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Comprehensive Profiling of Acral Lentiginous Melanoma Reveals Downregulated Immune Activation Compared to Cutaneous Melanoma

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ABSTRACT

Acral lentiginous melanoma (ALM) is a rare and insufficiently understood subtype of melanoma lacking in effective treatment options. Recent work has demonstrated that the response of ALM to immune checkpoint blockade is inferior to that of cutaneous melanoma (CM). Here we performed bulk genomic and transcriptomic sequencing of tumor tissue from 28 ALM and 5692 CM cases. Similar to prior studies, ALM was associated with a significantly lower incidence of point mutations, including in the *TERT* promoter and *BRAF*, but increased numbers of gene amplifications, notably of *CCND1*, *HMGA2*, and *MDM2*. Reactome pathway analysis revealed enhancement of keratinization and PI3K/AKT signaling pathways. Overall immunogenicity was decreased in ALM, which possessed lower IFNγ ($p < 0.001$) and T-cell inflammatory ($p = 0.03$) pathway scores than CM. Despite higher computationally inferred levels of myeloid dendritic cells ($p = 0.006$), neoantigen load independent of predicted HLA binding affinity was lower ($p < 0.01$) in ALM versus CM. Assessment of classical and nonclassical HLA mRNA levels revealed upregulation of HLA-G, suggesting alternative ALM immune evasion pathways in the setting of lower PD-L1 expression ($p = 0.005$). Additional research is needed to better understand and therapeutically target signaling networks in the ALM tumor microenvironment.

1 | Introduction

Acral lentiginous melanoma (ALM) is a rare subtype of melanoma found in the palms, soles, and nailbeds, and histologically characterized by an atypical radial growth pattern of melanocytes along the dermal-epidermal junction. ALM often presents at a later stage and is associated with worse survival than superficial spreading melanoma, nodular melanoma, and other non-acral cutaneous melanoma (CM) subtypes independent of stage (Bradford et al. 2009; Wang et al. 2016; Howard

Summary

- Acral lentiginous melanoma (ALM) responds poorly to anti-PD1 therapy alone and in combination with anti-CTLA4 and lacks reliable targets for molecularly guided therapy, underscoring the need for novel therapeutic approaches in this rare melanoma subtype.
- Our multi-omic analysis consolidates findings from other groups describing ALM's immunosuppressive tumor microenvironment compared to cutaneous melanoma (CM), with downregulated immune activation revealed by key biomarkers such as lower PD-L1 expression, neoantigen load, and T-cell inflammatory transcriptional signatures.
- This work points us toward new hypotheses for targeting additional pathways, such as those involving HLA-G, to improve immunotherapy responses in ALM.

et al. 2020; Huang et al. 2020; Bian et al. 2021). ALM represents only about 5% of melanoma diagnoses in individuals of European descent, but can constitute more than 50% of cases in populations of Latin American, African, and Asian descent (Basurto-Lozada et al. 2021). Because of its overall rarity and although it accounts for most melanoma cases in populations underrepresented in US- and Europe-based trials, ALM remains insufficiently understood compared to the most common forms of CM.

Previous work has investigated the genetic drivers behind ALM pathogenesis. ALM is known to possess more chromosomal aberrations and genomic signatures distinct from those associated with ultraviolet radiation on sun-exposed skin, including less frequent *BRAF V600* mutations and lower tumor mutational burden (TMB; Curtin et al. 2005; Hayward et al. 2017; Yeh et al. 2019; Newell et al. 2022; Basurto-Lozada et al. 2024). More recent sequencing data across multiple cancer types revealed the existence of tyfonas—complex genomic rearrangements with large regions of inversions—associated with *MDM2* and *CDK4*, and seen frequently in acral but not CMs (Hadi et al. 2020). Exome sequencing of sequential ALM biopsies further demonstrated that hailstorms, which are clustered copy number transitions similar to tyfonas, often appeared as early as ALM in situ and were frequently associated with *TERT* alterations (Wang et al. 2024). It appears that these tumor-initiating events are only later followed by driver mutations in the MAPK pathway, suggesting that the genetic evolution of ALM occurs in an alternative order compared to that of CM.

