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# HLA Genotype is Associated With Clinical Outcomes to anti-PD1 Therapy in Advanced Melanoma

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# Abstract

**Background:**

Predictive biomarkers of response to immune checkpoint inhibitors (ICI) are currently being evaluated in many studies. We evaluated whether germline Human Leukocyte Antigen (HLA) status can influence the response to immunotherapy.

**Materials and Methods:**

Advanced melanoma patients undergoing treatment with pembrolizumab were recruited between August 2014 to January 2020. 121 patients were recruited, with a median follow up of 40.1 months. We dichotomised the cohort based on homozygosity in one or more HLA-I alleles (n=29) and those with maximal heterozygosity (n=84).

**Results:**

Our analysis did not show any association between HLA-I homozygosity and PFS (HR 1.06, 95% CI: 0.60-1.87, p = 0.83) or OS (HR 0.98, 95% CI: 0.51-1.191, p = 0.97). Similarly, we did not find any impact of HLA-II homozygosity (46 homozygous and 67 heterozygous) on PFS (HR 1.08, 95% CI: 0.66-1.79, p = 0.741) or OS (HR 1.52, 95% CI: 0.80-2.85, p = 0.19). Notably, the HLA-B27 supertype was associated with reduced PFS (HR 2.19, 95% CI: 1.19-4.02, p=0.02), and OS (HR:2.20, 95% CI:1.85-4.47, p=0.0075). Multivariate analyses confirmed that the HLA-B27 genotype is an independent predictor of shorter PFS and OS. The HLA-B27 association with reduced OS was validated in an independent cohort of 114 cases from the MSK-IMPACT cohort (HR 3.17, 95% CI: 1.01-10.01, p=0.01).

**Conclusions:**

Patients with HLA-B27 supertype have worse prognosis compared to non-carriers and may predict reduced response to ICI therapy.


# Introduction

Immune checkpoint inhibitor therapy (ICI) with anti-programmed death 1 (anti-PD1) and anti-cytotoxic lymphocyte antigen 4 (anti-CTLA4) monoclonal antibodies have improved the outcomes of patients with advanced melanoma with five-year survival rates approaching 50% [1–3]. However, biomarkers predictive of response to these novel therapies are lacking. Currently, there is no biomarker available in routine clinical practice that can help facilitate decision making for the use of these drugs in patients with advanced melanoma.

Various tumour and host related biomarkers predictive of response to ICI are currently being evaluated [4–7]. However, most research into predictive biomarkers has focused on tumour immune phenotype, somatic genomic mutations and gut microbiome in advanced melanoma patients treated with ICI therapy

[8–11]. The impact of germline genetic effects on response to ICI is an area of intense research globally[12, 13]. However, studies looking at their predictive value in melanoma patients undergoing anti-PD1 therapy are limited [13, 14].

Human leukocyte antigen (HLA) molecules are expressed on both somatic as well as immune cells. The HLA encoding genes are located on the short arm of chromosome 6 [15]. HLA genes are one of the most polymorphic in the human genome, with thousands of alleles encoding for polypeptides [16, 17]. These antigens play an important role in the regulation of the host's immune response by presenting self or foreign peptides to T cell receptors (TCRs). This leads to initiation of self-tolerance or T cell responses [18]. The HLA genes are divided into classical and non-classical categories. Classical HLA genes are well characterised and associated with presentation of antigen to immune cells [19]. These include HLA class I (HLA-A, HLA-B and HLA-C) and class II (HLA-DR, HLA-DQ and HLA-DP). The nonclassical HLA genes include HLA-E, HLA-F, HLA-G, HLA-DM and HLA-DO [19]. HLA alleles can also be grouped based on their peptide-binding repertoire into supertypes [20, 21].

Chowell et al. evaluated whether germline HLA-I genotype influences response to ICI therapy in advanced melanoma and lung cancer patients [14]. They concluded from their research that HLA-I heterozygosity was associated with better survival than homozygosity for one or more HLA-I genes. Hence, maximal heterozygosity could be associated with higher diversity of antigen presentation to the T cells compared to other patients with homozygosity at one or more HLA loci. These findings could have implications for future research into predictive biomarkers of response to ICI therapy as well the design of epitopes for cancer vaccines and ICI therapies. The cohort of Chowell et al. included advanced melanoma and lung cancer patients treated with both anti-PD1 and anti-CTLA4 therapies [14]. They did not evaluate the impact of HLA-II genotype on response to ICI therapies.

