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Expression of immune checkpoint molecules TIGIT and TIM-3 by tumor-infiltrating lymphocytes predicts poor outcome in sinonasal mucosal melanoma

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ABSTRACT

Background: Sinonasal mucosal melanoma (SNMM) is a rare but aggressive tumor with a poor prognosis. The co-inhibitory receptors T cell immunoglobulin and mucinodomain containing-3 (TIM-3), lymphocyte activation gene-3 (LAG-3) and T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) are promising new targets in anti-cancer immunotherapy. The expression profiles of these immune checkpoint molecules (ICMs) and potential prognostic implications have not been characterized in SNMM yet.
Methods: Immunohistochemical staining for TIGIT, LAG-3 and TIM-3 was performed on tumor tissue samples from 27 patients with primary SNMM. Associations between ICM expression and demographic parameters, AJCC tumor stage, overall survival, and recurrence-free survival were retrospectively analyzed.
Results: SNMM patients with low numbers of TIGIT+ and TIM-3+ tumor infiltrating lymphocytes (TILs) in the primary tumor survived significantly longer than patients with a high degree of TIGIT+ and TIM-3+ TILs. High infiltration with TIM-3+ or TIGIT+ lymphocytes was associated with the higher T4 stage and decreased 5-year survival.
Conclusion: We identified high densities of TIM-3+ and TIGIT+ TILs as strong negative prognostic biomarkers in SNMM. This suggests that TIM-3 and TIGIT contribute to immunosuppression in SNMM and provides a rationale for novel treatment strategies based on this next generation of immune checkpoint inhibitors. Prospective studies with larger case numbers are warranted to confirm our findings and their implications for immunotherapy.

1. Introduction

Sinonasal mucosal melanoma (SNMM) is a rare but highly aggressive tumor entity with a poor prognosis [1]. Accounting for only 1.4 % of all melanomas, SNMM remains poorly understood in terms of risk factors and pathogenesis and evidence regarding treatment strategies is primarily derived from small retrospective case series [2,3]. The current standard of care involves a multimodal approach, comprising radical local tumor resection followed by adjuvant radiotherapy [4]. As diagnosis is usually delayed due to nonspecific symptoms, local infiltration into neighboring anatomical structures such as the large neck vessels, the orbita or the brain is often already present at the time of diagnosis [3]. In addition, approximately 20 % of patients initially present with lymph node metastases, and 10 % suffer from distant metastases [5]. The advanced local and systemic spread of the tumor frequently impedes curative resection, necessitating drug-based systemic therapy [2,6]. While cytotoxic chemotherapy previously constituted the mainstay of medical treatment for advanced SNMM, immune checkpoint inhibitors (ICIs) are now increasingly being used and often serve as the treatment of choice in both adjuvant and metastatic settings [3].

ICI therapy, specifically with inhibitors of programmed cell death protein 1 (PD-1), its ligand (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), is well established in cutaneous malignant melanoma (CM), which is the most common type of melanoma, and has significantly improved the prognosis of CM patients [7]. However, SNMMs are genetically and clinically distinct from CM and limited data exists regarding the effectiveness of ICIs in SNMM [3,8,9]. Pooled analyses of clinical studies indicate notably lower response rates to current