Prior studies have delved deeply into the genomics of ALM, and more recent work has examined its transcriptome, particularly as it relates to the tumor microenvironment. Despite limited representation in clinical trials, multiple studies have shown that ALM is poorly responsive to immune checkpoint inhibitors compared to CM (Johnson et al. 2015; Namikawa et al. 2018; Tang et al. 2020; Bhave et al. 2022; van Not et al. 2022; Mori et al. 2023; Wolchok et al. 2024; Zheng and Jenkins 2025; McGillivray et al. 2025). Separate cohorts of bulk and single-cell transcriptomic data in ALM have independently examined its neoantigen burden and the cellular composition of its microenvironment, as well as tumor-intrinsic pathways associated with immune exclusion (Liang et al. 2017; Li et al. 2022; Zhang et al. 2022; Augustin et al. 2023; Chiu et al. 2024; Choi et al. 2024; Minowa et al. 2024). These have shown, in isolation, that ALM TMB is correlated with neoantigen burden; acral melanomas possess a depleted and exhausted CD8+ effector T cell population; and the HGF, VEGF, and CCND1 signaling pathways, among others, are co-opted to suppress T cell inflammation. Importantly, a few of these reports have been limited by small sample sizes, and there remains a gap in examining the ALM tumor immune microenvironment compared to CM comprehensively within a single study.

We therefore aimed to use a multi-omic platform to better characterize the molecular landscape of ALM compared to CM, particularly with a focus on immunologic features. We expand upon previous work in this area in hopes of generating new hypotheses to enhance immune checkpoint inhibitor efficacy and to identify novel therapeutic targets in this rare melanoma subtype.

2 | Methods

2.1 | Study Design

This was a retrospective study of 5720 melanoma cases with samples obtained for comprehensive profiling by Caris Life Sciences (Tempe, AZ). De-identified patient demographic, clinicopathologic, systemic treatment, and outcome data were obtained from the Caris CODEai data platform.

Of all assessed samples from within the Caris Life Sciences data platform, 28 were classified as ALM and 5692 were classified as CM. At least one type of sequencing data was available for each patient. 2105 samples (23 ALM, 2082 CM) were obtained from primary skin sites, and 3615 (5 ALM, 3610 CM) were from metastases. A total of 2293 (10 ALM, 2283 CM) formalin-fixed paraffin-embedded (FFPE) samples underwent next-generation sequencing (NGS) with a 592-gene NextSeq panel (Agilent Technologies, Santa Clara, CA), while 3427 (18 ALM, 3409 CM) underwent whole exome sequencing (WES) via NovaSeq (Illumina, San Diego, CA). This genomic data was used to determine the frequency of mutations, amplifications, fusions, and deletions within each melanoma subtype. Variants were detected with $>99\%$ confidence based on allele frequency and amplicon coverage, with an average sequencing depth of $>500\times$ and an analytic sensitivity of 5% variant frequency. Copy number alterations were determined by comparing the average sequencing depth of each sample along with the sequencing depth of each exon to a pre-calibrated value, with average copies of 6 or greater considered amplified. Whole transcriptome sequencing (WTS) via NovaSeq was performed using RNA isolated from 4219 (23 ALM, 4196 CM) samples. Differential transcript expression was determined between melanoma subtypes, then used to assess for differentially regulated pathways via Reactome, an open-source and open-access biological pathway database (Fabregat et al. 2018).

2.2 | Quantification of Key Signaling Pathway Activity

Activity of key signaling pathways was quantified using previously described gene signatures. A MAPK pathway activity score was calculated by taking the average z-score of the transcript levels of 10 MAPK target genes (*SPRY2*, *SPRY4*, *ETV4*, *ETV5*, *DUSP4*, *DUSP6*, *CCND1*, *EPHA2*, *EPHA4*) in each sample (Wagle et al. 2018). Separately, the IFNγ score used an 18-gene signature previously validated in a large cohort of pembrolizumab-treated patients across 9 tumor types (Ayers et al. 2017). Expression values of the 18 genes were normalized to housekeeping gene expression, then combined as a weighted sum to obtain an IFNγ score. Negative scores indicated lower IFNγ pathway activity, whereas positive scores indicated higher IFNγ pathway activity. Additionally, the T-cell inflamed score (TIS) was based on a 160-gene signature (Spranger et al. 2016; Bao et al. 2020). Expression of each gene was first normalized to the mean expression across the whole cohort, such that −1 reflected downregulation and +1 reflected upregulation. The z-scores of all genes were summed together to obtain a TIS ranging from −160 (if all genes were downregulated, deeming the sample non-T-cell inflamed) to +160 (if all genes were upregulated, deeming the sample T-cell inflamed).

2.3 | Assessment of Immune-Related Biomarkers, Cell Populations, and Neoantigen Load

TMB was defined as the number of somatic nonsynonymous missense mutations found within 592 genes and 1.4 megabases (Mb) sequenced per tumor. Samples were classified as having high TMB if they possessed greater than 10 mutations/Mb (Sha et al. 2020). To determine PD-L1 status, immunohistochemistry (IHC) using the SP142 anti-PD-L1 antibody (Spring Biosciences, San Francisco, CA) was performed on FFPE samples. Staining intensity was scored as 0 if none was present, 1+ if weak, 2+ if moderate, or 3+ if strong. Samples were classified as positive for PD-L1 if tumor cell stain intensity was at least 2+ and if at least 5% of tumor cells were positively stained (Massi et al. 2014; Robert et al. 2015; Larkin et al. 2015).

