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Germline genetic variants are associated with development of insulin-dependent diabetes in cancer patients treated with immune checkpoint inhibitors

Jasmine I Caulfield,[1] Lilach Aizenbud,[1] Ana Luisa Perdigoto,[2] Eric Meffre,[3] Lucia Jilaveanu,[1] Dominika A Michalek,[4] Stephen S Rich,[4] Yariv Aizenbud,[5] Adebowale Adeniran,[6] Kevan C Herold,[2] Matthew R Austin,[1] Harriet Kluger [1]

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JIC and LA contributed equally. MRA and HK contributed equally.

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For numbered affiliations see end of article.

Correspondence to
Dr Harriet Kluger;
Harriet.Kluger@Yale.edu

ABSTRACT

Background Immune checkpoint inhibitors (ICIs) have dramatically improved survival in patients with cancer but are often accompanied by severe immune-related adverse events (irAEs), which can sometimes be irreversible. Insulin-dependent diabetes is a rare, but life-altering irAE. Our purpose was to determine whether recurrent somatic or germline mutations are observed in patients who develop insulin-dependent diabetes as an irAE.

Methods We performed RNA and whole exome sequencing on tumors from 13 patients who developed diabetes due to ICI exposure (ICI-induced diabetes mellitus, ICI-DM) compared with control patients who did not develop diabetes.

Results In tumors from ICI-DM patients, we did not find differences in expression of conventional type 1 diabetes autoantigens, but we did observe significant overexpression of ORM1, PLG, and G6PC, all of which have been implicated in type 1 diabetes or are related to pancreas and islet cell function. Interestingly, we observed a missense mutation in NLRC5 in tumors of 9 of the 13 ICI-DM patients that was not observed in the control patients treated with the same drugs for the same cancers. Germline DNA from the ICI-DM patients was sequenced; all *NLRC5* mutations were germline. The prevalence of *NLRC5* germline variants was significantly greater than the general population ($p=5.98\times10^{-6}$). Although NLRC5 is implicated in development of type 1 diabetes, germline *NLRC5* mutations were not found in public databases from patients with type 1 diabetes, suggesting a different mechanism of insulin-dependent diabetes in immunotherapy-treated patients with cancer.

Conclusions Validation of the *NLRC5* mutation as a potential predictive biomarker is warranted, as it might improve patient selection for treatment regimens. Furthermore, this genetic alteration suggests potential mechanisms of islet cell destruction in the setting of checkpoint inhibitor therapy.

BACKGROUND

Immune checkpoint inhibitors (ICIs) are widely used for multiple indications in cancer care. These monoclonal antibodies block key molecules in inhibitory pathways of immune cell activation, and include drugs that act on cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed cell death-1 (PD-1), programmed death ligand-1, and leukocyte antigen-3.[1] Although ICIs are intended to enhance antitumor immune activity, normal tissues and biological processes can be affected, resulting in immune-related adverse events (irAEs). Up to 90% of patients treated with combined ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) experience at least one irAE, while grade ≥3 irAEs occur in 46% of patients on this regimen.[2] Anti-PD-1 alone results in lower irAE rates; 71% have irAEs of any grade and 10% grade ≥3 irAEs.[3]

ICI-induced diabetes mellitus (ICI-DM) was first described by our group in 2015 and is among the most serious life-altering and life-threatening irAEs.[4] ICI-DM is a unique form of autoimmune insulin-dependent diabetes. Compared with childhood onset of type 1 diabetes mellitus (T1DM), onset of ICI-DM occurs between 55 and 66 years old, typically coinciding with ICI therapy. The fulminant nature of ICI-DM also differs, with loss of beta cell function within weeks. Up to 80% present with diabetic ketoacidosis and most have low or undetectable C-peptide levels at presentation. The majority (95%) of T1DM patients have known antibodies to islet cells, compared with 40% of ICI-DM patients.[4–6]

The pathogenesis of ICI-DM is not fully understood. It involves various cellular components of the immune system, as well as the microbiome, as reviewed.[7] The pathogenesis of irAEs might differ by organ site. For example, cytotoxic and helper T-cell infiltrates were found in myocarditis, while B-cells are believed to contribute, at least in part, to the development of bullous pemphigoid.[7]