The aim of our research was to further evaluate and/or validate the findings by Chowell et al. in an independent cohort of patients with advanced melanoma, focusing on those undergoing treatment with anti-PD1 monotherapy (pembrolizumab). We also evaluated the influence of HLA-II (in addition to HLA-I) genotype and HLA-I supertypes on survival in these patients.

# Patients And Methods

**Patients:** Advanced melanoma patients undergoing ICI therapy with pembrolizumab were recruited prospectively from two hospitals in Perth, Western Australia from August 2014 to January 2020. Participants signed informed consent in accordance with protocols safeguarding patient rights. All procedures were approved Human Research Ethics Committee from Edith Cowan University (No. 11543 and No. 18957) and Sir Charles Gairdner Hospital (No. 2007 – 123 and No. 2013 – 246) in compliance with the Declaration of Helsinki. Experiments were performed according to institutional and national guidelines and regulations.

**Treatment Response Assessment:** Tumour responses were assessed radiologically by computed tomography (CT) and/or positive emission tomography (PET) scans at two to three monthly intervals.

Scans were reported by radiologists that were blinded to patient HLA type. Patients were defined as responders if they had significant reduction in tumour size by RECIST 1.1 on CT or PET FDG avidity as per the treating clinician or stable disease lasting more than 6 months. Progression free survival (PFS) was defined as the time interval between the start of therapy and the date of first progression. Overall survival (OS) was defined as the time interval between the start of therapy and death.

**Genomic HLA-typing:** Blood samples were collected in EDTA blood tubes and white blood cell pellet separated and stored at − 80°C until analysis. QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany) was used for DNA extraction. Extracted DNA was used for high-resolution HLA typing at the Institute of Immunology and Infectious disease (IIID) at Murdoch University as previously reported[22]. HLA testing at IIID has been accredited by the American Society for Histocompatibility and Immunogenetics (ASHI) and the National Association of Testing Authorities (NATA) [23]. Genomic HLA-A and HLA-B alleles were classified into supertypes using the method described by Sidney et al. [20].

**Statistical analysis:** Progression free survival (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. Overall survival (OS) was defined as the time between the start of pembrolizumab treatment and death. The minimum follow-up time was 6 months. Those patients who did not experience disease progression or were still alive at the time of analysis (October 2021) were censored. For validation, data was extracted from MSK-IMPACT cohort reported by Chowell et al.[14], composed of 114 patients with melanoma, treated with anti-PD1 therapy.

Patients were grouped based on HLA homozygosity at one, two or three loci; or based on the presence of specific HLA-A and B supertypes. PFS and OS were compared between groups using log-rank (Mantel-Cox) test and Kaplan-Meier plots using GraphPad Prism version 8 (GraphPad Software, Inc., San Diego, CA).

Cox regression analyses were used to compare the effect of HLA homozygosity and HLA-B27 on PFS and OS outcomes using SPSS version 28 (IBM, Armonk, NY). Multivariate analyses were carried out to control for potential cofounder variables.

# Results

**Patient characteristics:** A total of 121 patients were recruited for the study, with a median follow up of 40.1 months. Seven patients were excluded as they did not complete at least three cycles of pembrolizumab to draw meaningful conclusions regarding the efficacy of therapy. The demographic and clinical data of the 113 patients included in the analysis is summarised in Table 1. Most of the patients were males, < 65 years age, ECOG PS 0–1 and *BRAF* wildtype. Liver and brain metastases were present in 12% and 19% of cases, respectively.

Table 1
Patient characteristics

| | N (%) |
|---|---|
| **Total** | **113** |
| **Age** | |
| <65 | 77 (68) |
| ≥65 | 36 (32) |
| **Sex** | |
| Male | 74 (65) |
| Female | 39 (35) |
| **ECOG** | |
| ≤1 | 82 (73) |
| >1 | 31 (27) |
| **BRAF status** | |
| BRAF mutant | 36 (32) |
| BRAF wild type | 77 (68) |
| **Line of treatment** | |
| First line | 83 (73) |
| Second line or more | 30 (27) |
| **Liver metastasis** | |
| Yes | 13 (12) |
| No | 100 (88) |
| **Brain metastasis** | |
| Yes | 21 (19) |
| No | 92 (81) |

The incidence of all grade irAEs was 67% overall during the whole follow-up period, with only 12% experiencing grade 3 irAEs. The most frequent irAEs were pruritus and rash, with an incidence of 29%. The severe grade irAEs mostly involved skin (16%), rheumatological (8%) or gastrointestinal disorders (8%).