HLA genotyping via arcasHLA and immune epitope prediction were performed using mRNA expression data (Orenbuch et al. 2020). To quantify neoantigen load, binding prediction for all HLA allele/peptide combinations for each tumor was performed using the NetMHCpan v4.0 in the Immune Epitope Database (Jurtz et al. 2017). Peptide affinity for HLA was categorized as high affinity if half-maximal inhibitory concentration (IC50) was < 50 nM, intermediate affinity if IC50 was between 50 and 500 nM, or low affinity if IC50 was between 500 and 5000 nM.

Relative fractions of individual immune cell populations within heterogeneous tissue samples were inferred using quanTIseq (Finotello et al. 2019). QuanTIseq is a deconvolution algorithm that uses a reference gene signature of 153 differentially expressed genes to estimate the proportions of 10 immune cell types from bulk RNA-seq data.

2.4 | Statistical Analysis

Patient demographics, as well as cancer- and tumor-specific data, were summarized with descriptive statistics. Overall survival (OS) was obtained from insurance claims data and assessed from the time of initial diagnosis to the date of death. Patients who were alive or lost to follow-up were censored at the date of last contact. Median OS was estimated via the Kaplan–Meier method, and hazard ratio and 95% CI were computed using the Cox proportional hazards model.

Categorical variables were compared using two-tailed Fisher's exact tests. Continuous variables were compared with two-tailed Mann–Whitney U tests. Statistical significance was declared based on the level set to 0.05. Q values were calculated by adjusting p values using the Benjamini-Hochberg method.

2.5 | Ethics Statement

This study was conducted in accordance with guidelines from and with approval by the Human Research Protection Program (HRPP) at the University of Southern California, under IRB number HS-16-00840.

3 | Results

3.1 | Demographic and Clinicopathological Features of the Study Cohort

The median age at diagnosis for patients within the study cohort was 67 (range, 5–89+) (Table 1). 2023/5720 (35.4%) patients were female and 3697 (64.6%) were male, with no significant difference in gender distribution between ALM versus CM. Compared to CM, a lower percentage of ALM patients were European/White (50.0% vs. 75.2%, p excluding unknown < 0.001), while more were Asian/Pacific Islander (7.1% vs. 0.8%, p excluding unknown = 0.02), Black/African-American (21.4% vs. 2.7%, p excluding unknown < 0.001) or Hispanic/Latino (21.4% vs. 5.4%, p excluding unknown = 0.003). There were no significant differences in stage at diagnosis, administration of radiation therapy, or distribution of systemic therapies received between groups. Median OS for ALM patients was 66.4 months, not significantly different from the median of 82.8 months for CM patients (HR = 1.26; 95% CI: 0.76–2.10; p = 0.37) (Figure S1).

3.2 | Genomic Differences Between ALM and CM

The most common genes possessing mutations classified as pathogenic and presumed pathogenic variants in ALM were *NRAS* (28.6%), the *TERT* promoter (12.5%), *CDKN2A* (9.5%), *ATRX* (7.1%), *NF1* (6.3%), and *RB1* (6.3%) (Figure S2A). Within our cohort, *KRAS*, *HRAS*, and *KIT* mutations were each present in 4.8% of patients, and 43.8% were triple wild-type (i.e., lacking mutations in *BRAF*, *RAS*, and *NF1*) (Akbani et al. 2015). The most commonly amplified genes in ALM were *CCND1* (23.1%), *FGF4* (15.4%), *MDM2* (15.4%), *FGF19* (15.4%), *HOOK3* (11.1%), and *HMGA2* (9.1%) (Figure S2B).

TABLE 1 | Descriptive statistics of patients with ALM vs. CM.

