Polygenic risk for skin autoimmunity impacts immune checkpoint blockade in bladder cancer

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Edited by Lawrence Steinman, Stanford University School of Medicine, Stanford, CA, and approved April 16, 2020 (received for review December 31, 2019)

PD-1 and PD-L1 act to restrict T cell responses in cancer and contribute to self-tolerance. Consistent with this role, PD-1 checkpoint inhibitors have been associated with immune-related adverse events (irAEs), immune toxicities thought to be autoimmune in origin. Analyses of dermatological irAEs have identified an association with improved overall survival (OS) following anti–PD-(L)1 therapy, but the factors that contribute to this relationship are poorly understood. We collected germline whole-genome sequencing data from IMvigor211, a recent phase 3 randomized controlled trial comparing atezolizumab (anti–PD-L1) monotherapy to chemotherapy in bladder cancer. We found that high vitiligo, high psoriasis, and low atopic dermatitis polygenic risk scores (PRSs) were associated with longer OS under anti–PD-L1 monotherapy as compared to chemotherapy, reflecting the Th17 polarization of these diseases. PRSs were not correlated with tumor mutation burden, PD-L1 immunohistochemistry, nor T-effector gene signatures. Shared genetic factors impact risk for dermatological autoimmunity and anti–PD-L1 monotherapy in bladder cancer.

Author contributions: Z.K., F.D.N., A.K., C.H., S.M., V.R., J.C., M.F., S.L.A., E.G., H.C.-H., T.B., I.M., J.R., T.P., J.H., G.S.C., and M.L.A. designed research; Z.K., A.K., C.H., S.M., V.R., J.C., M.F., S.L.A., E.G., H.C.-H., T.B., I.M., J.R., T.P., J.H., G.S.C., and M.L.A. performed research; Z.K., C.H., V.R., J.C., and M.L.A. analyzed data; and Z.K., I.M., J.R., T.P., G.S.C., and M.L.A. wrote the paper.

Competing interest statement: The authors declare a competing interest. Z.K., F.D.N., A.K., C.H., S.M., V.R., J.C., M.F., S.L.A., E.G., H.C.-H., T.B., I.M., J.R., T.P., J.H., G.S.C., and M.L.A. are inventors on a pending patent filed by Genentech/Roche on the use of polygenic risk scores for dermatological autoimmune diseases as methods for patient selection for treatment with immune checkpoint inhibitors. J.R. reported receiving personal fees and other support from Seattle Genetics, Astellas, Merck, Genentech/Roche, Bayer, AstraZeneca, Chugai, Quid, and Bristol-Myers Squibb; personal fees from UpToDate, Eli Lilly, Inovio, BioInvision, Addict Bio, Sensei Biotherapeutics, Pharmacyscics, GSK, Janssen, and Western Oncolytics; as well as other support from illumina. In addition, J.R. has a patent to predicting cisplatin sensitivity pending. T.P. has received research funding/honoraria from AstraZeneca, Genentech/Roche, Bristol-Myers Squibb, Merk, MSD, Pfizer, Exelixis, Astellas, and Johnson & Johnson. M.L.A. was previously an employee of Genentech/Roche and is currently an employee of insitro.

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Data deposition: An R package with anonymized data and code to reproduce the computational methods in the manuscript is available at http://research-pub.genome.com/CTISkinSurvival.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/ doi:10.1073/pnas.1922867117/-/DCSupplemental.

First published May 19, 2020.
data from safety-evaluable patients \((n = 459)\) receiving atezolizumab in IMvigor211 (13). We grouped irAEs using an organ and system-based classification (see Methods). Skin irAEs were the most common, followed by gastrointestinal and endocrine irAEs (Fig. 1). We confirmed a similar distribution of irAE in IMvigor210 \((n = 429)\), a phase 2 single-arm trial for treatment of mUC (14, 15). We focused on low-grade irAEs as they are typically managed without systemic corticosteroids and rarely lead to discontinuation of treatment (16). To address survival bias, we used a time-dependent covariate in a Cox proportional hazards model, including baseline factors as additional covariates (see Methods). In agreement with previous reports linking dermatological irAEs to survival (8–12), we found that OS was associated with low-grade skin irAEs in IMvigor211 \((P = 0.024; \text{HR} 0.66; 95\% \text{CI} 0.45–0.95)\) and IMvigor210 \((P = 0.0023; \text{HR} 0.53; 95\% \text{CI} 0.35–0.80; \text{Fig. 1B})\). To verify the robustness of our results, we conducted a landmark analysis. We selected land marks at the point at which 90% patients in the low-grade skin irAE group had experienced their event (Fig. 1C) and confirmed an association with improved OS in both trials (Fig. 1D).

