9-1-2022

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10.1016/j.ejca.2022.05.021
Abed, A., Law, N., Calapre, L., Lo, J., Bhat, V., Bowyer, S., ... & Gray, E. S. (2022). Human leucocyte antigen genotype association with the development of immune-related adverse events in patients with non-small cell lung cancer treated with single agent immunotherapy. European Journal of Cancer, 172, 98-106. https://doi.org/10.1016/j.ejca.2022.05.021

European Journal of Cancer 172 (2022) 98–106

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Human leucocyte antigen genotype association with the development of immune-related adverse events in patients with non-small cell lung cancer treated with single agent immunotherapy

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Received 12 December 2021; received in revised form 23 March 2022; accepted 17 May 2022

KEYWORDS
Human leucocyte antigens;
Non-small cell lung cancer;
Anti-PD1;
Immunotherapy;
Adverse drug events;
Patient outcomes assessment

Abstract ***Introduction:*** Biomarkers that predict the risk of immune-mediated adverse events (irAEs) among patients with non-small cell lung cancer (NSCLC) may reduce morbidity and mortality associated with these treatments.
Methods: We carried out high resolution human leucocyte antigen (HLA)-I typing on 179 patients with NSCLC treated with anti-program death (PD)-1/program death ligand (PDL)-1. Toxicity data were collected and graded as per common terminology criteria for adverse event (CTCAE) v5.0. We used 14.8-week for landmark analysis to address lead-time bias to investigate the correlation between HLA-I/II zygosity, supertypes and alleles with irAE. Furthermore, we assessed the association for irAE with clinical benefit rate (CBR), progression-free survival (PFS) and overall survival (OS).
Results: Homozygosity at one or more HLA-I loci, but not HLA-II, was associated with a

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https://doi.org/10.1016/j.ejca.2022.05.021

reduced risk of irAE (relative risk (RR) = 0.61, 95% CI 0.33–0.95, P = 0.035) especially pneumonitis or any grade 3 toxicity. Patients with HLA-A03 supertype had a higher risk of developing irAE (RR = 1.42, 95% CI 1.02–2.01, P = 0.039). The occurrence of any irAE was significantly associated with improved CBR (RR = 1.48, P < 0.0001), PFS (HR = 0.45, P = 0.0003) and OS (HR = 0.34, P < 0.0001).

Conclusions: Homozygosity at one or more HLA-I loci may serve as biomarker to predict patients who are unlikely to experience severe irAEs among patients with NSCLC and treated with anti-PD1/PDL1, but less likely to derive clinical benefit. Patients with HLA-I homozygous might benefit from additional therapy.

1. Introduction

The introduction of immune checkpoint inhibitors has revolutionised the treatment landscape of non-small cell lung cancer (NSCLC) and resulted in improved clinical outcome [1–3]. However, at least 74% of treated patients will experience immune-related adverse events (irAEs) following treatment [4]. Although mortality associated with irAE due to anti-PD1/PDL1 inhibitors is minimal (0.6% of patients), 14–21% of patients will experience grade 3 or 4 toxicity [4]. This can be associated with significant lifelong complications which can affect quality of life. Moreover, the management of some of these irAEs require the involvement of a large medical team of multiple health care specialists [4,5]. While steroid and immune suppressing agents form the backbone to treat irAEs [5], they are associated with side-effects themselves. In addition, development of grade 3 or 4 irAE can lead to the discontinuation of treatment. This could result in an increased risk of disease progression and preclude rechallenge with an effective therapy [5].

Alternatively, there is growing evidence that patients who experience irAE will more likely have favourable clinical outcomes when compared to those who do not experience any form of adverse event (AE) related to immunotherapy [6]. Therefore, predicting patients who will develop irAE will allow for closer monitoring and early management of irAE. This may avoid the development of grade 3/4 toxicity that usually leads to treatment discontinuation. Multiple biomarkers to predict irAE has been investigated [7,8]. None of those showed enough accuracy to reach clinical practice. While tumour PDL1 expression is the only FDA approved biomarker to predict clinical outcome among patients with NSCLC, it has no role to predict toxicity as it is related to the tumour microenvironment. Finding a non-invasive and easily obtained biomarker that can serve to predict clinical outcome as well as predict the possibility of developing irAE will improve the management of patients with NSCLC.