**Effect of HLA homozygosity on survival**: We dichotomised the cohort based on homozygosity in one or more HLA-I alleles (n = 29) and those with maximal heterozygosity (n = 84). Our analysis did not show any association between HLA-I homozygosity and PFS (HR 1.06, 95% CI: 0.60–1.87, p = 0.83) (Fig. 1A) or OS (HR 0.98, 95% CI: 0.51–1.191, p = 0.97) (Fig. 1B). Similarly, we did not find any impact of HLA-II homozygosity (46 homozygous and 67 heterozygous) on PFS (HR 1.08, 95% CI: 0.66–1.79, p = 0.741) or OS (HR 1.52, 95% CI: 0.80–2.85, p = 0.19) (Fig. 1C and D). We also evaluated each of the HLA-I and II genes on their allelic heterozygosity in relation with the patient survival (Table 2). No significant association was found for any of the comparisons. There was no association of HLA homozygosity with response to therapy.

Table 2
HLA-I and II loci zygosity and association with PFS and OS

| | PFS (HR (95% CI)) | OS (HR (95% CI)) |
|---|---|---|
| HLA-A | 0.866 (0.461–1.627) | 1.030 (0.511–2.077) |
| HLA-B | 1.108 (0.477–2.576) | 1.038 (0.371–2.904) |
| HLA-C | 1.112 (0.548–2.257) | 1.084 (0.459–2.563) |
| HLA-DPB1 | 0.982 (0.556–1.736) | 1.213 (0.645–2.279) |
| HLA-DQA1 | 1.248 (0.677–2.302) | 1.609 (0.828–3.125) |
| HLA-DQB1 | 1.210 (0.596–2.457) | 1.449 (0.673–3.120) |
| HLA-DRB1 | 1.384 (0.657–2.916) | 1.682 (0.749–3.774) |

**Prognostic value of HLA-I supertypes**: To evaluate the effect of HLA-I supertypes on survival in the advanced melanoma cohort, we grouped patients based on whether they carry or not specific supertypes and compared their survival outcomes (Table 3). Notably in our study, HLA-B27 was associated with shorter PFS and OS but the supertypes (HLA-B62 and HLA-B44) previously reported to be associated with ICI treatment outcomes [14], had no statistically significant association with survival. Nonetheless, carriers of the HLA-B62 had a trend towards shorter survival, while carriers of HLA-B44 had a trend for longer survival (Fig. 2A-D). HLA-B27 positive carrier status was associated with reduced PFS (HR 2.19, 95% CI: 1.19–4.02, p = 0.02), and OS (HR:2.20, 95% CI:1.85–4.47, p = 0.0075) (Fig. 2F and E).

Table 3
Cox regression survival analysis of HLA-I supertypes

| | PFS | | OS | |
|---|---|---|---|---|
| | **HR (95% CI)** | **p-value** | **HR (95% CI)** | **p-value** |
| A01 | 0.76 (0.46–1.25) | 0.272 | 0.95 (0.53–1.7) | 0.875 |
| A02 | 1.14 (0.69–1.88) | 0.610 | 1.01 (0.57–1.81) | 0.961 |
| A03 | 1.11 (0.67–1.83) | 0.683 | 0.89 (0.5–1.61) | 0.711 |
| A24 | 1.42 (0.8–2.52) | 0.225 | 1.41 (0.73–2.73) | 0.306 |
| B07 | 1.07 (0.65–1.76) | 0.804 | 1.07 (0.6–1.91) | 0.820 |
| B08 | **0.5 (0.27–0.94)** | **0.032** | 0.58 (0.28–1.2) | 0.144 |
| B27 | **2.22 (1.32–3.73)** | **0.003** | **2.24 (1.22–4.12)** | **0.009** |
| B44 | 0.6 (0.36–1.02) | 0.060 | 0.86 (0.48–1.55) | 0.616 |
| B58 | 0.73 (0.32–1.71) | 0.472 | 0.31 (0.08–1.3) | 0.110 |
| B62 | 1.72 (0.87–3.39) | 0.118 | 1.76 (0.84–3.67) | 0.132 |

