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Original Research

Human leukocyte antigen variation is associated with adverse events of checkpoint inhibitors

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Abstract ***Background:*** Checkpoint inhibitors (CIs) are highly effective but can induce severe immune-related adverse events (irAEs), which cannot be predicted. We investigated whether human leukocyte antigen (HLA) genes predispose to developing of irAEs during therapy and thus hold a predictive role.
Methods: We established a prospective observational single-centre study and collected data from patients with either metastatic non–small cell lung cancer (NSCLC) or metastatic melanoma, who were treated with anti–PD-1 (programmed cell death receptor 1), anti-CTLA4 (cytotoxic T-lymphocyte–associated protein 4) or both CIs combined. Data include irAEs and ranges from 15th July 2016 until 10th May 2018. In addition, we performed HLA typing via next generation sequencing.
Results: We enrolled 102 patients (median [range] age, 68 [62–74] years) with metastatic cancer in our study who received CI therapy. Of these patients, 59 (58%) developed one or more irAEs, among which pruritus (n = 32 (54%)) and rash (n = 24 (41%)) had the highest rates. We did not find evidence for a single HLA gene being associated with all irAEs (all $P > .05$). When assessing each irAE individually, we found a significant association between HLA-DRB1*11:01 and pruritus (OR = 4.53, $X^2_{1,95} = 9.45$, $P < .01$) as well as a nominally

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significant additive association between HLA-DQB1*03:01 and colitis (OR = 3.94, $X^2_{1,95} = 5.67$, $P = .017$).

Conclusions: The presence of two HLA alleles that are known to predispose to autoimmune diseases were associated with the development of pruritus or colitis during therapy, suggesting a genetic aetiology of irAEs. Larger genome-wide association studies should be performed to confirm our findings.

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1. Introduction

Checkpoint inhibitors (CIs) have significantly prolonged overall survival in metastatic cancer patients and have ushered a new treatment era in clinical oncology [1,2]. However, all CIs entail immune-mediated adverse events (irAEs), also referred to as toxicities, that can affect all organs and range from a mild skin rash managed by topical corticosteroids to fulminant colitis or myocarditis, requiring hospitalisation [3–7]. Although the exact mechanisms remain unclear, it is suspected that irAEs are caused by interference of CIs with tolerance mechanisms mediated by cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) [8,9] and the programmed cell death protein (PD-1) [10,11]. Currently, there are no markers before therapy to predict if an irAE will develop during treatment, except patients suffering from preexisting autoimmune disease, who may experience flares of their respective condition during therapy [12,13]. Severe irAEs require pausing CI therapy and are treated with immunosuppressive drugs [5,14–16].

Interestingly, some irAEs resemble well-characterised autoimmune diseases with defined human leukocyte antigen (HLA) risk alleles. Examples are generalised pruritus and atopic dermatitis [17,18], colitis and inflammatory bowel diseases [19], thyroiditis and Grave's disease [20,21] as well as arthritis and rheumatoid arthritis [22].

The aim of this study was to explore whether HLA genes known to mediate susceptibility for autoimmunity are also associated with development of irAEs in metastatic cancer patients during CI therapy.

2. Materials and methods

2.1. Study population

We established a monocentric prospective observational study and enrolled patients with metastatic non-small cell lung cancer (NSCLC) or metastatic melanoma, who received CI treatment with either anti-PD-1, anti-PD-ligand-1 (PD-L1), anti-CTLA4 or a combination anti-PD-1 and anti-CTLA4. The end of the follow-up period was 10th May 2018. The study was approved by the local ethics committee (Project ID 2016–009918) and was conducted accordingly.

All participants were from Eastern Switzerland and of European ancestry. Patients were at least 18 years of age and presented with either NSCLC or metastatic melanoma. Treatment consisted of CIs including ipilimumab (anti-CTLA4), pembrolizumab (anti–PD-1), nivolumab (anti–PD-1) or atezolizumab (anti-PD-L1) for at least one cycle. IrAE development was monitored from therapy start and was included into this study if onset was before 11th May 2018 (end of data collection). Monitoring was conducted by oncologists and categorised as follows, with respect to CTCAE V4.03: rash,

Table 1
Characteristics of patients with NSCLC or melanoma receiving checkpoint inhibitor therapy.

