Melanoma and Human Leukocyte Antigen (HLA): Immunogenicity of 69 HLA Class I Alleles With 11 Antigens Expressed in Melanoma Tumors

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ABSTRACT: Host immunogenetics play a critical role in the human immune response to melanoma, influencing both melanoma prevalence and immunotherapy outcomes. Beneficial outcomes that stimulate T cell response hinge on binding affinity and immunogenicity of human leukocyte antigen (HLA) with melanoma antigen epitopes. Here, we use an in silico approach to characterize binding affinity and immunogenicity of 69 HLA Class I human leukocyte antigen alleles to epitopes of 11 known melanoma antigens. The findings document a significant proportion of positively immunogenic epitope-allele combinations, with the highest proportions of positive immunogenicity found for the Q13072/BAGE1 melanoma antigen and alleles of the HLA B and C genes. The findings are discussed in terms of a personalized precision HLA-mediated adjunct to immune checkpoint blockade immunotherapy to maximize tumor elimination.

KEYWORDS: Melanoma, neoantigens, human leukocyte antigen, major histocompatibility complex, immunogenicity

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Introduction

Melanoma incidence has been increasing for decades and, despite being the least common form of skin cancer, melanoma accounts for a disproportionately high number of skin cancer related deaths.[1,2] Therapeutic advances such as checkpoint blockade immunotherapy have improved melanoma survival rates; however, checkpoint blockade immunotherapy outcomes are partially dependent on host immunogenetics. To that end, a recent study documented extended survival in patients who possessed certain human leukocyte antigen (HLA) Class I B alleles (B*18:01, B*44:02, B*44:03, B*44:05, B*50:01) and poor outcomes in those with another HLA allele (B*15:01).[3] Remarkably, immunogenetic epidemiological findings demonstrated congruence between the population frequency of those HLA alleles that influence checkpoint blockade immunotherapy outcomes and the population prevalence of melanoma, suggesting that certain HLA alleles may similarly influence overall melanoma risk and protection as well as treatment outcomes.[4] In addition, that study evaluated the influence of a large number of HLA alleles on the population prevalence of melanoma and identified robust protective effects associated primarily with alleles of the HLA Class I B gene, extending HLA-melanoma associations beyond those that are already known to influence melanoma checkpoint blockade immunotherapy outcomes.[4] Taken together, these and other studies highlight the influence of HLA on melanoma population prevalence and individual survival and treatment response and suggest broad HLA-melanoma associations.[3-6] Here we evaluate those associations in the context of the role of HLA in antigen elimination.

HLA plays a critical role in immune surveillance and host protection by facilitating identification and elimination of foreign antigens including cancer neoantigens.[7] Optimally, melanoma antigen-derived peptides bound by HLA class I are exported to the cell surface for presentation to CD8+ T lymphocytes signaling destruction of tumor cells, a process that depends on both binding affinity and immunogenicity. However, the highly polymorphic nature of HLA contributes to variation in the binding groove; consequently, HLA alleles vary tremendously with respect to peptide binding capability, immunogenicity, and subsequent antigen elimination.[8-16] This variability also has implications for checkpoint blockade immunotherapy outcomes. Various immune-escape mechanisms exploited by tumors may disrupt the host immune response, permitting unchecked tumor cell proliferation.[17-19] Immune checkpoint inhibitors are aimed at improving the ability of the host's immune system to detect and eliminate tumor antigens; however, that rests on the assumption that host HLA composition complements that of the tumor antigen to permit antigen binding with sufficient affinity and immunogenicity to support

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Table 1. The 11 melanoma-related antigens. The listing is from the UniProt Consortium[22].

	UNIPROTKB ID	PROTEIN	GENE	N AMINO ACIDS
1	O75767 · O75767_HUMAN	Tyrosinase-related protein-2	TRP-2	237
2	P04271 · S100B_HUMAN	Protein S100-B	S100B	92
3	P14679 · TYRO_HUMAN	Tyrosinase	TYR	529
4	P17643 · TYRP1_HUMAN	5,6-dihydroxyindole-2-carboxylic acid oxidase	TYRP1	537
5	P40967 · PMEL_HUMAN	Melanocyte protein PMEL	PMEL	661
6	P43355 · MAGA1_HUMAN	Melanoma-associated antigen 1	MAGEA1	309
7	P43358 · MAGA4_HUMAN	Melanoma-associated antigen 4	MAGEA4	317
8	P78358 · CTG1B_HUMAN	Cancer/testis antigen 1	CTAG1A, B	180
9	Q13072 · BAGE1_HUMAN	B melanoma antigen 1	BAGE	43
10	Q16385 · SSX2_HUMAN	Protein SSX2	SSX2	188
11	Q16655 · MAR1_HUMAN	Melanoma antigen recognized by T-cells 1	MLANA	118

tumor antigen elimination. Indeed, differential immunotherapy outcomes have been attributed to variability in melanoma neoantigen binding.[3] Thus, both host immune response to cancer neoantigens and success of immunotherapy depends on binding affinity and immunogenicity of a given HLA allele-melanoma antigen combination; yet, relatively little is known about binding affinity and immunogenicity of specific HLA alleles with melanoma antigens.

We have previously characterized the immunogenetic profile of melanoma with regard to 69 Class I HLA alleles.[4] In that study, we determined the association between allele prevalence and melanoma prevalence at the population level and documented negative (protective) and positive (susceptibility) scores. Here, to test the hypothesis that those protective and susceptibility effects are related to melanoma antigen elimination, we use an in silico approach[20] to characterize binding affinity and immunogenicity of those 69 Class I HLA alleles to 11 known melanoma antigens.[21]

Materials and Methods

Melanoma antigens

Eleven melanoma antigens (Table 1) were used based on their known occurrence in melanoma tumors[21]; their amino acid (AA) sequences were retrieved from the Uniprot Database[22] and are given in Supplemental Appendix A.