Table 4
Univariate and multivariate Cox regression survival analysis

| | PFS | | | | OS | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariate | | Multivariate | | Univariate | | Multivariate | |
| | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value |
| B27 | **2.22 (1.32–3.73)** | **0.003** | **2.49 (1.39–4.48)** | **0.002** | **2.24 (1.22–4.12)** | **0.009** | **2.89 (1.4–5.99)** | **0.004** |
| HLA-I homozygosity | 0.92 (0.51–1.67) | 0.788 | 0.97 (0.52–1.83) | 0.934 | 1.02 (0.52–2.01) | 0.955 | 1.06 (0.5–2.25) | 0.888 |
| HLA-II homozygosity | 1.07 (0.64–1.78) | 0.790 | 1.08 (0.62–1.87) | 0.793 | 1.44 (0.81–2.58) | 0.215 | 1.9 (0.96–3.77) | 0.066 |
| Female | 1 (0.77–1.31) | 0.980 | 1.06 (0.8–1.42) | 0.665 | 0.92 (0.67–1.26) | 0.605 | 1.01 (0.7–1.46) | 0.945 |
| Age | 1.06 (0.63–1.76) | 0.830 | 1.55 (0.87–2.75) | 0.133 | 1.3 (0.71–2.38) | 0.398 | **2.7 (1.29–5.67)** | **0.008** |
| ECOG ≥ 2 | **2.65 (1.55–4.51)** | **> 0.001** | **3.67 (2.06–6.56)** | **> 0.001** | **5.01 (2.8–8.98)** | **> 0.001** | **7.99 (4.16–15.33)** | **> 0.001** |
| Stage M1c/d | 1.54 (0.92–2.6) | 0.103 | 1.24 (0.66–2.32) | 0.503 | 2.24 (1.15–4.34) | 0.017 | 1.68 (0.76–3.72) | 0.198 |
| BRAF mut | **0.72 (0.56–0.93)** | **0.011** | **0.7 (0.53–0.94)** | **0.016** | **0.71 (0.53–0.95)** | **0.022** | **0.71 (0.51–0.99)** | **0.044** |
| Brain mets | 0.9 (0.66–1.23) | 0.513 | 1.22 (0.84–1.78) | 0.295 | 0.82 (0.58–1.16) | 0.263 | 1.23 (0.81–1.86) | 0.332 |
| NLR ≥ 5 | 1.85 (0.94–3.66) | 0.076 | **2.19 (1.05–4.59)** | **0.037** | **2.76 (1.36–5.6)** | **0.005** | **3.59 (1.57–8.22)** | **0.002** |
| Lymph ≥ 3 | 1.14 (0.52–2.51) | 0.741 | 0.69 (0.29–1.64) | 0.401 | 1.27 (0.54–3.01) | 0.581 | 1.14 (0.43–2.98) | 0.796 |

* maximal heterozygosity as reference group

| | PFS | | | | OS | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariate | | Multivariate | | Univariate | | Multivariate | |
| | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value |
| irAE | 0.57 (0.34–0.95) | 0.031 | 0.41 (0.23–0.71) | 0.002 | 0.52 (0.29–0.94) | 0.030 | 0.31 (0.16–0.61) | 0.001 |
| * maximal heterozygosity as reference group | | | | | | | | |

We carried out multivariate analyses to assess the association between HLA-B27 and HLA homozygosity and survival while controlling for sex, age, ECOG performance status, disease stage, BRAF mutation, the presence of brain metastases, neutrophil to lymphocyte ratio (NLR), lymphocyte count and the presence of irAE. Results underscored that melanoma patients treated with pembrolizumab and carrying HLA-B27 genotype have poor PFS and OS compared to HLA-B27 negative patients (Table 5). Additional independent predictors of poor survival included ECOG performance status equal or above 2, BRAF wt, NLR equal or above 5, and the absence of irAE.

**Validation cohort**: To confirm our findings we analysed a freely available data set from the MSK-IMPACT cohort (Chowell et al[14]) of 114 melanoma patients treated with anti-PD1 therapy. In this cohort, HLA supertype B27 was also found to be associated with shorter OS (HR:0.31 95% CI 0.09–0.99, p = 0.0135), while neither HLA supertypes B44 or B62, nor HLA-I homozygosity were prognostic similar to the findings from our cohort (Fig. 3).