Characteristic by group	Total, *n* (%)	ALM, *n* (%)	CM, *n* (%)	*p*
No. of individuals	5720 (100.0%)	28 (0.5%)	5692 (99.5%)	
Age at diagnosis (years)				
Range	5–89+	30–85	5–89+	0.75
Median	67	65	67	
Gender				
Female	2023 (35.4%)	12 (42.9%)	2011 (35.3%)	0.43
Male	3697 (64.6%)	16 (57.1%)	3681 (64.7%)	
Race				
Asian/Pacific Islander	48 (0.8%)	2 (7.1%)	46 (0.8%)	<0.001[a]
Black/African-American	157 (2.7%)	6 (21.4%)	151 (2.7%)	
European/White	4294 (75.1%)	14 (50.0%)	4280 (75.2%)	
Other	248 (4.3%)	2 (7.1%)	246 (4.3%)	
Unknown	973 (17.0%)	4 (14.3%)	969 (17.0%)	
Ethnicity				
Hispanic/Latino	315 (5.5%)	6 (21.4%)	309 (5.4%)	**0.003**[a]
Not Hispanic/Latino	4438 (77.6%)	17 (60.7%)	4421 (77.7%)	
Unknown	967 (16.9%)	5 (17.9%)	962 (16.9%)	
Stage at diagnosis				
I	38 (0.7%)	0 (0.0%)	38 (0.7%)	0.31[a]
II	228 (4.0%)	2 (7.1%)	226 (4.0%)	
III	787 (13.8%)	5 (17.9%)	782 (13.7%)	
IV	1480 (25.9%)	4 (14.3%)	1476 (25.9%)	
Unknown	3187 (55.7%)	17 (60.7%)	3170 (55.7%)	
Radiation therapy				
Yes	1892 (33.1%)	9 (32.1%)	1883 (33.1%)	1
No	3828 (66.9%)	19 (67.9%)	3809 (66.9%)	
Systemic therapies				
Chemotherapy	1054 (18.4%)	6 (21.4%)	1048 (18.4%)	0.63
Targeted therapy	1145 (20.0%)	4 (14.3%)	1141 (20.0%)	
Immunotherapy	3536 (61.8%)	13 (46.4%)	3523 (61.9%)	

[a]Excluding unknown.

We found that ALM was associated with a lower overall incidence of point mutations compared to CM (both with $q<0.05$ shown in Figure S2C, Table S1). ALM had significantly less frequent *TERT* promoter mutations (12.5% vs. 85.8%, $p<0.001$, $q<0.001$), as well as *BRAF* mutations within coding regions (4.8% vs. 45.5%, $p=0.001$, $q=0.03$) but not amplifications. ALM was enriched in gene amplifications, fusions, and deletions (all with $q<0.05$ shown in Figure S2D–F, Table S1); notably, none of these assessed genetic changes were differentially enriched in CM. Compared to CM, ALM possessed more amplifications in *SMARCE1*, *ECT2L*, *ETV5*, *JAK1*, *PAX5*, *HOOK3*, *ERBB3*, *MRE11*, *FOXP1*, *CCND1*, *HMGA2*, and *MDM2*; fusion genes involving *EGFR*, *PHF3*, *TYR*, and *TERT*; and deletions of *ATR*, *MEN1*, and *KMT2A* ($q<0.05$ for *FOXP1*, *CCND1*, *HMGA1*, *MDM2* amplifications and *TERT* fusion; otherwise all $q<0.001$). Of note, the differentially amplified genes listed in Table S1 were seen across eight patients with ALM.

3.3 | Differentially Regulated Pathways in ALM

We sought to generate additional hypotheses regarding differences between ALM and CM by assessing for differentially regulated

pathways via Reactome (Fabregat et al. 2018). We evaluated differential expression from WTS and found that pathways related to keratinization and epidermis formation were most enriched in ALM (Table 2). This was due to overexpression of the genes encoding keratin 6, 14, and 16, among others. ALM was also significantly associated with enhanced PI3K/AKT signaling.

TABLE 2 | Differentially regulated pathways between ALM vs. CM via Reactome.

Pathway name	*Q* value
Formation of the cornified envelope	1.50E-13
Keratinization	2.12E-11
Developmental biology	1.12E-10
Differentiation of keratinocytes in interfollicular epidermis in mammalian skin	1.12E-10
Developmental cell lineages	1.12E-10
Constitutive signaling by aberrant PI3K in cancer	2.19E-10
PI3K/AKT signaling in cancer	2.44E-10
Negative regulation of the PI3K/AKT network	2.07E-09
PI5P, PP2A and IER3 regulate PI3K/AKT signaling	2.47E-09
Diseases of signal transduction by growth factor receptors and second messengers	3.26E-09

Further, the activity of select signaling pathways was determined by comparing gene signatures described in other manuscripts. For example, the MAPK pathway activity score, which has previously been shown to vary independently of *RAF* and *RAS* mutational status, was quantified for our study cohort (Wagle et al. 2018). ALM was associated with lower MAPK activity compared to CM (0.81 vs. 2.68; $p=0.02$) (Table 3). Comparisons of other previously validated gene signatures revealed that ALM possessed lower IFNγ pathway activity (median, −0.43 vs. −0.20; $p<0.001$) and TIS (median, −40 vs. 35; $p=0.03$) (Spranger et al. 2016; Ayers et al. 2017; Bao et al. 2020).