Human leucocyte antigen (HLA) is expressed on cell surface including immune and/or somatic cells. The HLA locus is polygenic and highly polymorphic with each locus having multiple alleles [9]. HLA-A and HLA-B alleles has been grouped into nine supertypes based on their peptide-binding repertoire [10]. Multiple HLA-I and -II alleles have been found to be associated with different autoimmune diseases [11–25] or increased risk of developing specific irAE in patients treated with immunotherapies [23,26–28]. Thus, the relation of HLA genotype and irAEs in patients treated with immunotherapy ought to be investigated in detail.

It has been shown that HLA-I homozygosity is associated with a shorter overall survival (OS) among patients with NSCLC treated with single agent immunotherapy in the first- or second-line setting [29,30]. We report here the correlation between HLA-I/II homozygosity and the development of irAEs. We correlated different HLA-I/II genotypes and toxicity and investigated the relationship between HLA-A and -B supertypes and toxicity. Moreover, we examined the correlation between toxicity and clinical benefit rate (CBR), progression-free survival (PFS) and OS.

2. Methods

2.1. Patients

Patients were recruited from two major teaching hospitals in Western Australia. All procedures were approved by the Human Research Ethics Committees at Edith Cowan University (ECU) (No. 18957) and Sir Charles Gairdner Hospital (No. 2013-246 and RGS0000003289) in compliance with the Declaration of Helsinki. All participants signed a consent form which is saved at ECU research database. Recruited patients were 18 years or older, diagnosed with unresectable locally advanced or metastatic NSCLC and treated with single agent pembrolizumab, atezolizumab or nivolumab in the first-line or the second-line setting. Given

that genomic HLA would not be altered during treatment, patients were recruited both prospectively (before starting treatment) and retrospectively (after starting treatment). DNA extraction and HLA typing were performed as previously published [29].

Patient demographics and clinicopathological features that may affect the outcome were collected from the clinical records and included age, Eastern Cooperative Oncology Group (ECOG) performance status and smoking status, histopathology and tumour program death ligand-1 (PDL1) expression pre-treatment laboratory variable (neutrophils, lymphocytes, neutrophil lymphocyte ratio, platelet, platelet lymphocyte ratio and albumin). Only patients who were exposed to 10 or more pack year smoking were considered to be smokers. Information about irAE was collected. This included all immune-related toxicities starting between the initiation of immunotherapy and the time of collecting data. The median follow-up was 17 months. IrAE were categorised based on the involved system and graded from 1 to 5 according to the common terminology criteria for AEs v5.0 (CTCAE v5.0). Steroid usage to treat grade 2 or higher toxicity was recorded.

2.2. Statistical analysis

To avoid lead-time bias, a 14.8-week (103 days) landmark analysis was used to exclude patients who might die before developing irAE when correlating irAE and HLA-I/II zygosity. This landmark was used based on the results of a pooled analysis [31] which indicated that median time to onset of irAE in patients treated with anti-PD1/PD-L1 inhibitor was 8.4 weeks, with the latest irAE category presenting at 14.8 weeks. Patients were dichotomised based on having HLA homozygosity at one or more loci and heterozygosity; based on the presence of specific HLA-A and -B supertypes or based on carrying specific HLA-I/II alleles or haplotypes. HLA-I loci included HLA-A, -B and -C. HLA-II loci included HLA-DPB1, -DQA1, -DQB1, -DRB1 and -DRB3. All correlations with irAE were evaluated using Fisher's exact test. Binomial logistic regression models were used to study the factors associated with irAE using SPSS version 28.

CBR was defined as those patients who had complete response, partial response or stable disease for 6 or more months. The response assessment was based on clinician choice of imaging modality including computerised tomography or fluorodeoxyglucose-positron emission tomography scans. PFS was defined as the time interval between the start of therapy and the date of first progression/death. Progression was determined by clinician assessment based on both radiological and clinical presentation of the patient. OS was defined as the time between the start of immunotherapy and death. The minimum follow-up time was 9 months. Those who did not experience disease progression or were still alive at the time of analysis (April 2021) were censored.