A potential mechanism is shared antigens between tumor and normal tissue that may stimulate autoreactive cells in the presence of ICIs. This pathophysiology is well established in infectious diseases, such as group A streptococcus and rheumatic fever,[8] and has been proposed in others such as Epstein-Barr virus and multiple sclerosis.[9] This is also suspected in various cancers, as suggested by the reversible association between thymoma and myasthenia gravis,[10] and paraneoplastic syndromes.[11] A similar phenomenon has also been implicated in other irAEs. Tumor and myocardial tissue of patients with ICI-induced myocarditis and myositis showed shared T expanded cell clones, implying induction by mutual antigens.[12] Moreover, muscle-specific transcripts were present in these tumors, strengthening the evidence for specific autoreactive T-cell stimulation.[13] Vogt-Koyanagi-Harada (VKH) syndrome, an autoimmune condition where T-cells target melanocytes, featuring ocular, cutaneous and neurological symptoms, is observed in melanoma patients receiving ICIs, with vitiligo and uveitis.[14] One report demonstrated T-cell clones from a VKH patient targeting melanoma cells, suggesting similarity between epitopes.[15]

Here, we studied RNA expression and mutations, and whole exome tumor and germline sequencing of tumor samples, to determine the possible contribution of tumor overexpression, tumor neoantigens, or germline genetic mutations to the development of ICI-DM.

METHODS

Study design and sample selection

With approval of a Yale University Institutional Review Board, and after obtaining patients' written consent, we collected tumor tissue and peripheral blood samples from 13 patients diagnosed with ICI-DM who had no prior history of diabetes, had new-onset hyperglycemia on ICI requiring exogenous insulin, and continued requiring insulin for ≥1 month with evidence of insulin deficiency. Thirteen control patients with cancer were selected who did not develop ICI-DM, matched for age, sex, tumor type, and type of ICI. H&E slices of biopsy tissue were reviewed with a pathologist to identify areas of tumor, and 1 mm core samples were collected for sequencing of RNA and DNA.

Sequencing and data analyses

All methods are described in detail in online supplemental methods. RNA and DNA extraction, library preparation, and RNA sequencing (RNA-seq), whole exome sequencing (WES), and genome analysis toolkit (GATK) variant calling[16] for mutation analysis in RNA were performed by the Yale Center for Genomic Analysis. Tumors were subjected to RNA-seq and GATK variant calling, and normal tissue or peripheral blood mononuclear cell (PBMC) samples were analyzed by WES. Analysis of variance, t tests, and χ^2 tests were performed with GraphPad Prism V.9.4.1. Mann-Whitney U tests with the Bonferroni correction were used to determine the relationship between mutation presence and ICI-DM-onset time. To compare general population prevalence with mutation prevalence in ICI-DM patients, Fisher's exact test was employed with the Bonferroni correction for multiple comparisons, and sample label shuffling was performed. RNA-seq results were considered significant with an adjusted $p<0.05$. For other tests performed, a $p<0.05$ was considered significant.

Table 1 Demographic and clinical characteristics of study population

	ICI-DM (n=13)	Controls (n=13)
	No (%) or value±SD	No (%) or value±SD
Sex		
Female	8 (62%)	8 (62%)
Male	5 (38%)	5 (38%)
Mean age at biopsy (years)	66±9.5	65.5±7.2
Tumor type		
Renal cell cancer	4 (30.8%)	4 (30.8%)
Non-small cell lung carcinoma	4 (30.8%)	4 (30.8%)
Melanoma (cutaneous)	3 (23.1%)	3 (23.1%)
Melanoma (uveal)	1 (7.6%)	1 (7.6%)
Pancreatic adenocarcinoma	1 (7.6%)	1 (7.6%)
Treatment regimen		
Nivolumab+ipilimumab	5 (38.4%)	6 (46.2%)
Pembrolizumab	3 (23.1%)	4 (30.8%)
Atezolizumab	3 (23.1%)	2 (15.4%)
Nivolumab	2 (15.4%)	1 (7.6%)

ICI-DM, immune checkpoint inhibitor-induced diabetes mellitus.

RESULTS

Patient characteristics

Twenty-six patients (13 ICI-DM patients, 13 controls) were included with 5 cancer types: cutaneous melanoma, renal cell carcinoma, non-small cell lung carcinoma, uveal melanoma, and pancreatic adenocarcinoma. ICI regimens included nivolumab with or without ipilimumab, pembrolizumab, and atezolizumab (table 1). Patients' autoantibodies to known T1DM antigens were assessed and are included in online supplemental table 1.