HLA alleles and their supertypes

We used 69 HLA Class I alleles (Table 2), consisting of 20 gene A, 36 gene C, and 13 gene C alleles. Alleles of A and B genes were assigned to supertypes.[23] More specifically, of a total of 56 alleles of A and B genes, 53 alleles could be assigned to supertypes, namely all 20 A gene alleles and 33/36 B gene alleles; B*13:02, B*47:01 and B*49:01 were unassigned (Figure 2 in Sidney et al[23]). The assignment of alleles to supertypes is given in Table 2 and their distribution in Table 3.

Melanoma-HLA protection/susceptibility (P/S) scores

The P/S scores[4] are the Pearson correlations, r, between melanoma prevalence and HLA allele frequency across 14 Westerns European Countries, after Fisher z-transformation to normalize their distribution:

$$\text{Melanoma} - \text{HLA P / S score } = r' = \text{ arctanh } (r) \quad (1)$$

A negative score stems from a negative correlation between melanoma prevalence and allele frequency and, hence, indicated a protective role of that allele; conversely, a positive score stems from a positive correlation between melanoma prevalence and allele frequency, indicating a susceptibility role. Given the continuous nature of r', its absolute value could serve as a measure of the strength of the melanoma protection or susceptibility effect of a particular allele. The 69 alleles evaluated here and their Melanoma-HLA P/S scores protective/susceptibility (PS) HLA scores are given in Table 4.

Determination of immunogenicity of HLA Class I alleles

The INeo-Epp method[20] was used for T-cell receptor (TCR) epitope prediction using the INeo-Epp web tool via the INeo-Epp web form interface.[24] For that purpose, we split a given melanoma antigen to all possible 9-mer AA residue epitopes using a sliding window approach[10-12] (Figure 1) and submitted each epitope to the web-application together with a specific HLA allele (Table 2). More specifically, we paired all epitopes with all alleles and obtained for each pair its percentile rank, a measure of

Table 2. The 69 HLA Class I alleles used and their gene and supertype assignments.

INDEX	ALLELE	GENE	SUPERTYPE
1	A*01:01	A	A01
2	A*02:01	A	A02
3	A*02:05	A	A02
4	A*03:01	A	A03
5	A*11:01	A	A03
6	A*23:01	A	A24
7	A*24:02	A	A24
8	A*25:01	A	A01
9	A*26:01	A	A01
10	A*29:01	A	A24
11	A*29:02	A	A01 A24
12	A*30:01	A	A01 A03
13	A*30:02	A	A01
14	A*31:01	A	A03
15	A*32:01	A	A01
16	A*33:01	A	A03
17	A*33:03	A	A03
18	A*36:01	A	A01
19	A*68:01	A	A03
20	A*68:02	A	A02
21	B*07:02	B	B07
22	B*08:01	B	B08
23	B*13:02	B	Unassigned
24	B*14:01	B	B27
25	B*14:02	B	B27
26	B*15:01	B	B62
27	B*15:17	B	B58
28	B*15:18	B	B27
29	B*18:01	B	B44
30	B*27:02	B	B27
31	B*27:05	B	B27
32	B*35:01	B	B07
33	B*35:02	B	B07
34	B*35:03	B	B07
35	B*35:08	B	B07
36	B*37:01	B	B44
37	B*38:01	B	B27
38	B*39:01	B	B27
39	B*39:06	B	B27
40	B*40:01	B	B44
41	B*40:02	B	B44
42	B*41:01	B	B44
43	B*41:02	B	B44
44	B*44:02	B	B44
45	B*44:03	B	B44
46	B*44:05	B	B44
47	B*45:01	B	B44
48	B*47:01	B	Unassigned
49	B*49:01	B	Unassigned
50	B*50:01	B	B44
51	B*51:01	B	B07
52	B*52:01	B	B62
53	B*55:01	B	B07
54	B*56:01	B	B07
55	B*57:01	B	B58
56	B*58:01	B	B58
57	C*01:02	C	
58	C*03:03	C	
59	C*04:01	C	
60	C*05:01	C	
61	C*06:02	C	
62	C*07:01	C	
63	C*07:02	C	
64	C*07:04	C	
65	C*12:02	C	
66	C*12:03	C	
67	C*14:02	C	
68	C*15:02	C	
69	C*16:01	C	

binding affinity of the epitope-HLA allele complex; smaller percentile ranks indicate higher binding affinity. The web-application also gave a TCR predictive score for pairs with high binding affinities (percentile rank <2); scores >0.4 (called *s*) indicated positive immunogenicity and were analyzed further.

Table 3. Distribution of 56 Class I A and B alleles in supertypes.

SUPERTYPE	COUNT
A01	6
A02	3
A03	6
A24	3
A103	1
A124	1
B07	8
B08	1
B27	8
B44	11
B58	3
B62	2
Unassigned	3
Total	56

Application to individuals

Since every individual carries 6 classical HLA alleles (2 of each 3 HLA Class I genes), an average Melanoma-HLA P/S score can be calculated:

$$\xi = [r'(A_1)+r'(A_2)+r'(B_1)+r'(B_2)+r'(C_1)+r'(C_2)]/6 \quad (2)$$

where *A, B, C* denote HLA Class I genes. Similarly, we computed an average positive immunogenicity score:

$$\theta = [s(A_1)+s(A_2)+s(B_1)+s(B_2)+s(C_1)+s(C_2)]/6 \quad (3)$$

We obtained expected estimates of ξ and θ using a bootstrap procedure,[25] as follows. For each gene, 2 r' and θ scores were drawn randomly (with replacement) from the pool of available alleles of that gene and averaged to yield bootstrap values of ξ* and θ*for a simulated "individual" The procedure was repeated 1 million times for a total of 1 000 000 ξ* and θ* values. The association between P/S and immunogenicity scores was evaluated in the following 3 ways. (i) We computed the Pearson correlation between ξ* and θ*, as an overall measure of association. (ii) For each bootstrap iteration, we computed and retained the covariance between ξ* and θ* ($COV_{\xi^*\theta^*}$, $N=6$ allele-values per iteration), as a finer-grain measure of association. And (iii) we compared immunogenicity ξ* between protective ($\theta^* < 0$) and susceptibility ($\theta^* > 0$) Melanoma-HLA P/S groups, to find out whether it was higher in the protective group.