# Discussion

Several biomarkers like tumour mutational burden, PDL-1 expression, microsatellite instability (MSI) status, neoantigen load and HLA status have been recently evaluated to determine their utility to predict response to ICI therapy[6, 14, 22]. We have previously published on the utility of circulating tumour cell PDL1 expression [24] and serum vascular endothelial growth factor (VEGF) levels [25] as potential blood-based predictive biomarkers of response to anti-PD1 therapy in advanced melanoma. Here we report our findings of HLA genotyping as a predictive biomarker from a cohort of advanced melanoma patients treated with pembrolizumab. Overall, we found HLA-B27 to be a strong independent predictor of poor outcome to therapy.

Our results failed to demonstrate a predictive association between HLA Class I or II genotypes and survival in advanced melanoma cohort treated with pembrolizumab. Our findings are in contrast to the conclusions made by Chowell et al. [14], who found better treatment outcomes for patients with HLA Class I heterozygosity. This cohort was markedly heterogeneous, including patients with advanced melanoma or lung cancer in addition to some other tumour types. The sample size of their cohort was

substantially larger (n = 1535, with 269 melanoma patients in cohort 1) compared to our cohort (n = 113). Notably, the Chowell et al. study included different ICI therapies with patients receiving anti-PD1 and/or anti-CTLA4 therapy primarily in the 2nd or 3rd line. In contrast, we focused on melanoma patients treated with anti-PD1 (pembrolizumab), primarily in the 1st line setting (73%). This distinction is of clinical relevance as single agent anti-PD1 is administrated commonly as first line therapy for advanced melanoma as well as in the adjuvant settings[26, 27].

Studies evaluating the utility of HLA status as a predictive biomarker have yielded mixed results. In addition to the study by Chowell et al[14], two other studies have recently been published in advanced non-small cell lung cancer. The findings from Abed et al.[22] suggest that homozygosity at one or more HLA-I loci was associated with shorter OS and PFS in patients with advanced non-small cell lung cancer with PDL1 score $\geq$ 50%. The study also found that carriers of HLA-A02 supertype had better survival outcomes. However, in contrast to their findings, Negrao et al[28] found that HLA class I genotype did not correlate with survival in advanced non-small cell lung cancer patients treated with ICI therapy. They concluded that the impact of HLA class I diversity may be disease specific and that tumour genomic and immune markers might carry stronger predictive significance.

An important finding of our study was the association of the HLA-B27 supertype with pembrolizumab treatment outcomes. Albeit not statistically significant, HLA-B62 and B44 supertypes were found to be associated with shorter and longer PFS, respectively, similar to the report by Chowell et al.[14]. However, HLA-B27 positivity demonstrated a strong negative association with both PFS and OS, even after controlling for multiple cofounder variables. Notably, this novel finding was validated in an independent cohort[14], again focusing on anti-PD1 treated melanoma patients.

HLA-B27 is associated with the presence of seronegative spondyloarthropathies [29], such as ankylosing spondylitis (AS), psoriatic arthritis, and Reiter's syndrome (reactive arthritis). HLA B27 is also strongly associated with acute anterior uveitis[30]. These associations are amongst the strongest of any HLA antigen to a human disease, although the pathogenetic mechanism(s) involved remain unknown[31].

Another line of evidence suggests that HLA-B27 affects the composition of the gut microbiome, which in turn can modify the immune system and thereby affect health and disease [32]. This is particularly relevant, given the strong evidence that gut microbiota not only serves a biomarker of response to ICI [11], but its modification through faecal microbiota transplant can serve as a companion intervention to improve anti-PD1 therapy efficacy[33]. Thus, further studies should seek to evaluate patients' microbiomes and ICI outcomes in relation to HLA-27 supertype.

There are several limitations of our study. Our sample size is small meaning we might not have been able to able to appreciate small differences and determine the true magnitude of impact of HLA status on response to ICI therapy. Our multivariate analysis did not include other confounding factors reported to regulate anti-tumour immune response including tumour immune signature/score, tumour mutational burden or gut microbiome[6, 34]. We did not have access to tumour tissue and hence were unable to calculate the impact of somatic loss of HLA heterozygosity on survival outcomes. A quarter of our

patients were ECOG 2 + in contrast to the clinical trial population where the vast majority of the patients are ECOG ≤ 1. However, our cohort is more reflective of the real-world population.