RNA expression analysis

Looking at the entire mRNA expression dataset, we identified genes with the largest differential abundance in tumors from ICI-DM patients compared with controls (online supplemental figure 1, online supplemental table 2). Fifteen genes had greater expression in the ICI-DM samples, the highest of which was in ORM1, PLG, and DSG1 (figure 1A), while five genes had greater expression in control patients.

We interrogated data from the Human Protein Atlas[17] and found that only two of the genes abundantly

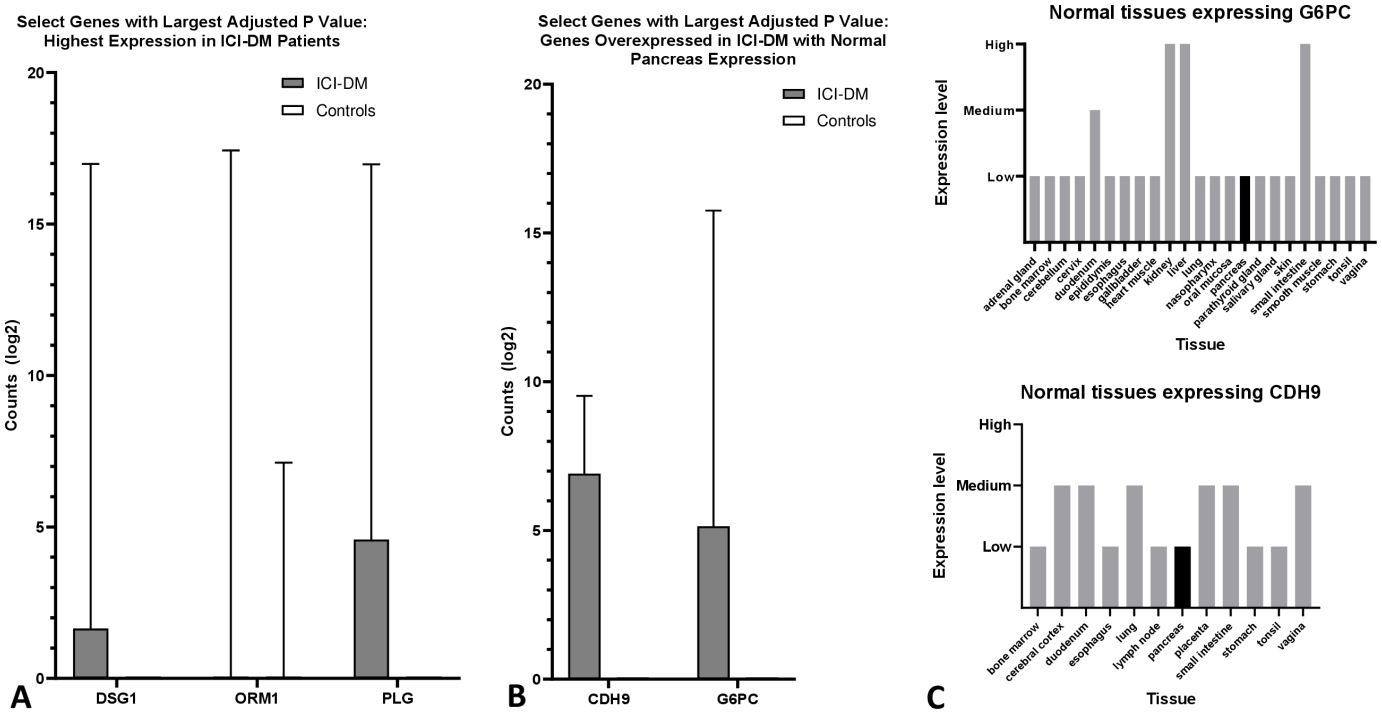

Figure 1 Select genes from the largest differentially expressed genes between ICI-DM and control patients with the largest adjusted p value. (A) Genes displayed here were selected to highlight those with the highest expression levels in the ICI-DM patients (DSG1, ORM1, PLG). (B:) Genes displayed here were selected to highlight those with normal pancreas expression (using Human Protein Atlas) among genes that were overexpressed in ICI-DM patients (G6PC and CDH9). (C:) Data from Human Protein Atlas indicating protein expression in normal pancreas for G6PC (top) and CDH9 (bottom). ICI-DM, immune checkpoint inhibitor-induced diabetes mellitus.

expressed in tumors from ICI-DM patients (CDH9 and G6PC) had protein expression in the normal pancreas (RNA-seq expression: figure 1B; Human Protein Atlas data: figure 1C).