3.4 | The ALM Tumor Immune Microenvironment

Given the decreased efficacy of immune checkpoint inhibitors in ALM compared to CM, we aimed to better characterize biomarkers associated with immunogenicity, including TMB and PD-L1. Overall, we found lower TMB in ALM (Table 3); only 1/20 (5.0%) assessed ALM samples possessed high TMB (greater than 10 mutations/Mb), compared to 3032/5266 (57.6%) assessed CM samples ($p<0.001$). The median TMB was 2 mutations/Mb for ALM, versus 12 mutations/Mb for CM ($p<0.001$).

TABLE 3 | Comparison of select gene signatures and immune-related biomarkers in ALM versus CM.

Characteristic by group	ALM	CM	*p*
MAPK activation score			
Value	0.81	2.68	0.02
No. of individuals	$n=17$	$n=3834$	
IFNγ score			
Value	−0.43	−0.20	<0.001
No. of individuals	$n=17$	$n=3834$	
T-cell inflamed score			
Value	−40	35	0.03
No. of individuals	$n=17$	$n=3830$	

Characteristic by group	Total, *n* (%)	ALM, *n* (%)	CM, *n* (%)	*p*
No. of individuals	5720 (100.0%)	28 (0.5%)	5692 (99.5%)	
TMB				
High	3033 (53.0%)	1 (3.6%)	3032 (53.3%)	<0.001[a]
Low	2253 (39.4%)	19 (67.9%)	2234 (39.2%)	
Unknown	434 (7.6%)	8 (28.6%)	426 (7.5%)	
PD-L1 IHC (SP142)				
Positive	2050 (35.8%)	3 (10.7%)	2047 (36.0%)	0.005[a]
Negative	2847 (49.8%)	20 (71.4%)	2827 (49.7%)	
Unknown	823 (14.4%)	5 (17.9%)	818 (14.4%)	

[a]Excluding unknown.

Additionally, only 3/23 (13.0%) ALM samples versus 2047/4874 (42.0%) CM samples were classified as PD-L1 positive, defined as having at least moderate tumor cell stain intensity with the SP142 antibody ($p = 0.005$).

Next, we quantified tumor immune epitopes with MHC-I binding for a subset of patients, including 18 with ALM and 699 with CM. This analysis demonstrated that neoantigen load was lower in ALM compared to CM, regardless of low ($p < 0.01$), intermediate ($p < 0.001$), or high ($p < 0.001$) HLA binding affinity (Figure 1A). Of the classical and non-classical MHC-I and MHC-II genes assessed, only HLA-G mRNA expression was significantly upregulated in ALM compared to CM (fold-change = 2.2 vs. CM; $q < 0.001$) (Figure 1B).

Finally, we used the quanTIseq deconvolution algorithm to calculate the relative fractions of 10 different immune cell types from bulk RNA-seq data of ALM vs. CM biopsies (Finotello et al. 2019). In total, immune cells comprised a median 30.1% and 33.9% of ALM and CM bulk tumor samples, respectively. Interestingly, ALM biopsies contained 1.6 times the relative proportion of myeloid dendritic cells (median, 6.2% vs. 3.9% in CM; $p = 0.006$) (Figure 2). In contrast, ALM possessed no CD8+ T cells compared to a median 0.7% in CM samples ($p = 0.01$). Proportions of neutrophils, M1 and M2 macrophages, B cells, NK cells, CD4+ and regulatory T cells, and monocytes were comparable between ALM and CM.

4 | Discussion

This retrospective cohort study of 5720 melanoma cases characterized the genomic and transcriptomic landscape of ALM compared to non-acral CM. Similar recent studies have reported

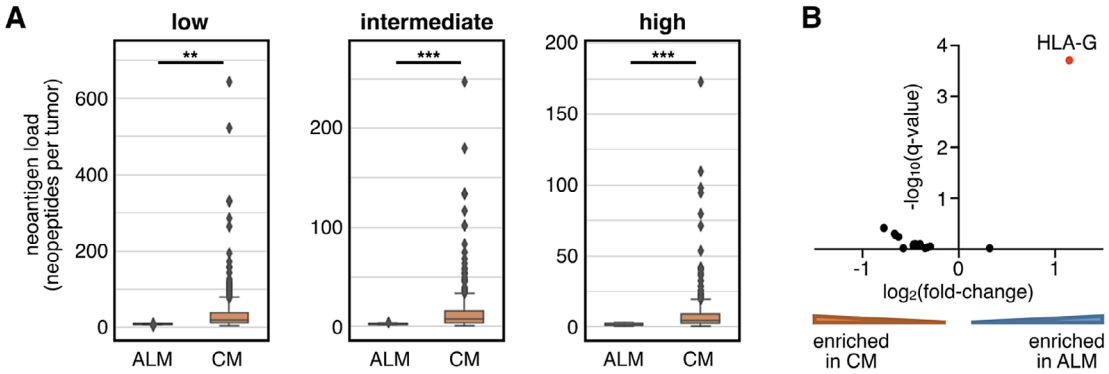

FIGURE 1 | (A) Neoantigen load with low, intermediate, or high binding affinities to HLA in ALM vs. CM. $^{**}p < 0.01$, $^{***}p < 0.001$. (B) Differential expression of HLA-family mRNA in ALM versus CM.