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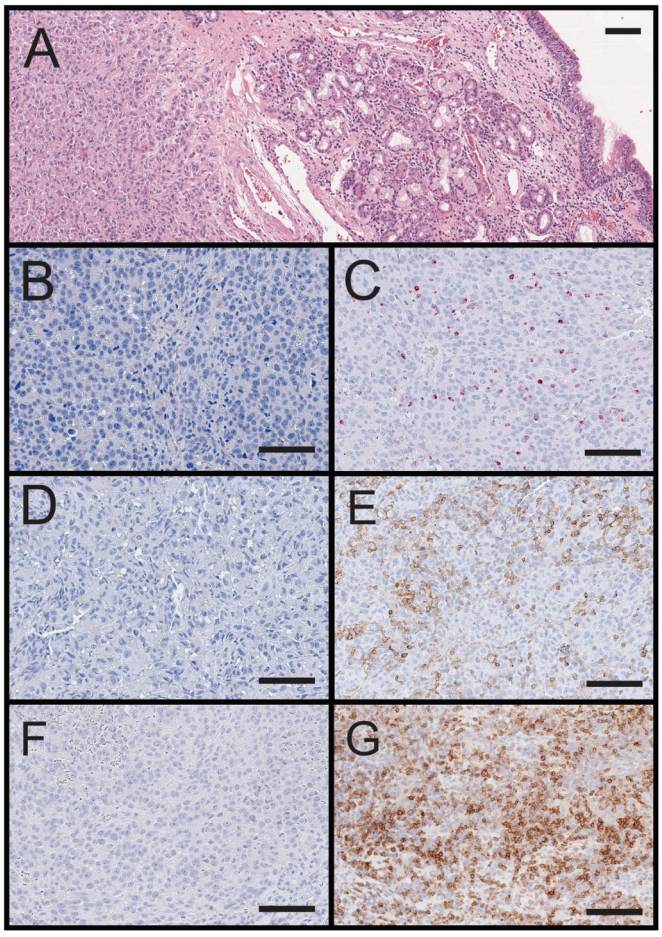

Fig. 1. Immune checkpoint molecule expression in sinonasal mucosal melanoma (SNMM). A Exemplary histomorphology of SNMM (H&E staining; x 10). B-G Representative images displaying infiltration of SNMMs with low (B, D, F) or high (C, E, G) numbers of LAG-3+ (B, C), TIM-3+ (D, E) and TIGIT+ (F, G) tumor infiltrating lymphocytes are shown (x 20; scale bars 200 μm).

anti-CTLA-4 and anti-PD-1 ICIs in SNMM patients compared to CM patients [10–12]. Furthermore, ICIs may be associated with immune-related adverse events, which can affect up to 60 % of SNMM patients [13–15]. There is therefore a need for characterizing other potential immuno-oncological targets in SNMM and identifying patients who may benefit from immunotherapy [8,16].

Recent advances in understanding the tumor microenvironment (TME) and tumor immunology have led to the identification of several new and promising targets for ICI therapy, including T cell immunoglobulin and mucinodomain containing-3 (TIM-3), lymphocyte activation gene-3 (LAG-3) and T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) [17,18]. These immune checkpoint molecules (ICMs) modulate the complex interactions between tumor-infiltrating lymphocytes (TILs) and tumor cells [17]. Increased expression of these ICMs on TILs contributes to suppression of the antitumor immune response and is associated with advanced tumor stages and worse outcomes in numerous malignant tumors, including CM [14,19,20]. Consequently, studies have demonstrated that pharmacological blockade of LAG-3, TIM-3 and TIGIT can enhance the antitumor immune response across various cancer types, resulting in reduced tumor size and an improved prognosis (reviewed in [17,21]). However, the expression patterns and prognostic significance of these newly identified immunoregulatory proteins have not been investigated in SNMM so far. In this study, we analyzed the expression of LAG-3, TIM-3 and TIGIT in SNMM and evaluated their prognostic value.

2. Materials and methods

The study was approved by the medical ethics committee of Ludwig Maximilian University (LMU) Munich and carried out in compliance with the principles outlined in the Declaration of Helsinki.

2.1. Patients

This retrospective study was conducted using a well-characterized cohort of 27 patients who underwent surgery for SNMM at the Department of Otorhinolaryngology, Head and Neck Surgery (LMU Munich) [22]. Exclusion criteria comprised extensive malignant infiltration of critical structures such as the brain, skull base, carotid artery or prevertebral space as well as a history of chemotherapy or immunotherapy prior to surgery. In addition, patients with malignant melanomas at other sites or distant metastases at the time of diagnosis were excluded. Staging was performed according to the TNM/AJCC classification for upper aero-digestive tract mucosal melanomas [23]. Post-surgery, all patients received adjuvant radiotherapy at varying doses. Chemotherapy or immunotherapy was administered inconsistently during the further course of the disease. Patient characteristics, clinicopathological parameters, recurrence-free survival time and overall survival time were documented and analyzed for association with LAG-3+, TIM-3+ and TIGIT+ TILs.