Standard parametric (mean, standard deviation, etc.) and nonparametric statistics (median, interquartile range, etc.) were used to evaluate the distribution of ξ* and θ*. We used Tukey's fences[26] to identify outlier and extreme values, as follows.

$$\text{Interquartile range: IQR} = Q3 - Q1 \quad (4)$$

$$\text{Lower fence} = Q1 - 1.5\ \text{IQR} \quad (5)$$

$$\text{Upper fence} = Q3 + 1.5\ \text{IQR} \quad (6)$$

where Q1, Q3 are the 25th and 75th percentiles, respectively. These fences demarcated outlier values, that is, values lying outside the fences.

Data analysis

Standard statistical methods were used to analyze the data, including descriptive statistics, analysis of variance (ANOVA), Pearson correlation, and the Wilson score and associated 95% confidence intervals (CI) for testing a proportion. These analyses were done using the IBM-SPSS statistical package (version 27). All reported statistical significance *P*-values are 2-sided. The bootstrap was implemented using FORTRAN (Geany, version 1.38, built on or after 2021-10-09). A 64-bit Mersenne Twister random number generator with a large random double-precision odd seed was used for the random sampling.

Results

Overall, there was a total of10 806 epitope-allele combinations with high estimated binding affinity (percentile rank < 2). Of those, 5817 (53.8%) had a positive immunogenicity score; this proportion (0.538 ± 0.005 SE, 95% CI 0.529-0.548) was statistically highly significant ($Z=7.96$, $P<.001$, Wilson score test for single proportion).

Assessment of predictive TCR (positive immunogenicity)

Detailed results are given in Tables 5 to 7 and are plotted in Figures 2 to 6. There was a total of 11 antigens ×´69 alleles = 759 antigen-allele combinations, of which 15 had no cases of positive immunogenicity (PI, "hits"). We analyzed the remaining 744 combinations using a univariate ANOVA where the proportion of PI per count of high binding affinity (for each antigen-allele combination) was the dependent variable and the Antigen ($N=11$) and Gene ($N=3$) were fixed factors. We found the following.

Effect of antigen

Antigen had a highly significant effect on PI ($F_{[10,711]}=36.7$, $P<.001$). It can be seen in Table 5 and Figure 2 that antigen BAGE had the highest PI score (proportion of hits), followed by PMEL and CTA. The remaining antigens had smaller effects, with SSX having the lowest.

Table 4. Melanoma-HLA P/S scores[4] of the 69 HLA Class I alleles. Negative and positive scores indicate protection or susceptibility, respectively.

INDEX	ALLELE	SUPERTYPE	P/S SCORE	INDEX	ALLELE	SUPERTYPE	P/S SCORE
1	A*01:01	A01	−0.2401	36	B*37:01	B44	1.1812
2	A*02:01	A02	0.2522	37	B*38:01	B27	−1.0951
3	A*02:05	A02	−0.4210	38	B*39:01	B27	−0.1741
4	A*03:01	A03	0.7791	39	B*39:06	B27	−0.2169
5	A*11:01	A03	−0.6140	40	B*40:01	B44	0.9572
6	A*23:01	A24	−0.7218	41	B*40:02	B44	0.2556
7	A*24:02	A24	0.2820	42	B*41:01	B44	−0.3617
8	A*25:01	A01	0.0823	43	B*41:02	B44	−0.7338
9	A*26:01	A01	−0.5901	44	B*44:02	B44	0.1075
10	A*29:01	A24	−0.0148	45	B*44:03	B44	−0.3647
11	A*29:02	A01 A24	−0.3711	46	B*44:05	B44	−0.5119
12	A*30:01	A01 A03	−0.0882	47	B*45:01	B44	−0.2990
13	A*30:02	A01	−0.3787	48	B*47:01	Unassigned	−0.3159
14	A*31:01	A03	0.9819	49	B*49:01	Unassigned	−0.8982
15	A*32:01	A01	−0.6739	50	B*50:01	B44	−0.6495
16	A*33:01	A03	−0.4220	51	B*51:01	B07	−0.7993
17	A*33:03	A03	−0.6069	52	B*52:01	B62	−0.3895
18	A*36:01	A01	−0.4347	53	B*55:01	B07	0.4939
19	A*68:01	A03	0.0872	54	B*56:01	B07	0.2318
20	A*68:02	A02	−0.1125	55	B*57:01	B58	−0.1386
21	B*07:02	B07	1.0125	56	B*58:01	B58	−0.4316
22	B*08:01	B08	0.4200	57	C*01:02		0.1016
23	B*13:02	Unassigned	0.1148	58	C*03:03		0.5415
24	B*14:01	B27	−0.4040	59	C*04:01		−0.7760
25	B*14:02	B27	−0.4484	60	C*05:01		0.1806
26	B*15:01	B62	1.0368	61	C*06:02		−0.0220
27	B*15:17	B58	−0.2184	62	C*07:01		0.1184
28	B*15:18	B27	−0.4948	63	C*07:02		1.2586
29	B*18:01	B44	−0.8552	64	C*07:04		−0.2449
30	B*27:02	B27	−0.1072	65	C*12:02		−0.5143
31	B*27:05	B27	0.5024	66	C*12:03		−0.7325
32	B*35:01	B07	−0.1010	67	C*14:02		−0.6617
33	B*35:02	B07	−0.5722	68	C*15:02		−0.5539
34	B*35:03	B07	−0.6984	69	C*16:01		−0.1165
35	B*35:08	B07	−0.7732				

Effect of Gene

Gene had also a highly significant effect ($F_{[2,711]}=44.0$, $P<.001$), with gene B having the highest PI score (Table 6 and Figure 3), followed by genes C and A.