We next evaluated mRNA expression of known autoantigens associated with T1DM[18] as tumor antigen expression could predispose patients to ICI-DM, although mRNA overexpression does not necessarily result in protein overexpression. No differential abundance of any of the known genes were found in ICI-DM patient tumors compared with controls (online supplemental table 3).

Mutation analysis

We applied quality filters described in online supplemental methods to identify mutations in tumors that were both unique to ICI-DM patients (not seen in control patients) and occurred in at least five patients. Using GATK variant calling on the RNA-seq data, we determined that the number of mutations per patient ranged from 5 to 413 (mean:191.2, online supplemental table 1).

We found 23 non-synonymous mutations that occurred in ≥5 tumors of the 13 ICI-DM patients: 15 missense mutations, 6 splice donor variants, 1 splice region variant, and 1 start lost variant (table 2). The most common recurring mutation in mRNA was a missense mutation in NLRC5, found in 9 of 13 ICI-DM patient tumors. Three mutations were unique to 7 of 13 patients: missense mutations in DNAJB11, PXN, and XRCC3. Two mutations were unique to 6 of the 13 ICI-DM patients: splice donor variant in HNRNPUL2 and a start lost mutation in ERCC6L2. There were 17 non-synonymous mutations that occurred in 5 ICI-DM patients: 11 missense mutations in ACCS, CEMIP2, HKDC1, ISG20L2, KIAA0100, MAP1S, MED16, MIA3, PON2, TANC1, and TEP1, one splice region variant in EIF4G2, and five splice donor variants in ABCA2, ANKRD36, ETHE1, MED25, and YTHDC1.

DNA extracted from PBMCs of ICI-DM patients was assessed using WES to determine whether the mutations found in the RNA were germline or somatic mutations (table 3). Mutations were germline for all patients in *NLRC5*, *DNAJB11*, *XRCC3*, *ERCC612*, *CEMIP2*, *EIF4G2*, *HKDC1*, *ISG20L2*, *MED16*, *PON2*, and *TEP1*. Mutations in *ACCS*, *KIAA0100*, *MAP1S*, *MIA3*, and *TANC1* each had one patient with a somatic mutation. The *PXN* missense mutation of interest from the RNA was not present in the WES of DNA, and we did not detect this mutation in online cancer genetic databases, including Catalog of Somatic Mutations in Cancer (COSMIC), cBioPortal for Cancer Genomics (cBioPortal), and The Cancer Genome Atlas (TCGA).

The Fisher's exact test with the Bonferroni correction was used to compare the prevalence of mutations that were uniquely or primarily germline (n=16) in the general US population, using the 1000 Genomes Project,[19] to the prevalence in the germline DNA of our ICI-DM cohort (table 3). NLRC5 germline mutations were found in 12.8% of the general population, significantly less than

Table 2 Unique mutations that occurred in tumors from five or more ICI-DM patients