Germline whole-genome sequencing (WGS) data from 465 individuals within the IMvigor211 study \((238 \text{ receiving atezolizumab and 227 chemotherapy treated})\) met strict filters for population and genotype data quality control (see Methods). We confirmed that OS and response rates were no different in these individuals, as compared to the intent-to-treat population of 931 individuals (SI Appendix, Fig. S1). We also confirmed that IC and TC staining of PD-L1 by immunohistochemistry (IHC) and tumor mutation burden (TMB) were similarly balanced across trial (SI Appendix, Fig. S2).

To test the hypothesis that genetic factors shared with dermatological autoimmunity impact anti–PD-L1 safety and efficacy, we used publicly available GWAS summary statistics to construct PRSs for PSO, AD, and VIT which were ascertained on independent case/control cohorts (see Methods and Fig. 2A and SI Appendix, Table S1 and Fig. S3) (17, 18). We used two

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**Fig. 1.** Low-grade dermatological irAEs in patients receiving atezolizumab are associated with longer OS. (A) Rate of irAEs, aggregated by system- and organ-based classification, across trials. Only irAE categories with occurrence rates >5% are shown. Low = grade 1 or 2 (orange stacking bar); High = grade 3, 4, or 5 (gray stacking bar). (B) Hazard ratios (HRs) with 95% CIs are shown as thick lines, comparing OS of individuals that experienced low-grade irAEs to those that did not experience irAE of a given classification. Individuals that experienced a high-grade irAE of the given classification were excluded from the analysis. A time-dependent covariate in a Cox proportional hazards model was used to estimate the HRs (see Methods). (C) Distribution of time to first skin irAE in days across trials. Ninety percent of patients that experienced skin irAEs lie to the left of the orange landmark line. (D) Kaplan-Meier survival curves comparing the OS of individuals in the atezolizumab arm of IMvigor211 and IMvigor210 after a defined landmark. Tick marks show censoring events. GI = gastrointestinal.
studies for PSO, Immunochip (PSO/IC) and the UK Biobank (PSO/UKBB). PSO/IC assessed genotypes curated for immunologically relevant variants, whereas PSO cases in PSO/UKBB were self-reported and assayed using genome-wide genotyping. We additionally constructed PRSs for Alzheimer’s disease (ALZ) to serve as negative controls. Controlling for genotype principal components (PCs) and sex, we identified associations at a false discovery rate (FDR) of 10% between the occurrence of skin irAEs and genetic risk for PSO within the atezolizumab arm of IMvigor211 (Fig. 2B and SI Appendix, Table S2). The associations were consistent across GWASs used to construct the PSO PRSs (Fig. 2C). Although the Nagelkerke pseudo-R² was only 0.12 for the PSO/IC (1e-08) PRS, we confirmed that the association observed was also reflected in the time to adverse event data (SI Appendix, Fig. S4).

Given the observations that dermatological irAEs are associated with longer OS, and germline genetic risk for PSO is associated with increased odds of skin irAEs during checkpoint blockade, we investigated whether dermatological autoimmune disease PRSs were associated with OS under atezolizumab treatment or chemotherapy (analyzed independently). PRSs for AD, PSO, and VIT were associated with OS in the atezolizumab arm, but not the chemotherapy arm, of IMvigor211 at an FDR of 10%, controlling for baseline clinical factors in addition to genotype PCs (Fig. 2D and SI Appendix, Table S2; also, see Methods). PRSs for ALZ, as expected, did not show any significant association. The GWAS P value cutoffs at which we observed significant associations differed between OS and skin irAEs, as well as across diseases, reflecting the differing degrees of genetic sharing and statistical power of the GWASs underlying the PRSs (see SI Appendix, Supplemental Discussion).

We also highlight that the T-effector gene signature, a measure of CD8+ T cell effector function within a tumor, was the unique

Fig. 2. Polygenic risk for skin autoimmunity is associated with the occurrence of dermatological irAEs and OS in the atezolizumab arm of IMvigor211. (A) PRSs were computed for each individual with whole-genome germline sequencing data in IMvigor211 for dermatological autoimmune diseases: AD, PSO, and VIT. PRSs were constructed for 6 P value cutoffs (see SI Appendix, Table S2 and Methods). (B) Negative log₁₀ P values for a given PRS testing for association with occurrence of skin irAEs controlling for five genotype principal components and sex by logistic regression. Orange circles show associations that were significant at an FDR of 10%. Gray circles did not meet statistical significance. (C) Odds ratios and 95% CIs were estimated for PRSs and skin irAE occurrence for PRSs that met a significance cutoff of FDR 10%. ORs are expressed in per-unit change of a normalized PRS. GWAS P value cutoff indicated within parentheses. (D) Negative log₁₀ P values for a given GWAS and P value cutoff PRS testing for association with OS using a Cox proportional hazards model controlling for five genotype principal components and baseline clinical factors (see Methods). (E) Adjusted HRs and 95% CIs for PRS and OS associations are shown, using significance cutoff of 10% FDR. HRs are expressed in unit change of a normalized PRS. IC = Immunochip; T-eff = CD8 T-effector gene expression signature score; IC IHC = PD-L1 expression on immune cells; TC IHC = PD-L1 expression on tumor cells.