Antigen x Gene interaction

There was a highly significant Antigen × Gene interaction ($F_{[20,711]}=4.5$, $P<.001$), indicating that the effect of gene differed among antigens, as can be seen in Figure 4.

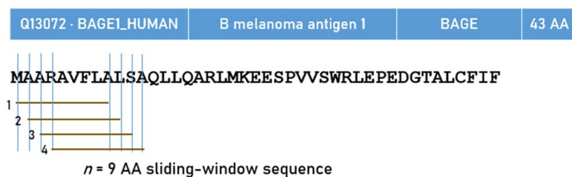

Figure 1. Schematic diagram to illustrate the exhaustive, sliding-window method for testing in silico all possible consecutive 9-AA residues.

Effect of alleles

This was assessed by a univariate ANOVA where the PI score (immunogenicity proportion) was the dependent variable and the allele was a fixed factor. We a highly significant allele effect ($F_{[68,675]}=2.58$, $P<.001$). The immunogenicity scores for each allele are given in Table 7 and are plotted ranked in Figure 5. Allele B*56:01 had the highest score of 0.743, and allele A*03:01 had the lowest (0.257). The frequency distribution of the immunogenicity scores and the corresponding box plot are shown in the left and right panels of Figure 6, respectively. The median was at 0.566, and there were 2 high (2.9%) and 6 low (8.7%) outliers.

Effect of supertype

This was assessed by a univariate ANOVA where the PI score (immunogenicity proportion) was the dependent variable and the supertype was a fixed factor. We found a highly significant effect ($F_{[12,589]}=8.29$, $P<.001$). The average scores for individual supertypes are plotted in Figure 7. It can be seen that

Table 5. Ranked mean proportions (and associated statistics) of sequences with positive nonamer immunogenicity (over all sequences with high binding affinity) for the 11 antigens tested.

RANK	PEPTIDE/ANTIGEN		N	MEAN PROPORTION	SEM	LOWER 95% CI	UPPER 95% CI
1	Q13072	BAGE	60	0.878	0.024	0.830	0.926
2	P40967	PMEL(17)	69	0.660	0.022	0.616	0.704
3	P78358	CTA	67	0.657	0.023	0.612	0.701
4	O75767	TRP2	69	0.561	0.022	0.517	0.605
5	P14679	TYR	69	0.500	0.022	0.456	0.544
6	Q16655	Melan-A, MART-1	67	0.498	0.023	0.453	0.543
7	P43358	MAGE4	69	0.474	0.022	0.430	0.518
8	P43355	MAGE1	69	0.473	0.022	0.429	0.517
9	P17643	TRP1	69	0.450	0.022	0.406	0.494
10	P04271	S100	67	0.399	0.023	0.355	0.444
11	Q16385	GAGE, SSX2	69	0.396	0.022	0.352	0.440

For a given antigen-allele combination, the immunogenicity of each nonamer of the antigen was evaluated with respect to the given allele. Let M be the number of nonamers that showed high binding affinity, and K the number of nonamers (out of M) that showed positive immunogenicity; the proportion Prop = K/M is the proportion (Prop) of nonamers with positive immunogenicity for an antigen-allele combination. The mean proportion in the table is the mean Prop across the contributing alleles (column 3, “N alleles,” ie, alleles associated with positive immunogenicity) for the corresponding antigen. SEM, standard error of the mean. N is the number of alleles with positive immunogenicity for the listed antigen. Results are from the ANOVA described in the text.

Table 6. Mean proportions of antigen-allele combinations with positive immunogenicity, ranked for the 3 HLA Class I genes. Conventions as in Table 3.

RANK	GENE	N	MEAN PROPORTION	SEM	LOWER 95% CI	UPPER 95% CI
1	B	392	0.609	0.009	0.592	0.626
2	C	142	0.540	0.014	0.512	0.568
3	A	210	0.473	0.012	0.449	0.496

Table 7. Mean proportions of antigen-allele combinations with positive immunogenicity, ranked for alleles.

RANK	ALLELE	SUPERTYPE	N ANTIGENS	MEAN PROPORTION	SEM	LOWER 95% CI	UPPER 95% CI
1	HLA*B56:01	B07	11	0.743	0.065	0.616	0.869
2	HLA*B45:01	B44	11	0.736	0.065	0.609	0.863
3	HLA*B49:01	Unassigned	11	0.725	0.065	0.599	0.852
4	HLA*B50:01	B44	11	0.709	0.065	0.583	0.836
5	HLA*B40:02	B44	11	0.708	0.065	0.581	0.834
6	HLA*B41:01	B44	11	0.691	0.065	0.564	0.818
7	HLA*B41:02	B44	11	0.687	0.065	0.561	0.814
8	HLA*B07:02	B07	10	0.672	0.068	0.539	0.805
9	HLA*A68:02	A02	11	0.665	0.065	0.539	0.792
10	HLA*B51:01	B07	11	0.654	0.065	0.527	0.780
11	HLA*B40:01	B44	11	0.640	0.065	0.513	0.767
12	HLA*B44:05	B44	11	0.640	0.065	0.513	0.767
13	HLA*B13:02	Unassigned	11	0.632	0.065	0.505	0.758
14	HLA*B55:01	B07	10	0.631	0.068	0.498	0.764
15	HLA*A24:02	A24	10	0.626	0.068	0.493	0.759
16	HLA*B37:01	B44	11	0.626	0.065	0.499	0.753
17	HLA*B35:02	B07	11	0.605	0.065	0.478	0.731
18	HLA*C05:01		11	0.603	0.065	0.476	0.729
19	HLA*B27:05	B27	11	0.601	0.065	0.474	0.728
20	HLA*C03:03		11	0.600	0.065	0.474	0.727
21	HLA*B38:01	B27	11	0.599	0.065	0.472	0.726
22	HLA*B35:03	B07	11	0.596	0.065	0.470	0.723
23	HLA*B39:06	B27	11	0.593	0.065	0.467	0.720
24	HLA*A23:01	A24	10	0.592	0.068	0.459	0.725
25	HLA*B39:01	B27	11	0.592	0.065	0.465	0.719
26	HLA*B58:01	B58	11	0.592	0.065	0.465	0.719
27	HLA*B57:01	B58	11	0.591	0.065	0.465	0.718
28	HLA*B15:17	B58	11	0.588	0.065	0.461	0.715
29	HLA*A02:01	A02	11	0.581	0.065	0.454	0.707
30	HLA*B18:01	B44	11	0.576	0.065	0.450	0.703
31	HLA*C04:01		11	0.572	0.065	0.445	0.699
32	HLA*B44:03	B44	11	0.571	0.065	0.444	0.698
33	HLA*A02:05	A02	11	0.568	0.065	0.441	0.695
34	HLA*B14:02	B27	11	0.568	0.065	0.442	0.695
35	HLA*B14:01	B27	11	0.566	0.065	0.439	0.693