The development of irAE was correlated with CBR using Fisher's exact test. PFS and OS were compared between patients who developed any form of irAE and those who did not use log-rank (Mantel–Cox) test and Kaplan–Meier plots. Statistical analyses and plotting were done using GraphPad Prism V.9 (GraphPad Software, Inc, San Diego, California). Multivariable Cox regression analysis of survival was performed using IBM SPSS version 28.

3. Results

3.1. Patients characteristics and toxicity incidence

A total of 193 patients with NSCLC were recruited between May 2018 and July 2020. These include 170 patients which were part of the previous study on the association between HLA-I homozygosity and survival [29]. Out of the 193 patients, 14 (7.3%) patients were excluded: 6 received targeted therapy or immunotherapy in combination with chemotherapy, 2 patients were lost to follow-up, 1 patient died from another cause before reaching response assessment, 1 was down staged and proceeded to surgery, 3 never had blood collected and 1 had inadequate blood volume. To reduce lead-time bias, a landmark analysis was conducted including only the 156 patients still alive at 14.8 weeks since treatment commencement (Table 1). The status of PDL1 was not known for 45/156 (28.8%) patients as this was not required when they received treatment with anti-PD1/PDL1 in the second-line setting. Among these 156 patients, 77 (49.4%) patients developed irAE (Table 2) with 23 of them (14.7%) experiencing more than one toxicity. A total of 102 toxicities were recorded, with skin rash the most commonly reported irAE followed by arthralgia. The frequency of grade 3 or more pneumonitis was (5/156) 5%. 17/156 (10.9%) of patients experienced grade 3 or more toxicity.

3.2. Correlation between HLA-I/II homozygosity and toxicity

Among the 156 patients included in the landmark analysis, we found that homozygosity at one or more HLA-I loci was associated with a reduced risk of developing any type of irAE, regardless of the grade, among patients with NSCLC treated with single agent anti-PD1/PDL1 (relative risk, RR = 0.61, 95% CI 0.33–0.99, P = 0.035) (Table 2). In contrast, no statistically significant correlation was found between HLA-II homozygosity and development of irAE RR = 0.83 (95% CI 0.57–1.16, P = 0.184). Further analysis of homozygosity at one or more HLA-I loci in relation to different types of irAEs showed statistically significant association with pneumonitis and ≥grade 3 irAE. None of the patients with NSCLC and with HLA-

Table 1
Patients demographic and clinical characteristics.

Patient characteristics	N (%)	N[a] (%)
Age		
≥65	107 (59.8)	95 (60.9)
<65	72 (40.2)	61 (39.1)
Sex		
M	101 (56.4)	87 (55.8)
F	78 (43.6)	69 (44.2)
ECOG		
≤1	151 (84.4)	137 (87.8)
>1	26 (14.5)	19 (12.2)
Unknown	2 (1.1)	0
Smoking		
Yes	146 (81.6)	125 (80.1)
No	22 (12.3)	21 (13.5)
Unknown	1 (6.1)	10 (6.4)
Histopathology		
Adenocarcinoma	122 (68.2)	104 (66.7)
SCC	48 (26.8)	42 (26.9)
Others	9 (5)	10 (6.4)
Molecular status[b]		
KRAS mutant	61 (46.6)	53 (46.5)
KRAS wild type	56 (42.7)	48 (42.1)
KRAS unknown	14 (10.7)	11 (9.6)
EGFR, ALK or ROS1 mutant	5 (3.8)	2 (1.8)
PDL1 Status		
≥50%	77 (43.0)	65 (41.7)
1–49%	27 (15.1)	22 (14.1)
<1%	26 (14.5)	24 (15.4)
Unknown	49 (27.4)	45 (28.8)
Line of treatment		
First-line	55 (30.7)	45 (28.8)
Second or more	124 (69.3)	111 (71.2)
Genomic HLA-I status		
Homozygous at one or more loci	34 (19)	27 (17.3)
Heterozygous at all loci	145 (81)	129 (82.7)
Genomic HLA-II status		
Homozygous at one or more loci	62 (34.6)	53 (34.0)
Heterozygous at all loci	117 (65.4)	103 (66.0)
Total	179	156