Gene	Gene name	Chromosome|mutation	Protein change	Top consequence	Number of ICI-DM Patients with mutation (n=13)	Cancer types represented (n=5)
NLRC5	NLR family CARD domain containing 5	chr16:57025515|SNP:C>T	Pro191Leu	Missense	9	4
DNAJB11	DnaJ heat shock protein family (Hsp40) member B11	chr3:186583914|SNP:A>G	Ile264Val	Missense	7	4
PXN	Paxillin	chr12:120216281|SNP:G>C	Pro160Ala	Missense	7	5
XRCC3	X-ray repair cross complementing 3	chr14:103699416|SNP:G>A	Thr241Met	Missense	7	4
ERCC6L2	ERCC excision repair 6 like 2	chr9:95876006|SNP:A>G	−64+4737A>G	Start lost	6	4
HNRNPUL2	Heterogeneous nuclear ribonucleoprotein U like 2	chr11:62715379|Del:ACT…AC>A	c.2163+1_2164-1del	Splice donor variant	6	4
ABCA2	ATP binding cassette subfamily A member 2	chr9:137016471|Del:TCT…>T	c.2923+1_2924-1del	Splice donor variant	5	4
ACCS	1-aminocyclopropane-1-carboxylate synthase homolog (inactive)	chr11:44083431|SNP:C>T	Pro421Leu	Missense	5	3
ANKRD36	Ankyrin repeat domain 36	chr2:97192885|Del:CGG…A>C	c.2376+2_2377del	Splice donor variant	5	4
CEMIP2	Cell migration inducing hyaluronidase 2	chr9:71745318|SNP:C>T	Arg245Lys	Missense	5	3
EIF4G2	Eukaryotic translation initiation factor 4 gamma 2	chr11:10806814|SNP:C>A	c.107+6G>T	Splice region variant	5	4
ETHE1	ETHE1 persulfide dioxygenase	chr19:43526349|Del:ACT…AC>A	c.226+1_227-1del	Splice donor variant	5	4
HKDC1	Hexokinase domain containing 1	chr10:69266754|SNP:C>A	Asn917Lys	Missense	5	2
ISG20L2	Interferon stimulated exonuclease gene 20 like 2	chr1:156727264|SNP:T>C	Asn130Ser	Missense	5	4
KIAA0100	KIAA0100	chr17:28628312|SNP:A>C	Val137Gly	Missense	5	4
MAP1S	Microtubule associated protein 1S	chr19:17726616|SNP:C>G	Ser411Cys	Missense	5	4
MED16	Mediator complex subunit 16	chr19:868115|SNP:C>T	Glu874Lys	Missense	5	5
MED25	Mediator complex subunit 25	chr19:49829947|Del:TGG…A>T	c.688+1_689-1del	Splice donor variant	5	3
MIA3	MIA SH3 domain ER export factor 3	chr1:222629034|SNP:A>G	Lys188Arg	Missense	5	4
PON2	Paraoxonase 2	chr7:95405463|SNP:G>C	Ser311Cys	Missense	5	4
TANC1	Tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 1	chr2:159097663|SNP:C>T	Pro30Ser	Missense	5	4
TEP1	Telomerase associated protein 1	chr14:20404722|SNP:G>T	Asn307Lys	Missense	5	4
YTHDC1	YTH domain containing 1	chr4:68337450|Del:TCT…C>T	c.459+1_460-1del	Splice donor variant	5	4

ICI-DM, immune checkpoint inhibitor-induced diabetes mellitus.

Table 3 Prevalence of mutations in germline DNA from ICI-DM patients and the general population

Index	Gene	Gene name	Chromosome|mutation	Protein change	Top consequence	ICI-DM Patients with germline mutation (Percentage, n=13)	Prevalence in 1000 genomes project, American (1000GP)	Fisher's Exact Test: 1000GP vs ICI-DM Mutation Prevalences (p value) *Significant with Bonferroni Correction
1	NLRC5	NLR family CARD domain containing 5	chr16:57025515|SNP:C>T	Pro191Leu	Missense	9 (69.2%)	12.80%	0.00000598*
2	CEMIP2	Cell migration inducing hyaluronidase 2	chr9:71745318|SNP:C>T	Arg245Lys	Missense	5 (38.5%)	4.60%	0.000284*
3	KIAA0100	KIAA0100	chr17:28628312|SNP:A>C	Val137Gly	Missense	4 (30.8%)	6.10%	0.007287
4	XRCC3	X-ray repair cross complementing 3	chr14:103699416|SNP:G>A	Thr241Met	Missense	7 (53.8%)	29.90%	0.016368
5	DNAJB11	DnaJ heat shock protein family (Hsp40) member B11	chr3:186583914|SNP:A>G	Ile264Val	Missense	7 (53.8%)	23.80%	0.01737
6	EIF4G2	Eukaryotic translation initiation factor 4 gamma 2	chr11:10806814|SNP:C>A	c.107+6G>T	Splice region variant	5 (38.5%)	66.10%	0.071673
7	MED16	Mediator complex subunit 16	chr19:868115|SNP:C>T	Glu874Lys	Missense	5 (38.5%)	20.70%	0.161434
8	PON2	Paraoxonase 2	chr7:95405463|SNP:G>C	Ser311Cys	Missense	5 (38.5%)	23.30%	0.199808
9	ERCC6L2	ERCC excision repair 6 like 2	chr9:95876006|SNP:A>G	−64+4737A>G	Start lost	6 (46.2%)	29.00%	0.217661
10	MAP1S	Microtubule associated protein 1S	chr19:17726616|SNP:C>G	Ser411Cys	Missense	4 (30.8%)	17.70%	0.265528
11	TEP1	Telomerase associated protein 1	chr14:20404722|SNP:G>T	Asn307Lys	Missense	5 (38.5%)	27.80%	0.368396
12	ISG20L2	Interferon stimulated exonuclease gene 20 like 2	chr1:156727264|SNP:T>C	Asn130Ser	Missense	5 (38.5%)	28.20%	0.53443
13	HKDC1	Hexokinase domain containing 1	chr10:69266754|SNP:C>A	Asn917Lys	Missense	5 (38.5%)	49.10%	0.578612
14	MIA3	MIA SH3 domain ER export factor 3	chr1:222629034|SNP:A>G	Lys188Arg	Missense	4 (30.8%)	39.80%	0.580397
15	TANC1	Tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 1	chr2:159097663|SNP:C>T	Pro30Ser	Missense	4 (30.8%)	24.90%	0.746313
16	ACCS	1-aminocyclopropane-1-carboxylate synthase homolog (inactive)	chr11:44083431|SNP:C>T	Pro421Leu	Missense	4 (30.8%)	30.40%	>0.999999