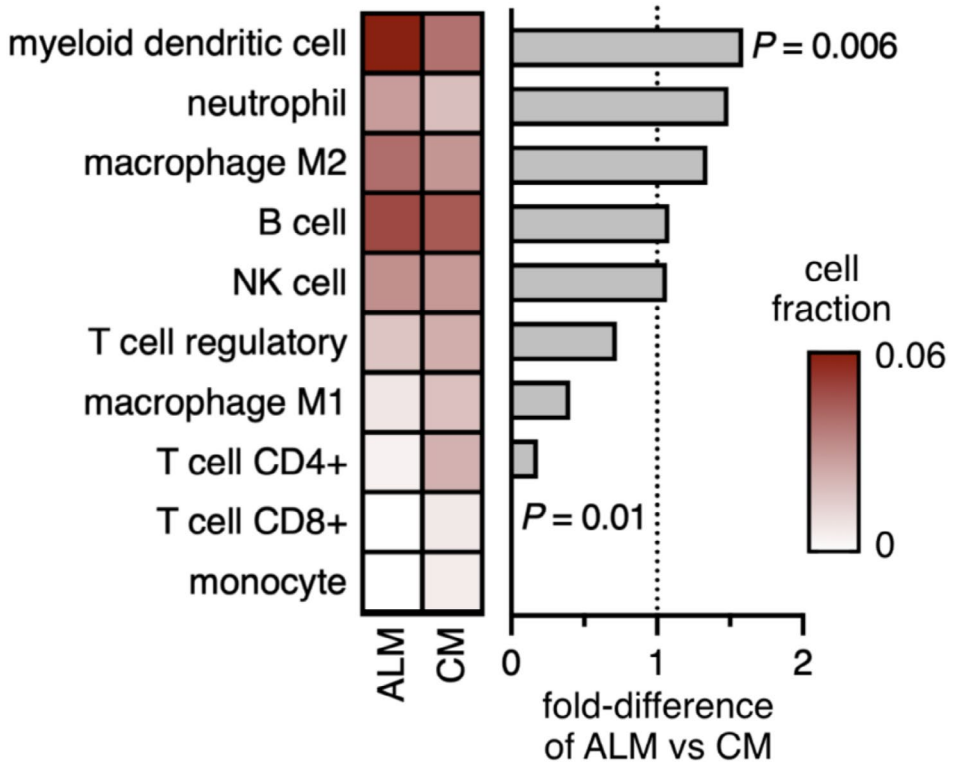

FIGURE 2 | Immune cell fractions in ALM versus CM, inferred via quanTIseq.

genetic alterations and differential pathway activity characteristic of ALM, but these largely have not translated into new therapeutic approaches, which are especially needed considering ALM's poor response to available immune checkpoint inhibitors. Our findings build upon existing literature by collectively detailing key features of the ALM tumor immune microenvironment including, to the best of our knowledge, being the first to demonstrate transcriptional upregulation of HLA-G in ALM compared to CM.

Our work is consistent with prior reports demonstrating increased copy number alterations and genomic rearrangements, but fewer point mutations and lower expression of MAPK pathway genes in ALM compared to CM (Curtin et al. 2005; Hayward et al. 2017; Wagle et al. 2018; Newell et al. 2022). We noted mutations in *BRAF* for 5% of cases (vs. 11%–24% in several larger cohorts and meta-analyses of either acral melanomas alone or combined with mucosal melanomas), *N/K/H-RAS* for 38% (vs. 19%–32%), *NF1* for 6% (vs. 7%), and *KIT* for 5% (vs. 8–21%), with 44% triple wild-type (vs 32% to over 60%) (Carvajal et al. 2011; Kong et al. 2011; Hayward et al. 2017; Millán-Esteban et al. 2021; Broit et al. 2022; Newell et al. 2022; M. Wang et al. 2022; Basurto-Lozada et al. 2024). While the incidence of *TERT* promoter mutations (13%), along with *CCND1* (23%), *FGF4* (15%), and *MDM2* (15%) amplifications, was also comparable to those reported by other groups, *TYRP1* mutations reported elsewhere were not seen in our cohort (Yeh et al. 2019; Broit et al. 2022; Wang et al. 2022). Differences between studies may partially be explained by wide variances associated with smaller cohort sizes, as well as inherent differences in racial and geographic groups.

