[71] and anti-PD1 therapy can rapidly induce tumor cytotoxicity.[57,74] Notably, cancers with higher HRD are more intrinsically responsive to DNA-damaging agents,[8,13] which can work with ICB to synergistically eliminate solid tumors in mouse models.[29,30] Our data suggest that *BRCA1/2*-deficient *PTEN*-loss and *BRCA1* promoter–hypermethylated cancers may respond to PARPi, whereas ICB may be optimal for patients *BRCA1/2*-deficient *PTEN*-WT cancer. Thus, combination therapy may be efficacious, as PARPi and ICB each treat a subgroup of *BRCA1/2*-deficient ovarian cancers.[75]

From pathway analysis, we found that *BRCA1/2*-deficient *PTEN*-WT ovarian cancers had higher expression of *ATM*-driven DNA repair pathways, consistent with previous studies demonstrating a prevalent role of *PTEN* in HR-based genomic repair.[76,77] Along with lower prevalence of LSTs in this group, these results indicate that partial retention of DNA repair activity may be less immunosuppressive than full loss. These findings also indicate that *BRCA1/2*-deficient ovarian cancers may represent another example in which aneuploidy and large CNVs[68,78] are important driving forces that regulate immune responses and may supersede TMB as the primary immune influence.[47]

Although nearly all *BRCA1/2*-deficient ovarian cancers in our study harbored a *TP53* mutation, p53 signaling in *BRCA1/2*-deficient *PTEN*-WT cancers was elevated relative to the remaining cancers by gene set variation analysis. *TP53* may be a key driver of immune responses in this subset of ovarian cancers, as a previous study found that pharmacologic activation of p53 in the tumor microenvironment enhances CD8-driven immunogenic cell death in mouse models.[79] Furthermore, our analyses of somatic CNVs identified a greater prevalence of deletions of *EIF2AK2*, *NCF2*, and *KPNA3*, all of which are either targets of or influence p53 signaling, in the *BRCA1/2 PTEN*-loss and *BRCA1* promoter–hypermethylated group.[80-82] In particular, *EIF2AK2* is a target of *TP53* activity, serving a proapoptotic role for tumor suppression.[83] The more prevalent deletion of *EIF2AK2* in *PTEN*-WT ovarian cancers in conjunction with heightened p53 signaling suggests an intricate proinflammatory mechanism of p53 in ovarian cancer with concomitant selection against the tumor-suppressive role of *EIF2AK2*.

Although the study benefited from inclusion of both TCGA and locally generated data, each sample set had limitations. TGCA data did not include linked immunohistochemistry data examining immune cells, and the Penn data derived from formalin-fixed paraffin-embedded tissues were limited in terms of expression analysis. Neither sample set had whole genome sequencing data, which most accurately characterize TMB and large-scale CNVs. Several findings warrant independent validation, in particular, the CyTOF analysis and association of the expression signatures with outcomes. In addition, a study of *BRCA1/2* breast cancer using publicly available TCGA and Wellcome Trust Institute data suggests that *PTEN* status modulates immunogenicity but, by contrast, found that *PTEN* loss was associated with a more T-cell–inflamed signature.[84] However, *PTEN* loss also correlates with *BRCA1* mutation status and triple-negative breast cancer status, and in their analysis, the latter may account for the enrichment of T-cell–inflamed signatures, which we and many others have found to be relatively more immunogenic than hormone-positive breast cancers.[65]

Taken together, our study gives novel insights into the genetic events that may contribute to immunosuppression in *BRCA1/2*-deficient ovarian cancers, defining a subset of immunologically cold tumors. This understanding may help craft more efficacious use of ICB in the clinic.

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DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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DATA SHARING STATEMENT

The WES data that support this study have been deposited in the National Center for Biotechnology Information (NCBI)'s Sequence Read Archive (SRA, https://www.ncbi.nlm.nih.gov/sra) with BioProject ID PRJNA38804 and can be accessed at http://www.ncbi.nlm.nih.gov/bioproject/388048. The TCGA data are available from the National Cancer Institute's Genome Data Commons (https://gdc.cancer.gov/). The remaining data are available within the article, and its Supplementary Information files are available from the authors upon request.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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