*Indicates significant difference in prevalence after the Bonferroni correction.
ICI-DM, immune checkpoint inhibitor-induced diabetes mellitus.

that in our ICI-DM patients (69.2%), p=5.98×10^{-6}. The general population prevalence of CEMIP2 (4.6%) was also significantly lower than in ICI-DM patients (38.5%), p=0.000284. The other 14 germline mutations were not significantly more prevalent than the general population.

To address the possibility that pre-existing germline mutations might be associated with earlier onset of ICI-DM, we employed Mann-Whitney U tests with the Bonferroni correction and evaluated germline mutations common to ≥5 ICI-DM patients. No associations were found between specific mutations and time to ICI-DM (online supplemental figure 2), (online supplemental table 4).

We determined whether individual genes had multiple mutations in the RNA. Genes with ≥10 individual mutations are listed in online supplemental table 5. Six unique mutations, the largest number of unique mutations, occurred in IVD, MTA2, WNK2, ALPK2, and MKI67. The only two genes that also recurred in ICI-DM patients (defined as germline mutations in ≥5 ICI-DM patients) were NLRC5 and MAP1S. NLRC5 missense mutation occurred in nine ICI-DM patients, and three patients had synonymous NLRC5 mutation. MAP1S missense mutation occurred in five ICI-DM patients, and six had synonymous mutations. The PolyPhen-2 program was used to predict how NLRC5 and MAP1S missense mutations may affect protein function.[20] NLRC5 (Pro191Leu) was predicted to be benign and not affect function, whereas MAP1S (Ser411Cys) is probably damaging to protein function. In the RNA-seq data, there were no significant differences in RNA counts of NLRC5 or MAP1S in ICI-DM patients with or without the missense mutations (online supplemental figure 3).

To further examine the contribution of NLRC5 (Pro191Leu) as a predisposing factor to T1DM, we queried prior published T1DM datasets[21 22] and the Type 1 Diabetes Genetics Consortium, a database consisting of majority European descent, African American, and Hispanic/Latino genomes for T1DM and controls, including some sibling pairs (https://repository.niddk.nih.gov/studies/t1dgc/). The prevalence of NLRC5 (Pro191Leu) was not significantly different between the T1DM and the controls.

DISCUSSION

Previous studies from our labs described the unique attributes of ICI-DM and aimed to map pathophysiological mechanisms that impact development of this life-altering irAE. Here, we explored the contribution of tumor tissue and germline characteristics as possible triggers or catalysts to ICI-DM.

We first examined genes associated with T1DM to explore the hypothesis that non-MHC genes implicated in the pathogenesis of T1DM would also play a role in ICI-DM, particularly if overexpressed in tumor cells and possibly antigenic.[18] We did not find overexpression of these autoantigens in tumors of ICI-DM patients.

Among the genes most abundantly expressed in tumors from ICI-DM patients compared with control patients, some have tangential relationships to the pancreas, insulin, and T1DM. CDH9, DSG1, G6PC, ORM1 and PLG had the highest expression in tumors from ICI-DM patients, and thus could be further investigated for associations with ICI-DM. ORM1 is an acute-phase reactant, which has been shown to be elevated in pancreatic tissue of patients with T1DM compared with controls.[23] There is no known direct relationship between PLG, DSG1, or CDH9 and T1DM. G6PC2, an isoform of G6PC with 50% overlap of its amino acids, is a T1DM-related autoantigen of interest because it is found almost exclusively in pancreatic islet cells. G6PC2, however, was not differentially expressed between ICI-DM and control patients in our cohort. These genes serve as a basis for further exploration of mechanisms of ICI-DM onset.