[a] 156 patients in 14.8 weeks landmark analysis.
[b] Molecular status was only examined in NSCLC with non-squamous cell carcinoma histology. ALK, echinoderm microtubule-associated protein like-4-anaplastic lymphoma kinase (*EML4/ALK*) fusion; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; F: female; HLA-I/II: human leucocyte antigen I/II, KRAS, Kirsten Rat Sarcoma GTPase; M: male; NSCLC, non-small cell lung cancer; PDL1: program death ligand-1; SCC: squamous cell carcinoma.

I homozygosity experienced pneumonitis (P = 0.044) or ≥grade 3 toxicity (P = 0.028) (Table 2). Although none of the HLA-I homozygous patients experienced endocrinopathy, nephrotoxicity or hepatotoxicity due to their lower occurrence in the cohort, these comparisons did not reach statistically significant results. Additionally, homozygosity at one or more HLA-I loci continues to show a statistically significant reduction in the development of any irAE requiring steroid including grade 2 irAE and ≥grade 3 (RR = 0.30, 95% CI 0.08–1.01, P = 0.039).

A multivariable logistic regression analysis considering other potential factors indicated a trend towards lower probability of developing irAE among patients with ECOG < 2 or those who are homozygous at all HLA-I loci. Low lymphocyte count ($\leq 2 \times 10^9$/L) was the only variable that reached statistical significance (Table 3). The analysis was performed among 155 patients after excluding one patient with unknown lymphocyte count. Moreover, Albumin [7] was only available for 151 patients, 13 (8.6%) of them have albumin <3.5 g/dL. It was not found to be associated with developing irAEs in both univariable and multivariable analysis.

3.3. Correlation between HLA supertypes and irAE

We found a statistically significant correlation between the development irAE and carrying the HLA-A03 supertype (RR = 1.42, 95% CI 1.02–2.01, P = 0.039) (Fig. 1). None of the other known 10 HLA-A and -B supertypes were found to correlate with the presentation of irAE.

3.4. Correlation between HLA-I/II alleles and irAE

An exploratory analysis was carried out to investigate HLA-I/II alleles and haplotypes previously reported to be associated with autoimmune diseases or irAEs (Supplementary Table 1) [11,13–15,17,19–22,26–28,32,33]. Of those, HLA-DRB1*0401 was found to be associated with the increased risk of developing irAE (RR = 1.55, 95% CI 1.10–2.06, P = 0.011) and HLA-DRB1*15:01 seems to be protective against developing arthralgia of any grade (RR = 0.18, 95% CI 0.03–0.94, P = 0.029) (Table 4). None of the patients who have HLA-DQB1*0301 experienced colitis (P = 0.048) (Table 4). Other reported alleles and haplotypes were not investigated in this cohort due to their low frequency, 5 or less out of the 156 patients (≤3.2%).

3.5. Correlation between irAE and clinical outcomes

We found a statistically significant correlation between the clinical outcomes and the development of irAE among the 156 patients with NSCLC and treated with single agent anti-PD1/PDL1 therapy included in the landmark analysis. Patients who experienced any type and grade of irAE had better CBR (43 versus 29%, RR = 1.48, 95% CI 1.23–1.85, P < 0.0001), as well as longer PFS (HR = 0.45, 95% CI 0.29–0.69, P = 0.0003) and OS (HR = 0.34, 95% CI 0.20–0.58, P < 0.0001), than those who did not experience any irAE (Fig. 2). Multivariable Cox regression analyses of PFS or OS confirmed our observations that irAE are associated with survival, after controlling known prognostic factors (Supplementary Table 2).