(Continued)

Table 7. (Continued)

RANK	ALLELE	SUPERTYPE	N ANTIGENS	MEAN PROPORTION	SEM	LOWER 95% CI	UPPER 95% CI
36	HLA*C01:02		11	0.566	0.065	0.439	0.693
37	HLA*A25:01	A01	11	0.562	0.065	0.435	0.688
38	HLA*B44:02	B44	11	0.561	0.065	0.434	0.688
39	HLA*B47:01	Unassigned	11	0.560	0.065	0.433	0.687
40	HLA*C15:02		11	0.558	0.065	0.432	0.685
41	HLA*A33:01	A03	11	0.553	0.065	0.426	0.680
42	HLA*B35:08	B07	10	0.553	0.068	0.420	0.685
43	HLA*C12:02		11	0.547	0.065	0.421	0.674
44	HLA*B27:02	B27	11	0.545	0.065	0.418	0.671
45	HLA*B35:01	B07	10	0.543	0.068	0.410	0.676
46	HLA*B52:01	B62	11	0.539	0.065	0.413	0.666
47	HLA*C07:04		11	0.536	0.065	0.409	0.662
48	HLA*C12:03		11	0.533	0.065	0.407	0.660
49	HLA*A33:03	A03	11	0.531	0.065	0.404	0.658
50	HLA*C16:01		11	0.524	0.065	0.398	0.651
51	HLA*C06:02		11	0.517	0.065	0.390	0.644
52	HLA*B15:18	B27	11	0.516	0.065	0.389	0.643
53	HLA*A30:01	A01 A03	11	0.500	0.065	0.373	0.626
54	HLA*C14:02		11	0.497	0.065	0.370	0.623
55	HLA*C07:02		11	0.495	0.065	0.368	0.622
56	HLA*A26:01	A01	10	0.486	0.068	0.353	0.619
57	HLA*A68:01	A03	11	0.461	0.065	0.335	0.588
58	HLA*B15:01	B62	11	0.452	0.065	0.326	0.579
59	HLA*B08:01	B08	11	0.448	0.065	0.321	0.574
60	HLA*A32:01	A01	11	0.439	0.065	0.312	0.566
61	HLA*A36:01	A01	10	0.423	0.068	0.290	0.556
62	HLA*C07:01		10	0.421	0.068	0.288	0.554
63	HLA*A31:01	A03	11	0.412	0.065	0.285	0.538
64	HLA*A01:01	A01	10	0.386	0.068	0.253	0.519
65	HLA*A30:02	A01	9	0.313	0.071	0.173	0.453
66	HLA*A29:01	A24	10	0.301	0.068	0.168	0.434
67	HLA*A29:02	A01 A24	10	0.301	0.068	0.168	0.434
68	HLA*A11:01	A03	11	0.297	0.065	0.170	0.424
69	HLA*A03:01	A03	10	0.254	0.068	0.121	0.387

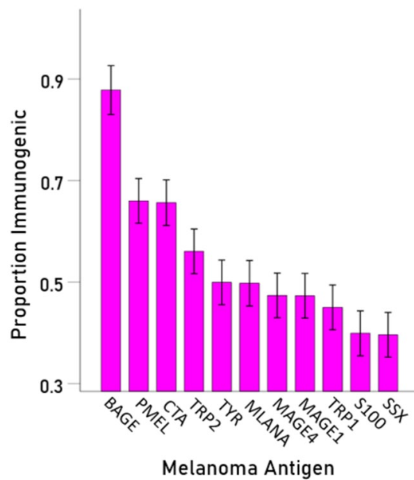

Figure 2. Mean PI (±95% CI) is plotted for each melanoma antigen, ranked from high to low.

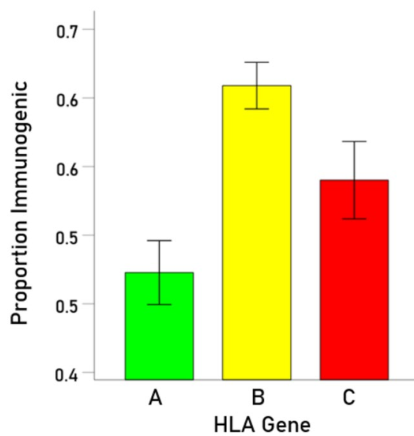

Figure 3. Mean PI (±95% CI) is plotted for each HLA gene.