Differential expression from WTS revealed that keratinization and epidermis formation pathways were enriched in ALM compared to CM. Two studies—including CM only—independently described a "keratin" cluster associated with overexpression of keratin-related genes including *KRT6*, *KRT14*, and *KRT16* seen here, distinct from an "immune" cluster enriched in lymphocyte infiltration, and characterized by worse survival (Akbani et al. 2015; Netanely et al. 2021). More recently, these findings were reproduced in acral melanoma via unsupervised gene expression clustering of 47 primary tumor samples from Mexican patients (Basurto-Lozada et al. 2024). Our findings, when considered alongside these studies, suggest that distinct keratin-related versus immune-related transcriptional clusters exist both within and across ALM and CM, though there may also exist other explanations for differential keratin expression. Spatially resolved transcriptomic analysis of acral melanomas demonstrated close spatial overlap between malignant melanocytes and keratinocytes in certain regions of interest within primary tumors (Choi et al. 2024). Previous "keratin" clusters in CM were enriched in primary rather than metastatic lesions, and 82% of ALM samples in our cohort were from primary sites (compared to 37% for CM), raising the possibility that the highlighted keratin signature may have simply resulted from keratinocyte contamination from thick-epidermis skin typically found at the palms, soles, fingers, toes, and nailbeds (Akbani et al. 2015; Netanely et al. 2021). In future studies, comparison of transcriptomic data from non-cutaneous metastases may help to differentiate between whether these keratin signatures represent true differences rather than sample contamination.

Our exploration of the ALM tumor immune microenvironment corroborates existing data demonstrating lower immunogenicity compared to CM, as evidenced by lower IFNγ pathway activity, TIS, TMB, and neoantigen load irrespective of HLA binding affinity. Diminished immune activity in ALM may, at least in part, account for the limited response to immune checkpoint blockade compared to CM (Namikawa et al. 2018; Tang et al. 2020; Bhave et al. 2022; van Not et al. 2022; Mori et al. 2023; Zheng and Jenkins 2025; McGillivray et al. 2025). Our findings are in agreement with those from other ALM cohorts, which showed a median TMB of 2.1 mutations/Mb for $n = 87$ ALM cases (compared to 36.2 mutations/Mb for $n = 303$ CM), as well as lower tumor-infiltrating lymphocyte levels, particularly of the CD8+ subtype (Castaneda et al. 2017; Nakamura et al. 2020; Li et al. 2022; Newell et al. 2022; Chiu et al. 2024). PD-L1 expression is geographically colocalized with CD8+ tumor-infiltrating lymphocytes in superficial spreading melanoma (SSM) and in melanomas associated with chronically sun-damaged skin, reflecting endogenous anti-tumor immunity and correlating with good response rates to anti-PD1 monotherapy (Taube et al. 2013; Kaunitz et al. 2017). In contrast, only 13% of our stained ALM samples demonstrated PD-L1 positivity by IHC, implying the presence of distinct immune signaling pathways. Decreased CD8, PD-1, and PD-L1 expression in acral melanoma has elsewhere been shown to correlate with decreased signaling via IL-2, IL-2R, CD274, and IFNg, all of which are related to T cell activity (Chiu et al. 2024).

ALM biopsies in our study were also enriched 1.6-fold for myeloid dendritic cells, and it is unclear what this represents when viewed in the context of ALM's thus far poor response to immune checkpoint blockade. It is possible that, without concomitant robust CD8+ T cell infiltration, there are limited cells for an increased myeloid dendritic cell population to present neoantigens to, or perhaps the presence of an immunosuppressive microenvironment shifts any tumor-associated dendritic cells towards a more tolerogenic phenotype. Single-cell RNA sequencing (scRNA-seq) of patient samples showed a higher proportion of myeloid cells—including macrophages, monocytes, and dendritic cells—in primary acral melanomas compared to metastatic sites (Li et al. 2022). Along these lines, multiplexed IHC of acral and CM-associated immune populations found increased conventional type 1 dendritic cells in earlier rather than more advanced stages (De Leon-Rodríguez et al. 2023). Most of the ALM samples in our cohort were from primary sites, compared to less than 40% for CM, potentially biasing our data towards overestimates of the dendritic cell population in ALM. Future work might involve evaluating dendritic cell subsets within ALM primary tumors compared to matched metastatic samples to shed increased light on intra-tumoral and intra-patient heterogeneity.