Characteristic	Patient count (N = 102)
	N (%)
irAE[a]	59 (58)
No irAE	43 (42)
Age (median [IQR[b]])	68 (62–74)
Sex	
Male	52 (51)
Female	50 (49)
Cancer type	
NSCLC	66 (65)
Melanoma	36 (35)
Treatment	
PD-(L)1[c] inhibitor	92 (90)
Nivolumab	38 (37)
Pembrolizumab	47 (46)
Atezolizumab	7 (7)
CTLA-4[d] inhibitor	10 (10)
Ipilimumab alone	4 (4)
Ipilimumab with nivolumab	6 (6)
Smoking (only NSCLC[e])	
Yes	60 (59)
Pack years (median [IQR])	40 (30–60)
No	5 (5)
Unknown	1 (1)
Sunburns (only melanoma)	
Never	1 (3)
Rare	32 (91)
Frequent	2 (6)
Unknown	1 (3)

[a]irAE, immune-related adverse event; [b]IQR, interquartile range; [c]PD(L)-1, programmed cell death protein (ligand)–1; [d]CTLA4, cytotoxic T-lymphocyte–associated protein 4; [e]NSCLC, non–small cell lung cancer.

generalised pruritus, vitiligo, colitis, pneumonitis, hepatitis, thyroiditis, hypophysitis, arthritis and others (asthenia, fatigue, nephritis and Guillain-Barré syndrome). Each irAE was documented via database technology (SecuTrial, version 5.3.0.10). If toxicity was equal to or exceeded common terminology criteria for adverse events (CTCAE) grade 3, it was independently verified by a physician trained in the respective medical speciality. High-resolution HLA haplotyping was performed using next generation sequencing (ProImmune Ltd., Oxford, UK and Histogenetics LLC, New York, USA). With this technology, complete HLA class I (A, B, C) and class II (DRB1, DPB1 and DQB1) genes were sequenced, resulting in second field (four digit) allele-level HLA types with no degeneracy.

2.2. Statistical analysis

Logistic regression models used for the main association analyses included several covariates that might potentially confound the risk for irAEs: patient age and sex, cancer type (NSCLC or melanoma) and specific CI used in therapy. When testing association of an entire HLA locus, we included all common variants of that locus as separate terms in the model. When testing for association of specific HLA variants, we included only that variant in the model. For the association test, we only analysed common variants (allele frequency >.1 in our cohort) and only included variants from the four HLA loci most frequently associated with autoimmune diseases: HLA-B ($N = 2$ alleles), HLA-C ($N = 3$), HLA-DRB1 ($N = 4$) and HLA-DQB1 ($N = 4$) [23]. We controlled for the false discovery rate (FDR) using a Bonferroni-corrected significance threshold ($P = .05/13 = .0038$). For the Cox proportional hazard regression models, we used the coxph function of the survival package in R. All analyses were conducted in R version 3.4.2.

3. Results

3.1. Study population

To identify HLA associations with irAEs, we enrolled a total of 102 patients (median [interquartile range] age, 68 [62–74] years) with metastatic cancer in our study who received CI therapy. Sixty-six (65%) had been