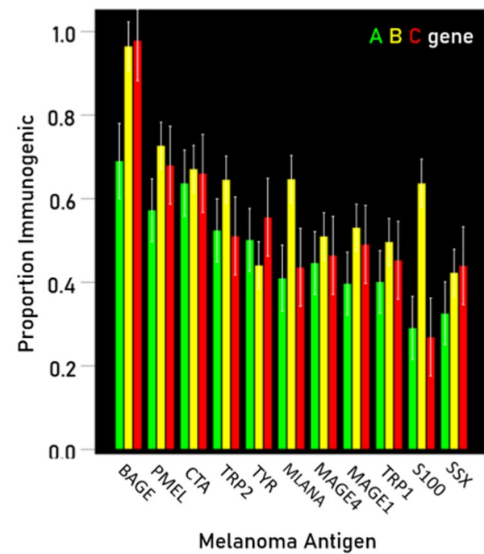

Figure 4. Mean PI (±95% CI) is plotted for each melanoma antigen – HLA gene combination.

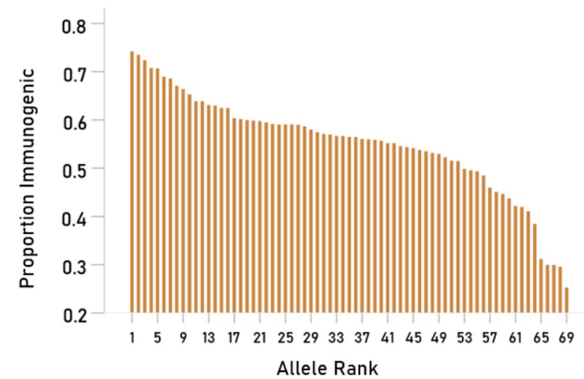

Figure 5. Immunogenicity scores of the 69 alleles are plotted against their rank. The numbers in the abscissa indicate the rank (first column in Table 7). The specific alleles corresponding to the ranks are also given in Table 7.

supertypes B44 and A03 A24 had the highest and lowest scores, respectively. Of special interest is the contrast between B44 and B62, since B44 has been found to be beneficial to the outcome of checkpoint blockade immunotherapy of melanoma, in contrast to B62 which had the opposite effect.[3] Indeed, the average scores of B44 and B62 were ranked first and ninth, respectively (Figure 7), reflecting a significantly higher immunogenicity score of B44 than B62 (Figure 8). For B44, the mean ± SEM was 0.650 ± 0.02, N = 121 antigen-allele combinations, whereas for B62 it was 0.496 ± 0.047, N = 22 antigen-allele combinations; the differences was highly statistically significant ($t_{[141]} = 3.16$, $P = .002$, independent samples t-test).

Evaluation of nonamers

The results above dealt with HLA allele immunogenicity at the antigen level. In this analysis we focused on the relations between individual immunogenic nonamers and HLA alleles. Detailed information about the counts of single nonamers with positive immunogenicity and alleles of the 3 HLA Class I genes is given in Supplemental Appendix B. There was a total 837 distinct nonamers with positive immunogenicity. Of those, 830 came each from a different antigen (peptide): although a given antigen comprised a number of immunogenic nonamers (Supplemental Appendix C), any specific immunogenic nonamer came from only one antigen. The remaining 7 nonamers were present in both MAGE1 (P43355) and MAGE4 (P43358) antigens, and, hence, are shown twice in Supplemental Appendices B and C.

Application to individuals: Association between positive nonamer immunogenicity and melanoma-HLA P/S scores

The bootstrap analysis was aimed to derive estimates of immunogenicity against the 11 melanoma antigens from sets of 6 HLA alleles (2 per each Class I gene), a realistic assessment at the organism level, given that each individual carries 6 such alleles. For the same set of randomly selected alleles, we computed (a) the average positive immunogenicity score ξ^*,

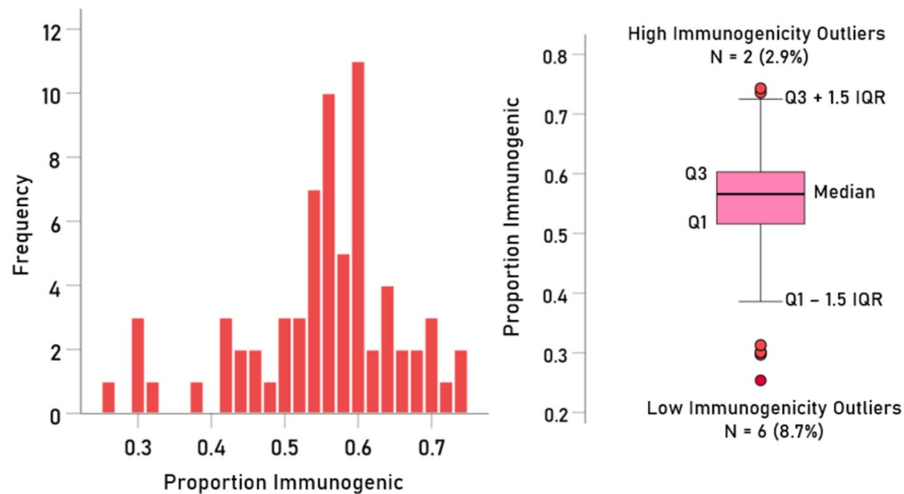

Figure 6. Frequency distribution (left panel) and corresponding boxplot (right panel) of the 69 immunogenicity scores. See text for detail.