Table 2
Summary of observed irAEs and prevalence of homozygosity at one or more HLA-I loci among patients developed irAE (N = 156).

irAE	Any grade N (%)	HLA-I Homozygous frequency N (%[a])	RR (95% CI)	P value	≥ Grade 3 N (%*)	HLA-I homozygous frequency N (%[a])	P value
Any event	**77 (49.4)**	**9/77 (11.7)**	**0.61 (0.33–0.99)**	**0.035**	17 (10.9)	**0/17**	**0.023**
Skin rash	34 (21.8)	4/34 (11.8)	0.61 (0.23–1.44)	0.213	3 (1.9)	0/3	
Arthralgia	20 (12.8)	4/20 (20)	1.19 (0.44–3.01)	0.552	0		
Pneumonitis	**15 (9.6)**	**0/15**		**0.044**	7 (4.5)	0/7	
Endocrinopathy	12 (7.7)	0/12		0.084	0		
Hepatotoxicity	10 (6.4)	0/10		0.130	4 (2.6)	0/4	
Colitis	7 (4.5)	2/7 (28.6)	1.83 (0.42–7.58)	0.397	3 (1.9)	0/3	
Lethargy	3 (1.9)	1/3 (33.3)	1.78 (0.32–4.69)	0.471	0		
Nephritis	1 (0.6)	0/1		0.810	0		

Statistically significant results has been bolded.
[a] Frequency of homozygosity among patients who developed specific irAE; RR, relative risk; HLA, human leucocyte antigen, irAE: immune-related adverse event. Grading is based on common terminology criteria for adverse events v5.0 (CTCAE v5.0). The correlation between individual ≥ Grade 3 irAE and homozygosity at least in one HLA-I loci was not done as all patients with ≥grade 3 irAE are heterozygous at all HLA-I loci. Note: 77 patients experienced 102 irAE, hence 23 patients experienced more than one irAE.

4. Discussion

Genomic HLA-I homozygosity has been associated with worse survival outcome among patients with NSCLC and melanoma and treated with immune checkpoint inhibitors [29,30]. Given that HLA is a host factor expressed on all tissues, HLA homozygosity may affect the overall immune response to immune checkpoint blockade. Here, we demonstrated that patients with advanced NSCLC and with homozygosity at one or more HLA-I, but not HLA-II, loci are less likely to develop irAEs. This relationship is more pronounced between HLA-I homozygosity and the development of any grade 3 or more irAEs and pneumonitis.

In this analysis, we reduce guarantee-time bias by conducting a conditioned landmark analysis at week 14.8 [34] based on the results of a pooled analysis of the pattern of time to onset of irAE in 23 clinical trials [31]. Multivariable logistic regression analysis failed to reach significance for the association of HLA-I homozygosity and irAE, showing lymphocyte counts as the major predictor of irAE in line with previous reports [7]. Notably, a major limitation is that we did not control PD-L1 status in this analysis as 45 patients did not have tumour PDL1 immunohistochemistry performed (Table 1), which would have greatly reduced our sample size.

We explored the association between different HLA-A and -B supertypes and only found that the presence of HLA-A03 to be associated with 1.4 increased the risk of developing irAEs. HLA-A03 supertype binds positively charged residues in the F pocket [35] and has been shown to present resistant species for HIV infection and may contribute to the control of retroviral infections [35]. This is the first report linking HLA-A03 to the development of irAE and will require validation in independent studies.

Among many different HLA-I/II alleles and haplotypes evaluated [11–28], we did not find statistically significant association with the increased risk of irAE, except for HLA-DRB1*04:01. HLA-DQB1*03:01, associated with reduced risk of developing gastrointestinal irAE and HLA-DRB1*15:01 which is probably

Table 3
Univariable and multivariable logistic regression analysis for association with development of irAE (N = 155).

Factors	Univariable				Multivariable			
	P value	OR	95% CI		P value	OR	95% CI	
			Lower	Upper			Lower	Upper
Sex (F versus M)	0.449	1.279	0.677	2.416	0.278	1.458	0.738	2.881
Age (≥68 versus < 68)	0.798	1.086	0.578	2.041	0.532	1.249	0.623	2.504
ECOG (≥2 versus < 2)	0.111	2.298	0.825	6.399	**0.059**	2.844	0.962	8.408
Line of therapy (>1 versus 1)	0.165	0.609	0.302	1.227	0.375	0.715	0.341	1.499
NLR (≤5 versus > 5)	0.860	1.061	0.548	2.055	0.797	1.107	0.511	2.401
Lymphocyte[a] (≤2 versus > 2)	**0.062**	0.469	0.212	1.038	**0.034**	0.336	0.122	0.921
PLR (≥180 versus < 180)	0.966	0.986	0.521	1.867	0.323	1.548	0.650	3.689
HLA-I (homozygous versus heterozygous)	**0.052**	0.424	0.178	1.009	**0.089**	0.455	0.184	1.127
HLA-II (homozygous versus heterozygous)	0.312	0.709	0.364	1.382	0.295	0.675	0.323	1.409