2.2. Immunohistochemical analysis

Immunoreactivity for LAG-3, TIM-3, and TIGIT in TILs was examined

Table 1
Correlation between expression of immune checkpoint molecules and demographic or clinicopathological parameters.

	LAG-3				TIM-3				TIGIT			
	low n = 23	high n = 4	OR high vs. low (95 % CI)	*p*	low n = 13	high n = 14	OR high vs. low (95 % CI)	*p*	low n = 10	high n = 17	OR high vs. low (95 % CI)	*p*
Age at diagnosis (years)				0.45†				0.31†				0.21†
mean	67	73			65	71			63	71		
median	70	82			65	74			63	74		
range	47–89	33–94			47–81	33–94			47–76	33–94		
Sex				0.29‡				0.05‡				0.42‡
male (%)	14 (61)	1 (25)			10 (77)	5 (36)			7 (70)	8 (47)		
female (%)	9 (39)	3 (75)			3 (23)	9 (64)			3 (30)	9 (53)		
Tumor Stage				0.600‡				**0.033**‡*				**0.046**‡*
T3 (%)	11 (48)	3 (75)	Reference		10 (77)	4 (28)	Reference		8 (80)	6 (35)	Reference	
T4 (%)	12 (52)	1 (25)	0.3 (0.03 – 3.4)		3 (23)	10 (71)	8.3 (1.5–47.2)		2 (20)	11 (65)	7.3 (1.2 – 46.2)	

†t test; ‡Fisher's exact test. OR: odds ratio; CI: confidence interval. *p<0.05.