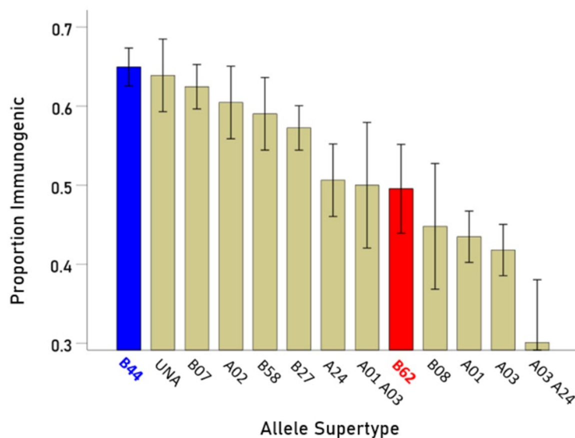

Figure 7. Mean PI (±SEM) for the 11 allele supertypes.

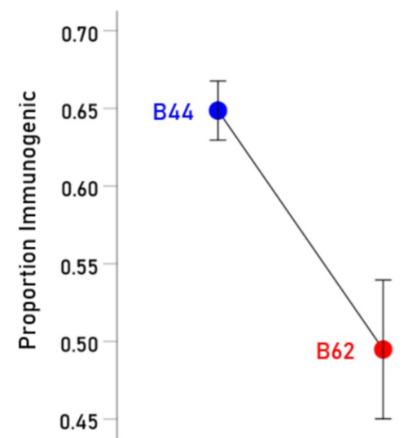

Figure 8. Mean PI (±SEM) for B44 and B62 supertypes.

(b) the average Melanoma-HLA P/S score θ*, and (c) the covariance $COV_{\xi^*\theta^*}$ between the 2 scores. We thus obtained 1 million triplets which enabled us to evaluate the distributions of ξ*and θ*, and their association. The frequency distributions of ξ*and θ* are plotted in Figure 9A and B, respectively. With respect to the distribution of ξ*(Figure 9A), the median was at −0.124, and there were 655 (0.06%) high- and 7525 (0.75%) low-immunogenicity outliers (Figure 10A). With respect to the distribution of θ*Figure 9B, the median was at 0.537, and there were 8093 (0.81%) high (susceptibility) and 40 (0.004%) low (protection) P/S score outliers (Figure 10B). These findings indicate a correspondence between high immunogenicity/high protection and low immunogenicity/low protection (susceptibility). Direct support for this association was provided by 3 additional findings. First, the Pearson correlation between ξ*and θ* was negative and highly significant (P= −.095, P < .001, N = 1 million). Second, the average covariance was significantly negative (P < .001, one-sample t-test). And third, the average immunogenicity ξ* was significantly higher in the group with protective (negative) θ* values: for protective ξ*, θ* = 0.537 ± 0.018 (mean ±SEM, N = 700257), and for susceptibility ξ*, θ* = 0.534 ± 0.021 (N = 299743; P < .001, independent samples t-test).

Discussion

Here we evaluated the immunogenicity of 69 HLA Class I alleles with 11 antigens expressed in melanoma tumors. Overall, a significant proportion of epitope-allele combinations were positively immunogenic, suggesting a propensity toward melanoma antigen elimination conferred by those HLA Class I alleles. The relatively low rates of melanoma may be partially attributable to the relatively high proportion of positively immunogenic epitope-antigen combinations. Further analyses documented positive immunogenicity varied across melanoma antigens and HLA Class I alleles with the highest proportions of positive immunogenicity found for the Q13072/BAGE1 melanoma antigen and alleles of the HLA B gene. Those findings and their implications are discussed further below.

Of the 11 melanoma antigens, Q13072 (BAGE1) had the highest proportion of positive immunogenicity (88%) followed by P78358 (CTG1B) and P40967 (PMEL/gp100) (each 66%). A prior study that reported BAGE1 expression in 22% of

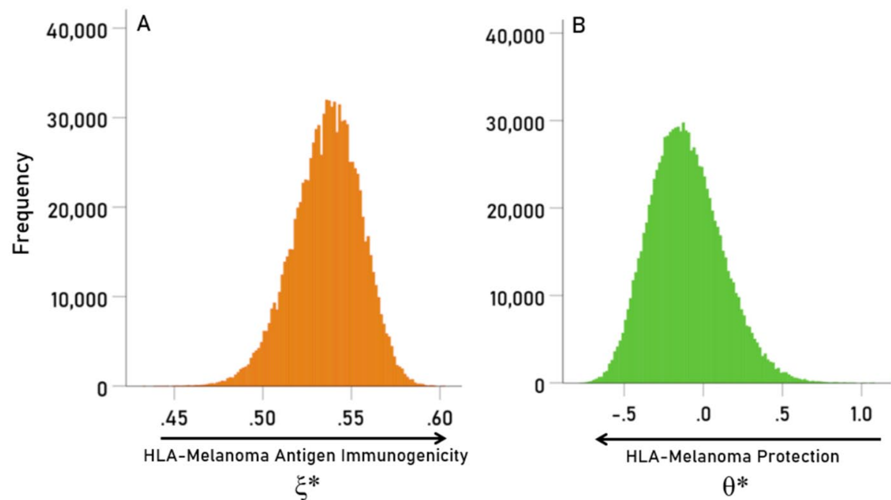

Figure 9. Frequency distribution of 1 million ξ* (A) and θ* (B) values. See text for details.