The underlined number represent the only statistically significant result.
[a] $\times 10^9$/L; CI: confidence interval; ECOG, Eastern Cooperative Oncology Group; F: female; HLA-I/II: human leucocyte antigen I/II; irAE: immune-related adverse event; line of therapy (0 = first-line, 1 = second-line); M: male; NLR: neutrophil to lymphocyte ratio; OR: odds ratio; PLR: platelet lymphocyte ratio. Note: P values <0.1 are bolded.

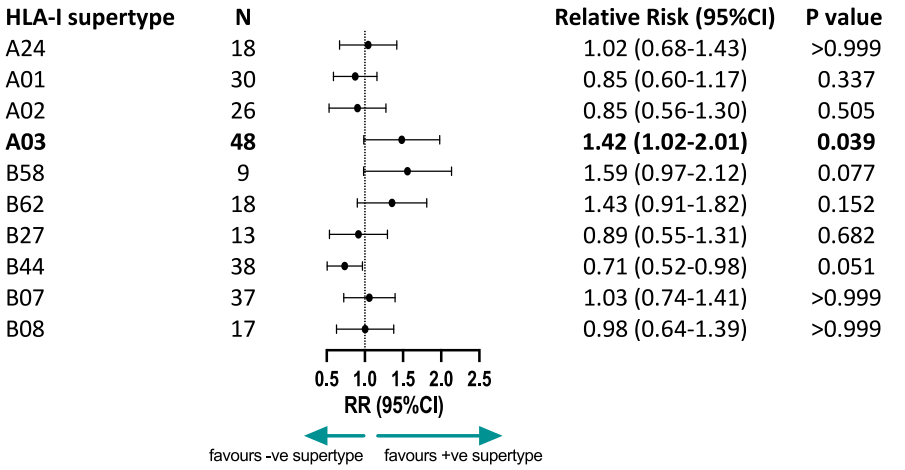

Fig. 1. Association of HLA-I supertypes with the development of any irAE. Forrest plot indicates relative risk (RR) with 95% confidence interval (CI) of developing immune-related adverse event (irAE). The number of patients with a specific supertype who developed irAE is indicated (N).

Table 4
Association between HLA-I/II alleles, haplotypes and development of any irAE or specific irAE.