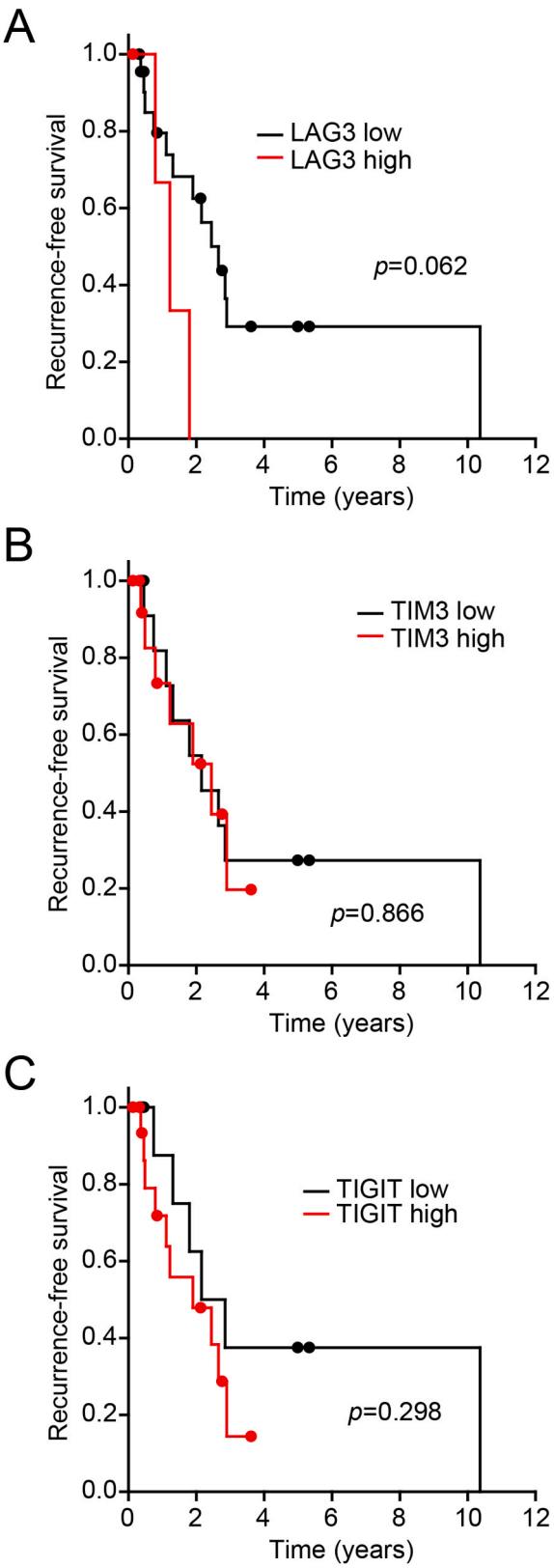

Fig. 2. LAG-3, TIM-3 and TIGIT expression by tumor infiltrating lymphocytes (TILs) does not affect recurrence-free survival in sinonasal mucosal melanoma (SNMM). SNMM patients were stratified according to the degree of infiltration with LAG-3+ (A; low: n = 23; high: n = 4), TIM-3+ (B; low: n = 13; high: n = 14) and TIGIT+ (C; low: n = 10; high: n = 17) TILs and recurrence-free survival was plotted for each group using the Kaplan-Meier method. Differences between groups were tested for statistical significance with the log-rank test.

immunohistochemically on formalin-fixed and paraffin-embedded (FFPE) tumor tissue samples from 27 patients with SNMM. Antigen retrieval was conducted via heat treatment using Target Retrieval Solution (Agilent Technologies, Santa Clara, CA, USA; S1699) for LAG-3 and TIM3 and Novocastra Epitope Retrieval Solution pH 8.0 (Leica Biosystems, Wetzlar, Germany; RE7116) for TIGIT staining. Slides were subsequently incubated with rabbit monoclonal anti-human primary antibodies against LAG-3 (1:600; Abcam, Berlin, Germany; EPR4392 (2)), TIGIT (1:150; Cell Signaling, Danvers, MA, USA; E5Y1W), or TIM-3 (1:80; Cell Signaling; D5D5R) at room temperature for 60 minutes. Bound antibodies were detected with ZytoChem Plus AP Anti-Rabbit Polymer Kit (Zytomed Systems, Berlin, Germany; ZUC-031; LAG-3) or ImmPRESS Anti-Rabbit IgG Polymer Kit (Vector Laboratories, Newark, CA, USA; MP-7401; TIGIT and TIM-3). LAG-3 staining results were visualized using Permanent AP Red (Zytomed, ZUC001) as a chromogen, while DAB+ (Agilent Technologies; K3468) was used as the chromogen for TIGIT and TIM-3 staining. Sections were finally counterstained with hematoxylin (Vector Laboratories; H-3401), dehydrated, and mounted. Positive controls for each biomarker were derived from tonsil tissue and included in every staining run.

2.3. Semiquantitative analysis of co-inhibitory receptor expression

The presence of LAG-3+, TIM-3+, and TIGIT+ TILs was examined by immunohistochemistry and quantified using the lymphocyte score (LScore) as previously described [16,24]. In brief, the LScore for each case was determined by adding the lymphocyte distribution score and the lymphocyte density score. The lymphocyte distribution score ranged from 0 to 3, indicating the extent of lymphocytes present within the tumor tissue: 0 = absent, 1 = < 25 %, 2 = 25–50 %, and 3 = > 50 %. Lymphocyte density, ranging from 0 to 3, was defined as follows: 0 = absent, 1 = mild, 2 = moderate, and 3 = high. LScores ranged from 0 to 6, where scores from 0 to 2 were classified as low while scores from 3 to 6 were considered high. TILs within areas of necrosis were excluded from the analysis. Representative images demonstrating various staining patterns are shown in Fig. 1.