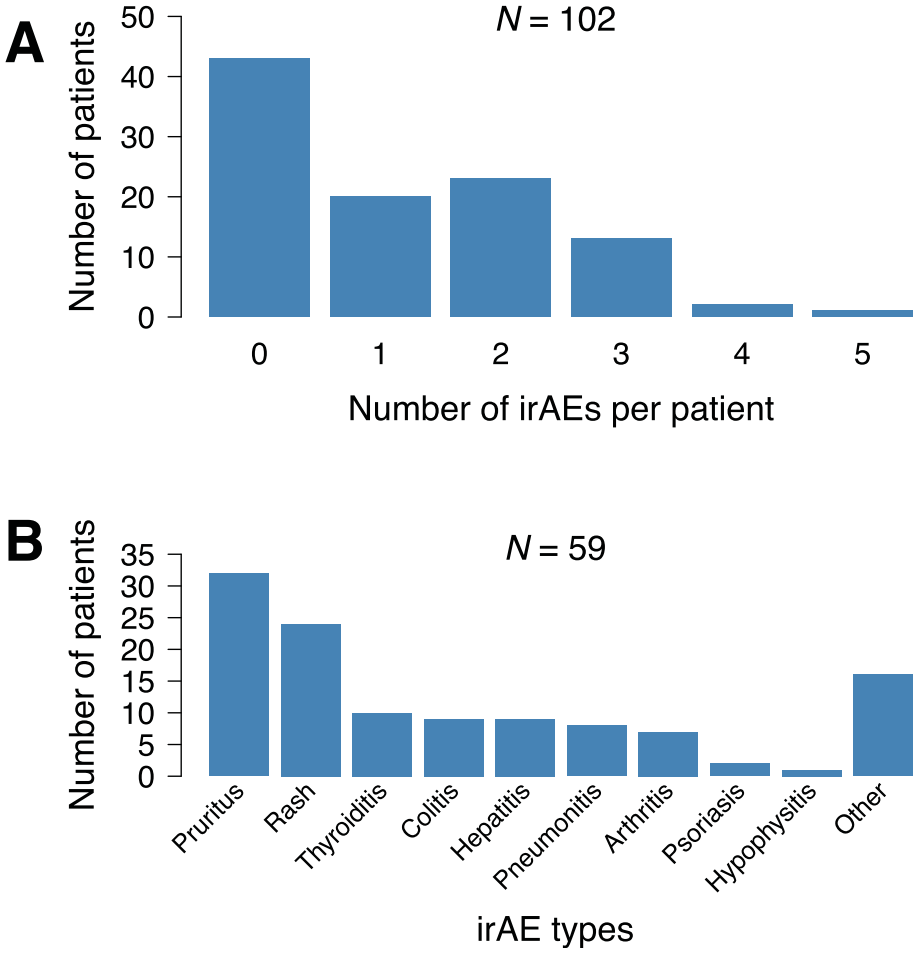

Fig. 1. Immune-mediated adverse events (irAEs) in cancer patients. Number and frequency of irAEs in non–small cell lung cancer and melanoma patients treated with checkpoint inhibitors. (A) Distribution of the number of irAEs per patient (0–5, $N = 102$). (B) A bar plot illustrating the numbers of patients affected with each irAE ($N = 59$).

diagnosed with stage IV NSCLC, and 36 (35%), with stage IV melanoma. Ninety-two (90%) patients were treated with anti–PD-1 (nivolumab or pembrolizumab) or anti-PD-L1 (atezolizumab) treatment alone. Six (6%) patients had dual treatment with anti-CTLA-4 (ipilimumab) and anti–PD-1 (nivolumab), and four (4%) patients received anti-CTLA-4 therapy (ipilimumab) only. The clinical characteristics are summarised in Table 1. Of these patients, 59 (58%) developed one or more irAEs (Fig. 1).

3.2. Associating irAEs with HLA

First, we tested whether a common genetic variation at the four investigated HLA loci (HLA-B, -C, -DRB1, -DQB1) was associated with the risk of developing any irAE, regardless of the affected organ. We did not find evidence for a general association between HLA and developing any irAE during CI therapy, regardless of the affected organ (all $P > .05$). This was expected because, as with irAEs, autoimmune disorders are organ-specific, and each is individually associated with certain HLA variants and loci. Next, we focused on the group of skin-related toxicities only (pruritus and rash). Our analysis revealed no effect of HLA class I genes ($P > .05$). However, the risk to develop one or more skin-related irAE was nominally associated with common variation in HLA-DRB1 ($X^2_{4,92} = 15.21$, $P = .004$) and HLA-DQB1 ($X^2_{4,92} = 13.35$, $P = .01$). Encouraged by these results, we proceeded to analyse pruritus and