HLA-I/II alleles	Frequency (%) any irAE	RR (95% CI)	P value	Specific irAE	Frequency (%) specific irAE	RR (95% CI)	P value
HLA-A*02:01 [28]	38/77 (49.4)	1.20 (0.87–1.65)	0.171	Skin toxicity	15/34 (44.1)	0.97 (0.53–1.74)	0.539
HLA-A*03:01 [16]	19/77 (24.7)	0.92 (0.61–1.30)	0.395	MS	0/0		
HLA-B*08:01 [15]	16/77 (20.8)	0.94 (0.61–1.35)	0.457	GI toxicity	1/7	0.60 (0.10–3.54)	0.525
HLA-B*35 [26]	14/77 (18.2)	1.06 (0.68–1.51)	0.471	Resp toxicity	2/15 (13.3)	0.74 (0.19–2.61)	0.500
HLA-C*06:02 [13]	15/77 (19.5)	1.16 (0.75–1.62)	0.310	Skin toxicity	9/34 (26.5)	1.72 (0.88–3.10)	0.093
HLA-DQB1*03:01 [27]	26/77 (33.8)	0.96 (0.67–1.33)	0.480	**GI toxicity**	**0/7**		**0.048**
HLA-DRB1*11 [26]	6/77 (7.79)	0.65 (0.31–1.13)	0.116	Resp toxicity	0/15		0.145
HLA-DRB1*11:01 [27]	5/77 (6.49)	0.70 (0.32–1.25)	0.215	Skin toxicity	2/34 (5.9)	0.63 (0.17–1.88)	0.373
HLA-DRB1*04:01 [18]	**22/77 (28.6)**	**1.55 (1.10–2.06)**	**0.011**	Rheum toxicity	5/20 (25)	1.29 (0.51–3.07)	0.391
HLA-DRB1*04:04 [18]	4/77 (5.2)	0.80 (0.33–1.43)	0.389	Rheum toxicity	2/20 (10)	1.62 (0.44–4.72)	0.375
HLA-DRB1*15:01 [18]	15/77 (19.5)	0.81 (0.51–1.18)	0.194	**Rheum toxicity**	**1/20 (5)**	**0.18 (0.03–0.94)**	**0.029**
HLA-DRB1*01:01 [18]	8/77 (10.4)	0.79 (0.42–1.26)	0.256	Rheum toxicity	2/20 (10)	0.76 (0.20–2.50)	0.510
HLA-DRB1*13:01 [12]	9/77 (11.7)	1.24 (0.72–1.78)	0.276	Rheum toxicity	3/20 (15)	1.66 (0.54–4.32)	0.297
HLA-DRB1*07 [14]	17/77 (22.1)	0.91 (0.60–1.30)	0.387	GI toxicity	2/7 (28.6)	3.27 (0.75–13)	0.169
HLA-DRB1*01 [14]	12/77 (15.6)	0.81 (0.49–1.22)	0.228	GI toxicity	0/7		0.230
HLA-DQB1*02:01 [11]	22/77 (28.6)	1.12 (0.78–1.55)	0.323	GI toxicity	0/7		0.113
HLA-DQB1*05:01 [25]	14/77 (18.2)	0.93 (0.59–1.35)	0.451	GI toxicity	1/7 (3.3)	0.70 (0.11–4.11)	0.597
HLA-DRB3*03:01 [24]	5/77 (6.5)	1.13 (0.54–1.75)	0.484	GI toxicity	0/7		0.544

irAE: immune-related adverse event, GI: gastrointestinal; Resp: respiratory; Rheum: rheumatology, MS: multiple sclerosis.
Statistically significant results has been bolded.

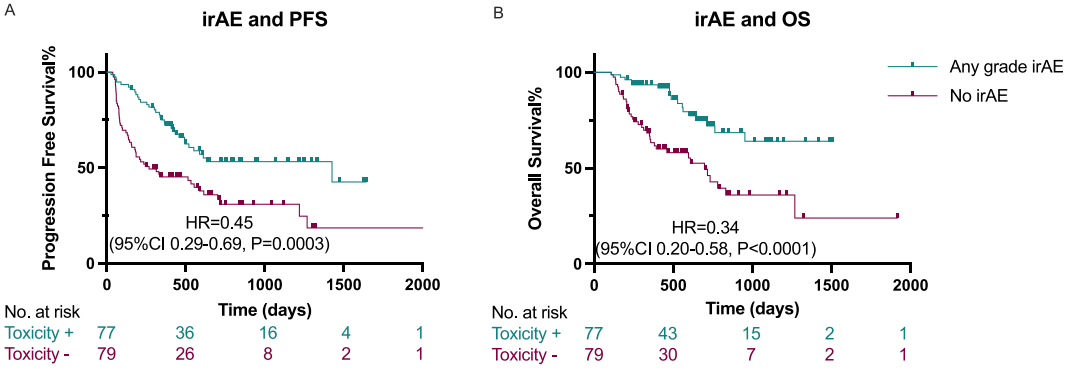

Fig. 2. Kaplan–Meier plots of progression-free survival (PFS, a) and overall survival (OS, b) with a 14.8-week landmark analysis of patients with and without immune-related adverse events (irAE). Hazard ratio (HR), 95% confidence interval (95% CI) and P-value of log-rank test are indicated for each comparison.

protective against developing immunotherapy-related arthralgia. The latest was reported to be protective against developing rheumatoid arthritis [18]. While these might be interesting findings, analysing the association of HLA alleles and haplotypes with irAEs requires very large cohorts (N > 1000) correcting for multiple comparisons to provide accurate conclusions.