tumor factor significantly associated with OS within the atezolizumab arm in the IMvigor211 [confirming prior studies (14)]. Both increased polygenic risk for VIT and PSO were associated with longer OS under treatment with atezolizumab, whereas decreased polygenic risk for AD was associated with longer OS under atezolizumab treatment (Fig. 2E). We confirmed that the OS associations were not simply due to correlation between the T-effector signature or strong intercorrelation among the disease-specific PRSs (SI Appendix, Fig. S5). We quantile-normalized the T-effector signature score to allow comparison to quantile-normalized PRSs. The HR benefit of a higher T-effector signature score per normalized unit was 0.81, similar to that of the PRSs per normalized unit for PSO, VIT, and the inverse of AD 0.78–0.83.

We then assessed whether PRSs were prognostic, informative of outcome regardless of treatment, or predictive, informative of the effect of experimental treatment. To formally test if PRSs were predictive, we incorporated both trial arms into a Cox proportional hazards model and assessed interaction between the PRS and trial arm. After controlling for baseline clinical covariates and genotype PCs, a significant trial arm by PRS interaction for each dermatological autoimmune disease. High VIT (HR = 0.0016; HR = 0.58; 95% CI 0.41–0.81) and high PSO risk (HR = 5.5 × 10−3; HR = 0.50; 95% CI 0.36–0.70) individuals had better OS under checkpoint blockade than chemotherapy, whereas low AD risk (HR = 0.0008; HR = 0.57; 95% CI 0.41–0.79) individuals had improved OS under atezolizumab treatment as compared to chemotherapy (Fig. 3B and SI Appendix, Figs. S7–S9). PRSs were uncorrelated tumor IC staining of PD-L1 by IHC, TMB, and across patients (Fig. 3C). To gain further insight into the PRSs, we compared high and low PRS subgroups within each treatment arm (Fig. 3D). High VIT risk reflected improved OS in the atezolizumab arm, whereas high PSO risk and low AD risk groups reflected both improved OS in the atezolizumab arm and worse OS within the chemotherapy arm. As response is a proxy for longer and shorter OS, we found that the response rates within these subgroups followed a similar numeric pattern (SI Appendix, Fig. S10).

Variants in the major histocompatibility complex (MHC) locus and specific human leukocyte antigen (HLA) alleles contribute significantly to genetic risk for PSO, VIT, and AD (SI Methods).

In the major histocompatibility complex (MHC) locus and specific human leukocyte antigen (HLA) alleles contribute significantly to genetic risk for PSO, VIT, and AD (SI Methods).
As our PRSs were generated using a reference panel that poorly approximated linkage disequilibrium (LD) in the MHC region, we excluded variants from this region in our association analyses. To address this caveat, we repeated our analysis including variants in the MHC region. Inclusion of this additional information did not strengthen the associations we observed (SI Appendix, Fig. S11). We also called HLA alleles on the basis of direct sequence evidence (see Methods). We found that HLA alleles previously found to be associated with risk of PSO, AD, or VIT were not associated with OS in the atezolizumab or the chemotherapy arm of IMvigor211 (SI Appendix, Table S5). As these alleles may contribute additively to risk of dermatological autoimmunity, we incorporated the risk conferred by these alleles to our PRSs (see Methods). Inclusion of this additional information had negligible impact (SI Appendix, Fig. S11).