We next studied mutations in tumor tissue of ICI-DM patients that were absent in controls. NLRC5 and CEMIP2 were significantly more prevalent in ICI-DM patients compared with controls. CEMIP2 does not have a known relationship to T1DM. We therefore focused on the missense mutation in NLRC5 (Pro191Leu). This mutation was also found in germline DNA from all 9 of 13 patients. We compared the prevalence in the ICI-DM patients to that of the general population, and found that it was significantly higher in patients who developed ICI-DM. Despite the prevalence of *NLRC5* mutations, this gene was not overexpressed at the mRNA level in ICI-DM patient tumors. NLRC5 is an important transactivator of HLA-I genes and is necessary for HLA expression. It has been implicated in multiple studies related to T1DM. For example, NLRC5 affects immune function in islet cells and it increases autoimmunity in patients with T1DM.[24] This mutation was not significantly more prevalent in a diverse population of T1DM genomes compared with controls, indicating it is not a risk factor for T1DM and further suggesting that the genetic predisposition may be unique to the mechanism of ICI-DM. *NLRC5* mutations could be used as a predictive biomarker for ICI-DM. Additional investigations are needed in ICI-DM patients to validate the finding of high prevalence of *NLRC5* germline mutations in this patient population, and functional studies are warranted to determine the mechanism by which NLRC5 mutations predispose ICI-treated patients to DM.

A missense mutation was found in *MAP1S* in germline DNA from 38% of our ICI-DM patients. Though the *MAP1S* mutation was expected to affect protein function by the PolyPhen-2 program, there are no known connections between mutated *MAP1S*, T1DM, and islet cell function.

A PXN missense mutation was found in tumors from ICI-DM patients. Interestingly, PXN was recently reported as associated with T1DM progression.[25] However, we did not find this *PXN* mutation in ICI-DM patients' germline DNA, nor did we find this somatic mutation in other large cancer datasets, including COSMIC, cBioPortal,

and TCGA. Noting that ICI-DM is rare, this somatic PXN mutation might therefore be a novel mutation unique to ICI-DM patients, and further interrogation of tumors from ICI-DM patients is warranted.

Time to onset of ICI-DM is highly variable, as reported in our previous studies.[6] Since the unique tumor mutations were mostly determined to be germline, we hypothesized that a genetic predisposition might lead to earlier onset ICI-DM. However, no statistically significant association between presence of germline mutations and time of onset of ICI-DM. Larger datasets might elucidate associations between specific germline variants and early-onset ICI-DM.

The biggest limitation of this study is the small sample size of tumors from ICI-DM patients. Given the rarity of ICI-DM, multi-institutional efforts are needed to further study this rare but serious irAE. Larger cohorts might reveal associations with specific tumor types or regimens. In addition, to compare to T1DM genetic data, we are limited by using WES rather than whole genome sequencing, which is often employed because the majority of genetic discovery in T1DM occurs in gene regulatory regions rather than coding regions.

In conclusion, in tumors from 13 ICI-DM patients, we identified differentially prevalent genetic variants, the majority of which were germline. Additional studies are warranted to verify the association between germline variants in genes, such as *NLRC5* and *CEMIP2*, and ICI-DM, as these might serve as biomarkers for patient selection for immunotherapy. Mechanistic studies are similarly warranted to identify potential drug targets to mitigate ICI-DM.

Author affiliations
[1]Medical Oncology, Yale School of Medicine, New Haven, Connecticut, USA
[2]Department of Medicine, Yale School of Medicine, New Haven, Connecticut, USA
[3]Department of Immunobiology, Yale School of Medicine, New Haven, Connecticut, USA
[4]Center for Public Health Genomics, University of Virginia, Charlottesville, Virginia, USA
[5]Department of Mathematics, Program in Applied Mathematics, Yale University, New Haven, Connecticut, New Haven, Connecticut, USA
[6]Pathology, Yale University School of Medicine, New Haven, Connecticut, USA

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Ethics approval This study involves human participants and was approved by Yale Institutional Review Board, HIC#0609001869 and HIC#0608001773. Participants gave informed consent to participate in the study before taking part.

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ORCID iD
Harriet Kluger http://orcid.org/0000-0002-4932-9873

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