Investigating the role of genotypes in developing irAE require the incorporation of ethnicity into the analysis to assess this correlation more accurately [21,36]. Although most of the patients in our cohort are Caucasian, the effect of specific genotype might be diluted due to the presence of Asian, Indian and Arabic minorities. However, our database does not have explicit information about ethnicity. Therefore, we are unable to assess the role of those alleles while taking in consideration the ethnic groups of patients.

Understanding the biomarkers to predict patients who will develop irAEs will aid in understating the mechanism of irAEs. Knowing the impact of developing irAEs on patient outcome is an important prognostic marker, especially if it will preclude patients from further rechallenge with immunotherapy. The correlation between the development of any irAE and favourable clinical outcome was very pronounced in our cohort and consistent with what has been found by multiple reviews [6,37–39].

One of the strengths of this study is the length of follow-up, with 90% of patients being followed up for at least 18 months. This is important as the development of irAE can take up to 60 weeks (15 months) [40] to appear. It is noted, however, that the rate of any grade irAE in our cohort (49.4%) was less than that recorded in clinical trials (69–79%) [4]. This could be explained in part by retrospective data entry and potentially the underreporting of low-grade toxicities in clinical practice. The incidence of grade 3 toxicities however was comparable to the known side-effect profile of single agent PD1 inhibitors.

5. Conclusion

In conclusion, our study is the first to report genomic HLA-I homozygosity as a predictive marker for the development of irAE among patients with NSCLC and treated with single agent anti-PD1/PDL1 therapy. Further analysis of the above correlation within the different ethnic groups will give a better understanding of the role of different HLA supertypes and genotypes in different population. Moreover, our data support the current evidence about the correlation between irAE and favourable clinical outcomes among patients with NSCLC and treated with anti-PD1/PDL1.

Ethics approval and consent to participate

Written informed consent was obtained from all patients and procedures were approved by Human Research Ethics Committees at Edith Cowan University (No. 18957) and Sir Charles Gairdner Hospital (No. 2013-246 and RGS0000003289) in compliance with Helsinki Declaration. Experiments were conducted per institutional and national guidelines and regulations.

Patient consent for publication

Not required.

Availability of data and material

All data is available upon reasonable request.

Funding

This work was supported by the clinical trial unit at Fiona Stanley Hospital, Murdoch, Western Australia; a research grant from the Lung Foundation Australia – Ellen Yates Memorial Grant in Aid for Lung Cancer Research; a fellowship to A.A. from the Western Australia Cancer Council Palliative Care Network; and a fellowship to E.G. from the Cancer Research Trust and Cancer Council WA.

Author contributions

Afaf Abed: Conceptualisation; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Project administration; Visualisation; Roles/Writing – original draft; Writing – review & editing. **Ngie Law**: Data curation; Writing – review & editing. **Leslie Calapre**: Conceptualisation; Formal analysis; Writing – review & editing. **Johnny Lo**: Formal analysis; Writing – review & editing. **Vikas Bhat**: Data curation; Writing – review & editing. **Samantha Bowyer**: Resources; Writing – review & editing. **Michael Millward**: Investigation; Methodology; Supervision; Roles/Writing – original draft. **Elin Gray**: Conceptualisation; Formal analysis; Investigation; Methodology; Resources; Supervision; Visualisation; Roles/Writing – original draft; Writing – review & editing.

Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Dr Millward sits on advisory boards for Merck Sharp and Dohme (MSD), Bristol-Myers Squibb (BMS) and AstraZeneca (AZ). Dr Bowyer reports advisory board role for Sanofi and virtual meeting sponsorship from Bristol-Myers Squibb and MSD Australia. Dr Gray has received travel support from MSD. All remaining authors have declared no conflicts of interest.

Acknowledgements

The authors would like to acknowledge the contribution of patients and their families whom without them we would not be able to perform the study. The authors would like to thank the members of the Edith Cowan University Research Group for sample receiving and processing, in particular Anna Reid, Michael Morici and Emmanuel Acheampong.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2022.05.021.

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