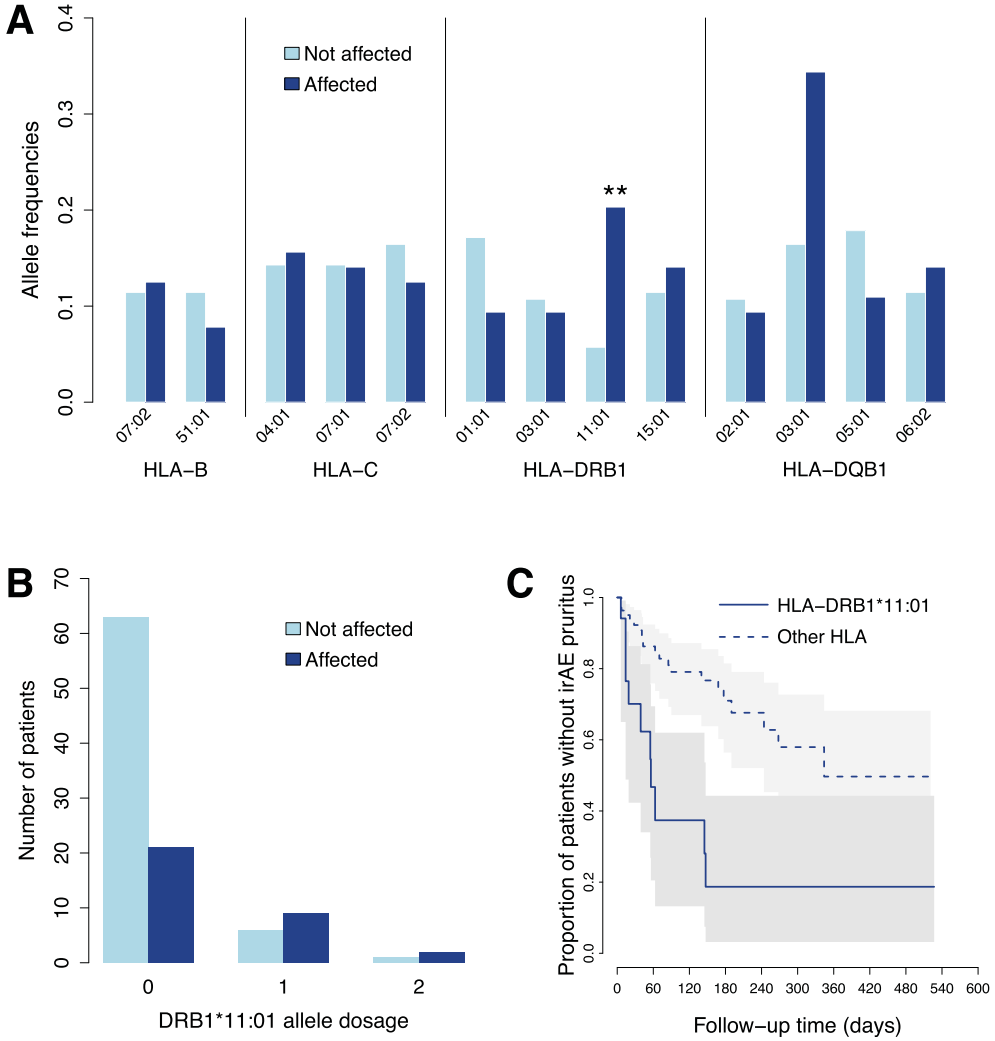

Fig. 2. Association between HLA variants and pruritus. (A) Allele frequencies of 13 common HLA variants among cancer patients treated with checkpoint inhibitors. They are shown separately for patients without (light blue bars) and with (dark blue bars) immune-mediated pruritus. Only the association of HLA-DRB1*11:01 and pruritus is significant (OR = 4.53, $X^2_{1,95} = 9.45$, $^{**}P = .0021$, $N = 102$). HLA-DQB1*03:01 is commonly associated with HLA-DRB1*11:01 and, by itself, shows no significant association with pruritus. (B) Patients carrying the HLA variant DRB1*11:01 are more likely to exhibit pruritus than patients with other HLA haplotypes ($N = 102$). (C) Concurringly, patients without pruritus are very likely not to carry HLA-DRB1*11:01 (interrupted line), whereas only very few carriers of this variant remain free of pruritus (full line). Kaplan–Meier survival curves with 95% log hazard confidence intervals (shaded areas). For visualisation, a patient with follow-up time >2000 days was excluded in this plot ($N = 101$). OR, odds ratio. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