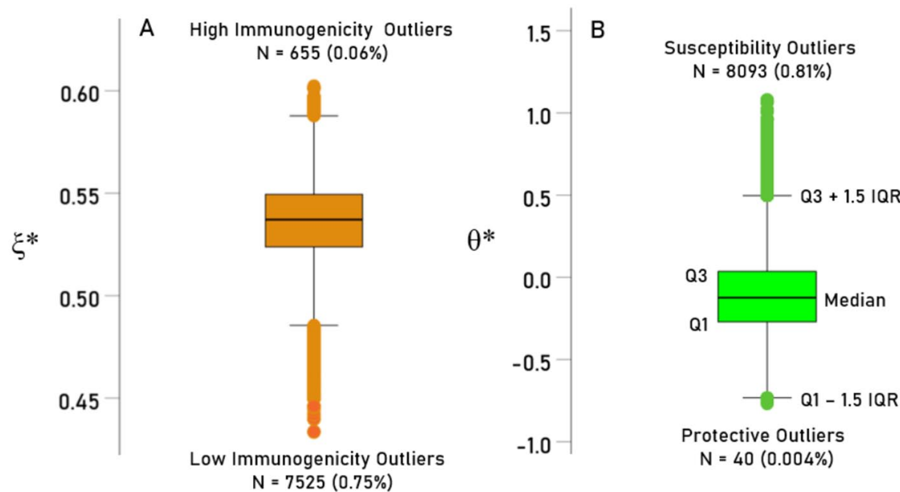

Figure 10. Boxplots of the data shown in Figure 9. See text for details.

melanomas suggested BAGE1 could serve as a target for T cells immunotherapy[27]; however, that has not been evaluated in clinical trials to date.[28] The present findings showing that the vast majority of BAGE epitope-HLA Class I allele combinations were highly immunogenic support the hypothesis that targeting BAGE may be beneficial. Of note, 9 of the 69 HLA alleles did not have any "hits" in terms of positive immunogenicity for BAGE, highlighting the specificity of antigen epitope-allele effects. We are not aware of clinical trials involving antigen P78358 (CTG1B); however, several clinical trials targeting gp100 have shown varying rates of clinical response.[28,29] Of course, clinical response is partially reliant on host HLA. Here, while 66% of the epitope-allele combinations resulted in positive immunogenicity for P78358 and P40967 antigens, 34% did not. Thus, for all 3 of these melanoma antigens, a majority of antigen epitope–HLA Class I allele combinations investigated here predict T cell response aimed at tumor elimination, although several HLA Class I alleles may be incapable of promoting an immune response to these antigens thereby permitting tumor proliferation.

With regard to HLA, the B gene had the highest proportion of positive immunogenicity with melanoma antigens both overall (Figure 3) and for many of the specific melanoma antigens (Figure 4); however, considerable variation in immunogenicity was documented across alleles within each of the Class I genes (Table 4). Remarkably, alleles of the B44 supertype had significantly higher immunogenicity scores than those of supertype B62 (Figures 7 and 8), corresponding to favorable versus poor outcomes associated with those supertypes, respectively, in melanoma checkpoint blockade immunotherapy.[3] This finding lends further support to the hypothesis that HLA-mediated melanoma antigen elimination underlies successful clinical response to the aforementioned immunotherapy.

Variable immunogenicity likely partially accounts for varying HLA associations with melanoma prevalence[4] and immunotherapy outcomes,[3] a conclusion supported by the significant congruence documented here between positive immunogenicity and population-derived immunogenetic scores with respect to melanoma.[4] This congruence was

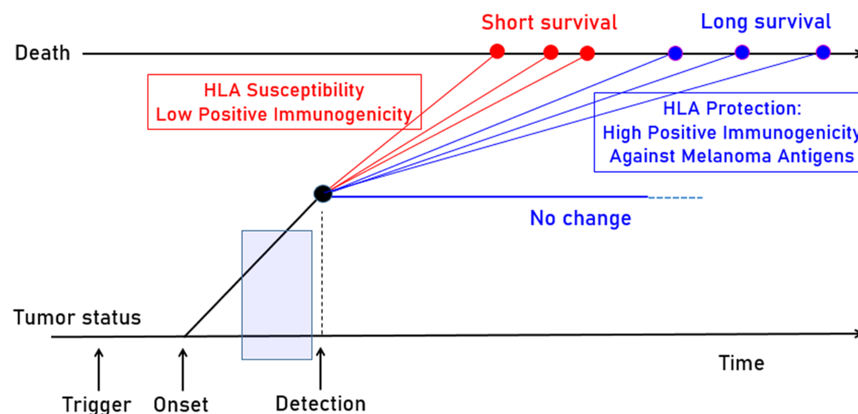

Figure 11. Schematic diagram to illustrate the hypothesized effects of antigen elimination by HLA molecules on melanoma prevalence and survival (blue, protective; red, susceptibility). HLA alleles with high positive immunogenicity will facilitate the elimination of cancer cells even prior to detection; therefore, those protective alleles would be associated with low prevalence of melanoma. In contrast, HLA alleles that are associated with susceptibility may be unable to sufficiently bind and eliminate melanoma neoantigens, thereby promoting continued proliferation of cancerous cells and reduced survival.

further supported by the results of the bootstrap analyses which took into account the fact that each individual carries 6 HLA Class I alleles, thus documenting the positive association between immunogenicity and melanoma-HLA P/S scores at this more realistic level. In summary, melanoma antigen binding and immunogenicity is presumed to be the mechanism through which certain HLA alleles protect against melanoma at the population level and improve treatment outcomes at the individual level. This idea is illustrated in the schematic diagram of Figure 11. To that end, we have proposed that sufficient immunogenic binding of melanoma antigens may eliminate cancerous cells, potentially even before their detection.[4] In contrast, HLA alleles with poor binding affinity and/or weak/negative immunogenicity may result in cancer proliferation, metastasis, poor checkpoint blockade immunotherapy response, and shortened survival. In the latter case that is characterized by the absence of protective HLA, we propose a novel cancer immunotherapy consisting of administering the mRNA blueprint for the synthesis of specific HLA molecules with the highest affinity and immunogenicity to a patient's tumor antigen(s). Coupled with immune checkpoint blockade immunotherapy, this personalized precision approach has the potential to maximize the effectiveness of HLA-mediated tumor elimination, and ideally, improve melanoma survival.

Author Contributions

SAC and MS retrieved the data. APG performed data analysis. LMJ and APG wrote and reviewed the paper. All authors edited the paper.

Ethical Approval

This article does not contain any studies with human participants performed by any of the authors.

Supplemental Material

Supplemental material for this article is available online.

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