2.4. Statistical analyses

Primary endpoints were overall survival and recurrence-free survival. Overall survival was defined as the time from primary surgery to the occurrence of death. Patients who were still alive at the end of follow-up were censored. Recurrence-free survival refers to the time from primary surgery to the onset of relapse, characterized by either locoregional recurrence or distant metastasis as determined by radiological imaging diagnostics. Patients were censored for recurrence-free survival if there was no evidence of metastasis or relapse at the end of follow-up or at the time of death. Survival curves were generated using the Kaplan-Meier method and compared by the log-rank test. Associations between LAG-3+, TIM-3+, and TIGIT+ TIL infiltration and demographic, clinicopathological, or survival parameters were assessed using Student's t-test for continuous variables and Fisher's exact test for categorical variables, and odds rations (OR) and 95 % confidence intervals (CI) were computed. P values <0.05 were considered statistically significant. All analyses were done with SigmaPlot 12.5 software (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Demographics and clinical characteristics

Patient and tumor characteristics have been published previously [22]. Briefly, tumors were classified as either stage T3, T4a or T4b. The mean age of all patients at the time of surgery was 68 (range: 33–94) years. All patients underwent surgery with curative intent followed by adjuvant radiotherapy. Chemotherapy or immunotherapy was

Table 2
Correlation between expression of immune checkpoint molecules and outcome.

	Checkpoint molecule expression						vs. low expression					
	LAG-3		TIM-3		TIGIT		LAG-3 high		TIM-3 high		TIGIT high	
	low n = 23	high n = 4	low n = 13	high n = 14	low n = 10	high n = 17	OR (95 % CI)	*p*	OR (95 % CI)	*p*	OR (95 % CI)	*p*
Recurrence-free Survival								1.00		0.326		0.128
>3 years (%)	4 (17)	0 (0)	3 (23)	1 (7)	3 (30)	1 (6)	Reference		Reference		Reference	
<3 years (%)	19 (83)	4 (100)	10 (77)	13 (93)	7 (70)	16 (94)	+∞ (– - +∞)	100	3.9 (0.4 – 43.4)		6.9 (0.6 – 78.0)	
Overall Survival								1.00		**0.004***		**0.004***
>5 years (%)	8 (35)	1 (25)	8 (62)	1 (1)	7 (70)	2 (12)	Reference		Reference		Reference	
<5 years (%)	15 (65)	3 (75)	5 (38)	13 (99)	3 (30)	15 (88)	1.6 (0.1 – 18.0)		20.8 (2.0–211.8)		17.5 (2.4–129.5)	

OR: odds ratio; CI: confidence interval.
* $p < 0.05$ (Fisher's exact test).

administered to five (18.5 %) patients during the further course of the disease. During follow-up, a total of 25 patients (93 %) died, 16 patients (59.3 %) experienced local recurrence, regional lymph node metastases occurred in 11.1 % and distant metastases were diagnosed in 40.7 %.

3.2. Associations between co-inhibitory receptor expression and demographics and clinicopathological characteristics

Expression levels of LAG-3, TIM-3 and TIGIT in SNMM infiltrating lymphocytes were scored as high or low and tested for associations with demographic and clinicopathological characteristics (Table 1). A strong infiltration with LAG-3+ TILs was observed in 4 tumors (14.8 %), with TIM-3+ TILs in 14 tumors (51.9 %), and with TIGIT+ TILs in 17 tumors (63.0 %), respectively. No significant associations were found between age or gender and the expression patterns of co-inhibitory receptors ($p = 0.05 – 0.45$). While SNMMs are advanced by definition (stage T3 or T4), high infiltration with TIM-3+ and TIGIT+ TILs were more likely among tumors of stage T4 ($p = 0.033$ and $p = 0.046$, respectively). No statistically significant associations were found between T stage and LAG-3+ TILs ($p = 0.600$).

3.3. Co-inhibitory receptor expression is not correlated with recurrence-free survival

The median recurrence-free survival time of all patients was 15.7 months (range: 1.5–124.8). No significant change in recurrence-free survival was found when comparing patients with high and low infiltration of LAG-3+, TIM-3+, and TIGIT+ TILs (Fig. 2). In agreement, there were no statistically significant differences in 3-year recurrence-free survival rates between SNMM patients with high and low infiltration of LAG-3+ (OR = +∞, CI = – - +∞), TIM-3+ (OR = 3.9, CI = 0.4 – 43.4), and TIGIT+ (OR = 6.9, CI = 0.6 – 78.0) TILs (Table 2).