rash separately to focus on their individual associations with specific variants in those two genes. With this approach, we found a significant association between HLA-DRB1*11:01 and pruritus that exceeded the study-wide FDR-corrected significance threshold (OR = 4.53, $X^2_{1,95}$ = 9.45, P = 0.002, Fig. 2A). This association became even stronger when testing for the presence/absence of the variant, rather than assuming an additive risk model ($X^2_{1,95}$ = 10.47, P = 0.0012). This might indicate a slight dominance effect of this variant on pruritus risk. The association is supported by literature, because an association between atopic dermatitis and DRB1*11:01 has been reported. During our analysis of the association between the presence of HLA-DRB1*11:01 and pruritus, we noticed that in our cohort HLA-DRB1*11:01 always co-occurs with the variant HLA-DQB1*03:01, indicating genetic linkage between HLA-DRB1 and HLA-DQB1. Such tightly linked haplotypes in the HLA region are a common and well-described phenomenon [24]. However, HLA-DQB1*03:01 co-occurred with other HLA-DRB1 variants as well, which enabled us to additionally control for a possible effect of HLA-DQB1 variation. Reassuringly, the association of HLA-DRB1*11:01 remained nominally significant after conditioning on the presence of common variants at the HLA-DQB1 locus ($X^2_{1,91}$ = 5.1, P = .024). Investigating the proportional hazard of developing pruritus with and without HLA-DRB1*11:01 revealed that the effect of this variant was even stronger than we initially assumed (proportional hazard: 5.39, $P < .001$; Fig. 2B and C). Carriers of this variant also presented earlier with pruritus than those without the variant. Furthermore, we find a possible trend for a protective association between HLA-DRB1*01:01 and skin rash (OR = .26 P = .054). Among the non−skin-related irAEs, colitis revealed a nominally significant additive association with the HLA class II variant HLA-DQB1*03:01 (OR = 3.94, $X^2_{1,95}$ = 5.67, P = .017, see Fig. 3). Although low-to medium-grade pruritus can be conveniently treated with