In conclusion, the results of our study underscore that prognostic biomarkers may be only relevant in very specific clinical context. In particular, we report on the poor outcomes observed in melanoma patients with the HLA-B27 supertype when treated with pembrolizumab monotherapy. This observation requires validation in an independent study, as it may inform the need to treat patients with certain HLA supertypes associated with poorer OS with combination therapy to improve their long-term outcomes. Additionally, HLA-B27 carriers might be better suited to treatment with single agent ICI to avoid the exaggerated risk of immune side effects with combination ICI given the association of this supertype with other autoimmune syndromes.

# Declarations

**Conflict of interest**: MM: advisory boards for Merck Sharpe and Dohme (MSD), Bristol-Myers Squibb (BMS) and Astra Zeneca (AZ); MAK: travel grant BMS, MSD, Merck; EG: travel grant MSD.

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**Authorship Contribution Statement:** MAK and ESG conceptualised and designed the study; MAK, OO, AG, ALR and AA collected data; MMo and AG developed database; ESG, LC and AC carried out testing and analysis of the data; MAK, TM and MMi provided and cared for study patients; MM and MZ served as scientific advisors; MAK and ESG drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

**Data availability statement:** Data are available upon reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information.

**Ethics statements:**

**Patient consent for publication:** Not applicable.

**Ethics approval:** This study was approved by the Human Research Ethics Committee from Edith Cowan University (No. 11543 and No. 18957) and Sir Charles Gairdner Hospital (No. 2007-123 and No. 2013-246).

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# Table

Table 5 is not available with this version.

# Figures

Figure 1.

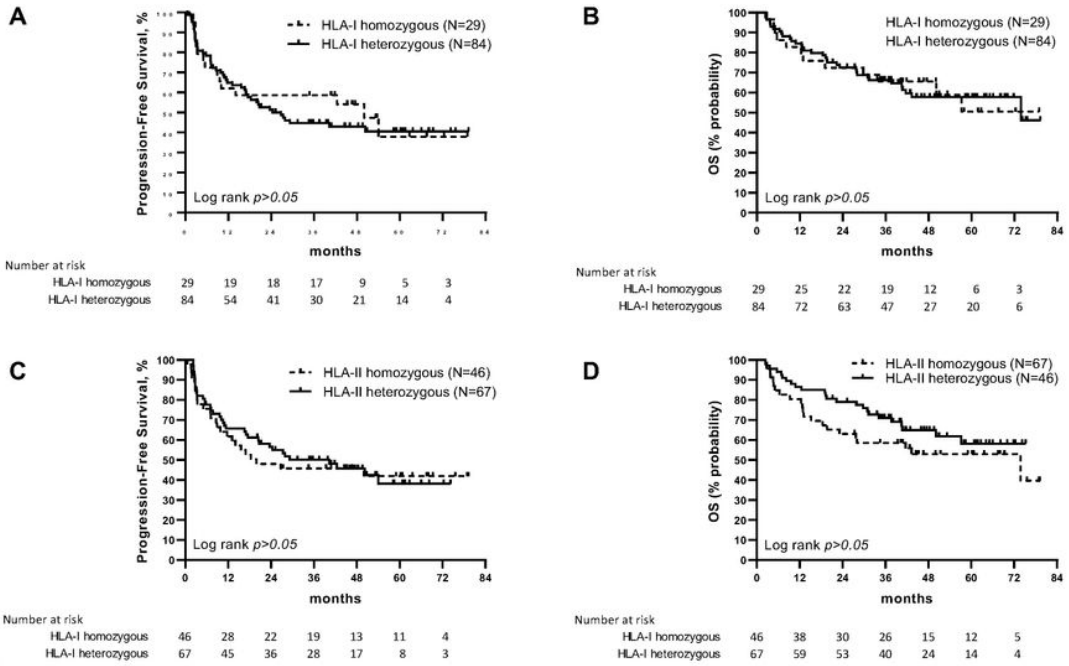
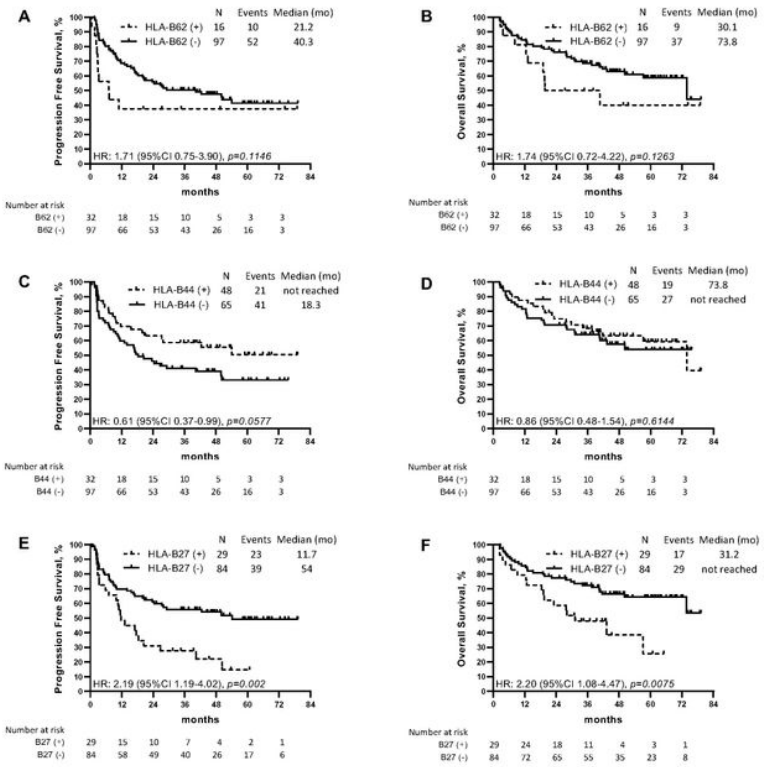