The tumor microenvironment (TME) consists of a complex mixture of immune cells, stromal, and tumor cells (22). Polygenic
risk may influence the composition of the TME, which in turn impacts patient survival. Using pretreatment, bulk tumor gene expression for \( n = 398 \) individuals with both RNA-seq and germline genetic data, we generated immune and stromal cell-type enrichment scores, and, to limit the multiple testing burden, we associated them with PRSs that had the strongest trial arm by risk score interaction for each dermatological autoimmune disease (23). We found no evidence for association between cell-type enrichment scores and PRSs (SI Appendix, Fig. S12). Alternatively, PRSs might be relevant only in certain tumor contexts. To address this question, we delineated four subgroups on the basis of high or low PRS and a high or low tumor factor. Specifically, we considered: PD-L1 expression measured by IHC and expressed on ICs or TCs, TMB, and the CD8 T-effector signature. We additionally considered the tumor expression of selected T-helper chemokines and cytokines involved in differentiation, recruitment, and response (SI Appendix, Table S6). We statistically assessed whether combining a PRS and a tumor factor was more informative of the treatment effect on OS than the PRS alone (see Methods). This would occur if the treatment effect depended on both the PRS value and the tumor factor value (SI Appendix, Fig. S13). We found high-PSO PRS was beneficial in immune-infiltrated tumors as reflected by survival benefit in tumors with high values of IC staining of PD-L1 by IHC and high expression of genes involved in CD8 T-effector function. Although little or no pretreatment \( IL17A/F \) expression was detected by bulk RNA-seq, above median expression of genes involved in Th17 function including \( IL23A \), \( CXCL2 \) and \( CCL20 \) when combined with the PSO PRS also delineated a subgroup that benefited from atezolizumab as compared to chemotherapy (Fig. 4). This subgroup existed in the absence of any correlation between PSO PRS and these tumor factors or overlap with patients with high T-effector scores (SI Appendix, Fig. S14). As both expression measures were uncorrelated, we found that the survival benefit of high-PSO PRS was strongest in patients with tumors that had both high \( IL23A \) expression and T-effector scores (SI Appendix, Fig. S15). We also observed a divergent pattern between PSO PRS and expression of subunits of IL-12 (\( IL12A \) and \( IL12B \)) in a manner consistent with preclinical observations of the divergent roles of IL-12 and IL-23 in autoimmunity (SI Appendix, Supplemental Discussion and Figs. S16 and S17).

**Methods**

**Patient Cohorts.** We conducted our analysis of irAEs in the safety-evaluable population from IMvigor211 (NCT02302807) and IMvigor210 (NCT02951767, NCT02108652). irAEs were captured using an Adverse Events of Special Interest strategy across these trials. Protocols, sites, participating investigators, and confirmation of an independent ethics committee approval of each protocol at each site is provided in the original IMvigor211 and IMvigor210 publications (13–15). Confirmation of informed consent for research on germline DNA for this study was provided by the Ethics Lead in Biosample & Repository Management at F. Hoffmann-La Roche AG.

**Germline WGS.** We sequenced genomic DNA isolated from blood samples from \( n = 479 \) individuals from IMvigor211 on the basis of availability and informed consent for research on germline DNA. DNA was extracted using the DNA Blood400 kit (Chemagic). DNA was sheared (Covaris LE220), and sequencing libraries were prepared using the TruSeq Nano DNA HT kit (Illumina, Inc.). Libraries were sequenced at Human Longevity. The 150-bp paired-end WGS data were generated to an average read depth of 30x using the HiSeq platform (Illumina X10). Joint genotyping was performed using Genome Analysis Toolkit followed by filters for variant and population data quality control. We used HLA*PRG5-LA (retrieved March 8, 2017, git commit SHA-1 hash prefixed by 7b9ba45) to infer HLA alleles at G group resolution. Details are provided in SI Appendix.

**Construction of PRSs.** All PRSs were constructed by pruning and thresholding using publicly available GWAS summary statistics. We used an LD reference panel, the EUR population from the 1000 Genomes Project, to approximate LD that would be present in the original case control populations for each of the GWAS of these diseases. Associations between irAE occurrence, OS, and PRSs were performed in R. Details are provided in SI Appendix.

**Code Availability.** An R package with anonymized clinical data; precomputed normalized PRSs; precomputed HLA calls for VIT, PSO, and AD risk alleles; and all R code to produce figures in the manuscript has been made available for download: http://research.pub gene.com/CIT55kinSurvival.

**Data Availability.** Anonymized patient-level clinical data and summary-level PRS data are available within the R package described above. All requests for genotype (VCF) or raw data (BAM/FASTQ) will be promptly reviewed by Roche/Genentech to verify if the request is subject to patient consent and confidentiality obligations. Any data that can be shared will be released through establishment of a data-sharing agreement with Roche/Genentech. Please contact the corresponding authors for any inquiries regarding the requests for data.

**ACKNOWLEDGMENTS.** We thank all of our Genentech colleagues involved in the Human Genetics Initiative. We acknowledge Elaine Murray for help with curating irAE datasets and Felix Arellano for comments on early drafts of this manuscript. We acknowledge the Cancer Immunotherapy Committee, Genito-Urinary-Global Development Team for their support, the IMvigor210 and IMvigor211 study teams, the investigators, and the patients that contributed their samples and data for this study.
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