The primary hypothesis generated from our study is that HLA-G, a non-classical MHC-I molecule, plays a crucial role in ALM's immune tolerance, at least in part via its role as a ligand for inhibitory NK receptors. HLA-G, initially shown to confer fetal protection from the maternal immune system, is overexpressed by immunohistochemistry in ALM compared to SSM, and has been shown to be an unfavorable prognostic factor in localized colorectal cancer (Carosella et al. 2015; Castillo et al. 2017). While efforts are under way to better understand the role of

HLA-G in other cancers and in the development and testing of HLA-G-targeted therapies (NCT04485013, NCT05672459), transcriptomic analyses in ALM have thus far primarily highlighted other HLA molecules (Xu et al. 2021; Anna et al. 2021; Carosella et al. 2021). For example, acral melanomas were notable for decreased expression of HLA-A, -B, -DMA, -DMB, and -K, but increased expression of HLA-E on tumor cells along with its receptor NKG2A in associated CD8 TILs (Chiu et al. 2024; Minowa et al. 2024). Using spatial transcriptomic analysis, Choi et al. demonstrated decreased expression of many HLA genes, including HLA-G, though this was in amelanotic compared to pigmented acral melanomas, rather than a comparison between acral versus non-acral subtypes (Choi et al. 2024).

Limitations of our study included the relative paucity of ALM compared to CM samples. While patient demographic data was better accounted for, with 17% of patients classified as unknown race or ethnicity, over half (61% of ALM and 56% of CM) of our cohort had unknown stage at diagnosis, which may have contributed to lack of overall survival differences between melanoma subtypes in this study. Future studies would ideally further distinguish between subtypes of non-acral CM (including superficial spreading, nodular, and lentigo maligna melanomas), along with mucosal and ocular melanomas, as they are known to be associated with different pathways of oncogenesis, clinical behaviors, and outcomes. Additionally, patients in our cohort had variable amounts of molecular information available, in part due to changes in commercial profiling techniques over time. Further, our reliance on post hoc computational inference of bulk transcriptional data restricted our ability to differentiate between tumor versus microenvironment expression of transcripts of interest. ScRNA-seq, as has been utilized in other studies of ALM at times coupled with single-cell T cell receptor sequencing, is preferred for capturing additional detail related to immune cell infiltrates (Li et al. 2022; Zhang et al. 2022; Chiu et al. 2024; Choi et al. 2024; Minowa et al. 2024).

Taken together, our data demonstrate that ALM possesses distinct immunologic features, including upregulation of HLA-G, as well as lower IFNγ and T-cell inflammatory activity compared to CM. Additional research in ALM is warranted to clarify the potential of HLA-G as an immunotherapeutic target, dissect whether the highlighted aberrant keratin-related and PI3K/AKT signaling networks converge to affect the tumor microenvironment, and determine the utility of targeting such pathways in combination with checkpoint blockade. Considering ALM's less immunogenic tumor microenvironment, increased efforts to discover non-immune therapeutic targets for ALM may also be worthwhile.

Author Contributions

S.J.W. contributed to conceptualization, formal analysis, investigation, methodology, visualization, and writing (original draft). J.X. contributed to project administration, data curation, formal analysis, investigation, software, and methodology. F.A., K.A.M., G.T.G., L.F.H., J.L., and J.M. contributed to conceptualization, methodology, resources, investigation, and supervision. K.M.B., B.K.D., G.H.K., and F.I. contributed to resources, investigation, and supervision. P.A.P. and C.D.R. contributed to conceptualization, methodology, and supervision. G.K.I. contributed to conceptualization, formal analysis, resources, investigation, methodology, supervision, and writing – original draft. All authors contributed to writing – review and editing.

Disclosure

Joanne Xiu and Farah Abdulla are employed by Caris Life Sciences. Katherine M. Butcher has received travel support from Replimune and Iovance. Fumito Ito has consulted for Otsuka Pharmaceutical Factory Inc. Gino K. In has consulted for Pfizer and participated on advisory boards for BMS, Merck, Regeneron, Sanofi, Replimune, Novartis, and Pfizer. Institutional research support funds have been received from Regeneron, Idera, Replimune, Xencor, InstilBio, Pfizer, Obsidian, Bicara, Georgiamune Immunocore, and Checkmate Pharmaceuticals.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available for replication and verification purposes from the corresponding author on reasonable request. The de-identified DNA and RNA sequencing data are owned by Caris Life Sciences and cannot be publicly shared due to the data usage agreement signed by Dr. Heinz-Josef Lenz at the University of Southern California Keck School of Medicine. Qualified researchers can apply for access to these data by contacting Joanne Xiu, PhD, at jxiu@carisls.com, submitting a brief proposal, and signing a data usage agreement.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.