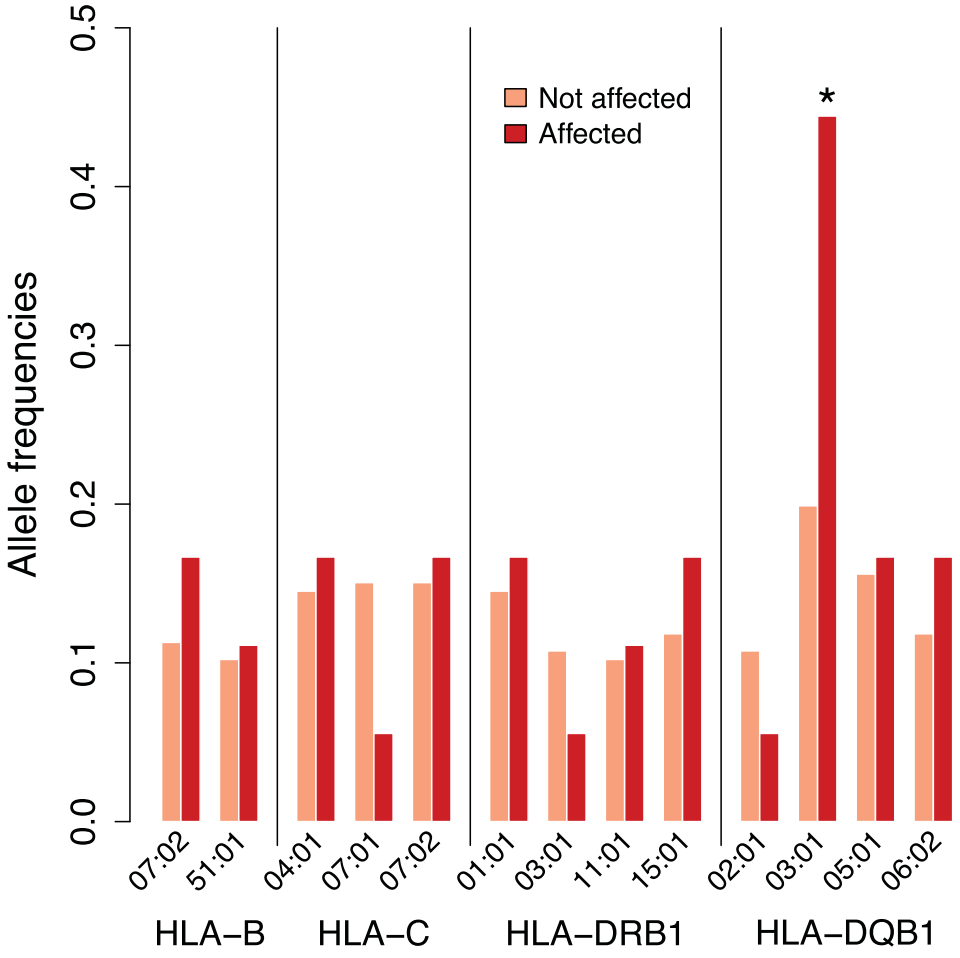

Fig. 3. Association between HLA variants and colitis. Allele frequencies of 13 common HLA variants among cancer patients treated with checkpoint inhibitors (N = 102). Orange bars show patients without immune-mediated colitis (N = 93), whereas red bars depict patients with colitis (N = 9). Despite the small sample size of 9 patients with colitis, it revealed a nominally significant additive association, with the HLA class II variant HLA-DQB1*03:01 (OR = 3.94, $X^2_{1,95}$ = 5.67, *P = .017). OR, odds ratio. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article).

topical corticosteroids, colitis presents a major clinical challenge and a medical emergency, as it can lead to colon perforation and death [25]. In addition, HLA-DQB1*03:01 has been strongly linked to inflammatory bowel diseases [19] further supporting its proposed link with irAE colitis and linking HLA and irAEs in general. All identified effects were independent of sex or age. Other irAEs did not show any evidence for association with specific HLA variants, potentially owing to the limited case numbers in our cohort (all $n \leq 10$).

4. Discussion

Our study reveals a previously unrecognised link between HLA alleles that are associated with autoimmune diseases and the risk of developing organ-specific irAEs during CI therapy. The findings are supported by the following: (1) we demonstrate a statistically significant association between developing pruritus and HLA-DRB1*11:01; (2) we identified a nominally significant association between HLA-DQB1*03:01 and colitis; (3) previous reports that patients with autoimmune diseases can exhibit flare-ups of their autoimmune disease during CI therapy. The fact that we had a fairly limited sample size for a genetic association study ($N = 102$) at our disposal further highlights the significance of the found associations. Even though the discovery of an association between HLA risk alleles and developing irAEs may aid in risk calculations, some limitations need to be addressed. First, we examined a cohort of rather limited size ($N = 102$). Second, irAEs during checkpoint inhibition may appear at any given time, sometimes after months, which may constitute a bias in the time frame of data collection [4]. The increasing use of CI therapies will allow recruiting a much larger cohort over longer time that could eventually facilitate a broader genetic investigation of these associations to provide a better understanding of the HLA-associated disease dynamics.

5. Conclusion

Our data suggest that a genetic HLA-mediated predisposition for autoimmune diseases may lead to the development of irAEs once the adaptive immune system loses its tolerance against self-antigens through treatment with CIs. Currently, severe irAEs lead to therapy interruption and are treated with systemic immunosuppression, which may counteract the desired therapy effect. Identifying patients at risk for developing irAEs prompts symptom monitoring and early interdisciplinary management among medical subspecialities. Our results may contribute to the development of a screening tool that is able to single out patients at risk for developing potentially high grade irAEs before CI therapy. Such a tool would allow physicians to differentiate between low- and high-risk patients with direct implications on patient monitoring and care.

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Conflict of interest statement

None declared.

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