**Figure 1**

Progression free survival (A and C) and overall survival (B and D) estimates based on homozygosity in at least one HLA-I (A and B) or HLA-II locus.

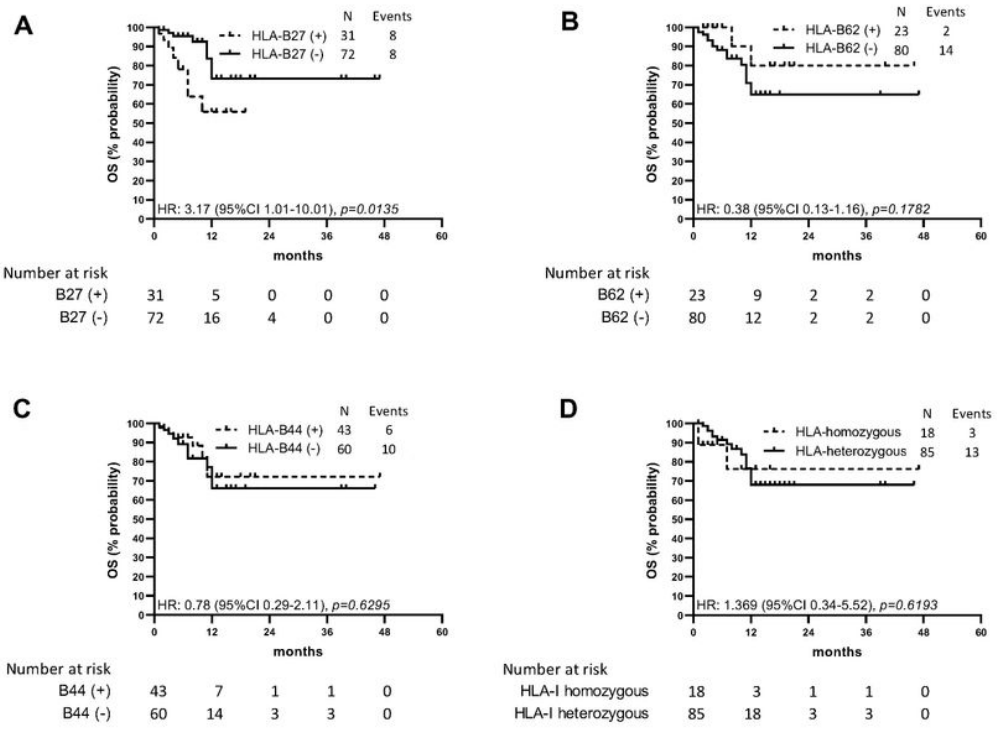
Figure 2




## Figure 2

Progression free survival (A, C and E) and overall survival (B, D and F) estimates based on HLA supertypes. HLA-B62 (A and B), HLA-B44 (C and D) and HLA B27 (E and F).

Figure 3.




## Figure 3

Overall survival estimates based on HLA-B62 (A), HLA-B44 (B), HLA-B27 (C) and HLA zygosity status (D) in the MSK-IMPACT cohort [14].