3.4. TIM-3 and TIGIT expressing TILs are negatively associated with overall survival

Median survival of all patients after initial surgery was 33.0 (range: 1.5–164.5) months. There was no significant correlation between LAG-3 expression patterns and overall survival ($p = 0.64$, Fig. 3A). However, low infiltration of TIM-3+ and TIGIT+ lymphocytes into the tumor was significantly correlated with prolonged overall survival (Fig. 3B-C). A high number of TIM-3+ TILs was significantly linked to overall survival of 5 years or less with an odds ratio of 20.8 (CI 95 % 2.0 – 211.8, $p = 0.004$). Similarly, significantly reduced 5-year overall survival rates were observed for tumors with high TIGIT+ lymphocytic infiltration as compared to tumors with a low degree of TIGIT+ TILs (OR = 17.5, CI 95 % 2.4–129.5, $p = 0.004$; Table 2).

4. Discussion

SNMMs represent highly malignant tumors with a very poor prognosis and reported 5-year survival rates of 25 % or less [6]. While some patients quickly undergo fatal tumor progression, others respond well to therapy and maintain a stable disease for many years [6]. Presently, predicting how individual patients will react remains challenging [6,16]. To avoid subjecting patients to overly aggressive treatments and to strike a balance between cancer control and therapy-related side effects, identification of new prognostic biomarkers is imperative [15,16]. Given the rapidly advancing landscape of immunotherapy, exploring corresponding target structures within tumor cells at the protein level has proven advantageous [25]. In our study, we describe the expression patterns of LAG-3, TIM-3, and TIGIT in SNMM and demonstrate that TIM-3 and TIGIT can be used as prognostic markers for survival in SNMM patients.

An effective antitumor immune response is crucial to prevent cancer development and progression [26]. ICMs, expressed by both tumor and immune cells, are important for orchestrating the adequate activation and suppression of immune cell functions [27,28]. Tumor cells often exploit these inhibitory mechanisms to evade immune surveillance [29]. ICIs are used to block those immunosuppressive pathways, thus preventing immune evasion and restoring the host's anti-tumor immune response [30]. The first and still most commonly used ICIs are directed against CTLA-4 and PD-1/PD-L1. While these drugs have been shown to be effective in numerous solid and liquid malignant neoplasms, not all patients benefit from these agents, and therapy resistance is common [25]. Additionally, severe side effects like encephalitis, myocarditis and pneumonitis can pose significant risks to cancer patients [25]. Therefore, considerable efforts are focused on identifying new ICMs to enhance treatment success, minimize toxicity and overcome therapy resistance [31]. Among these emerging ICMs, LAG-3, TIM-3 and TIGIT are considered the most promising targets for next-generation ICI therapy [17,32].

LAG-3 is expressed on the cell membrane of various TIL subtypes [17,33,34]. As a structural homolog of cluster of differentiation (CD) 4, LAG-3 interacts with major histocompatibility complex (MHC) class II proteins on antigen-presenting cells, interfering with the recognition and binding of MHCII-bound tumor antigens by CD4 and the T cell receptor (TCR) [35]. In addition, LAG-3 crosslinking with CD3 can impair T cell proliferation and cytokine secretion [36]. Thus, LAG-3 signaling inhibits sufficient T cell activation and impairs T cell effector functions. Consequently, studies have shown that LAG-3 contributes to immune evasion in various malignant tumor entities and can mediate resistance to ICI therapy even though the exact molecular signaling mechanisms are not completely clear [17,37–39]. LAG-3 expression was described as a negative prognostic factor in multiple cancers. For instance, a recent meta-analysis found significant associations between high LAG-3 expression and worse overall survival in patients with carcinomas of

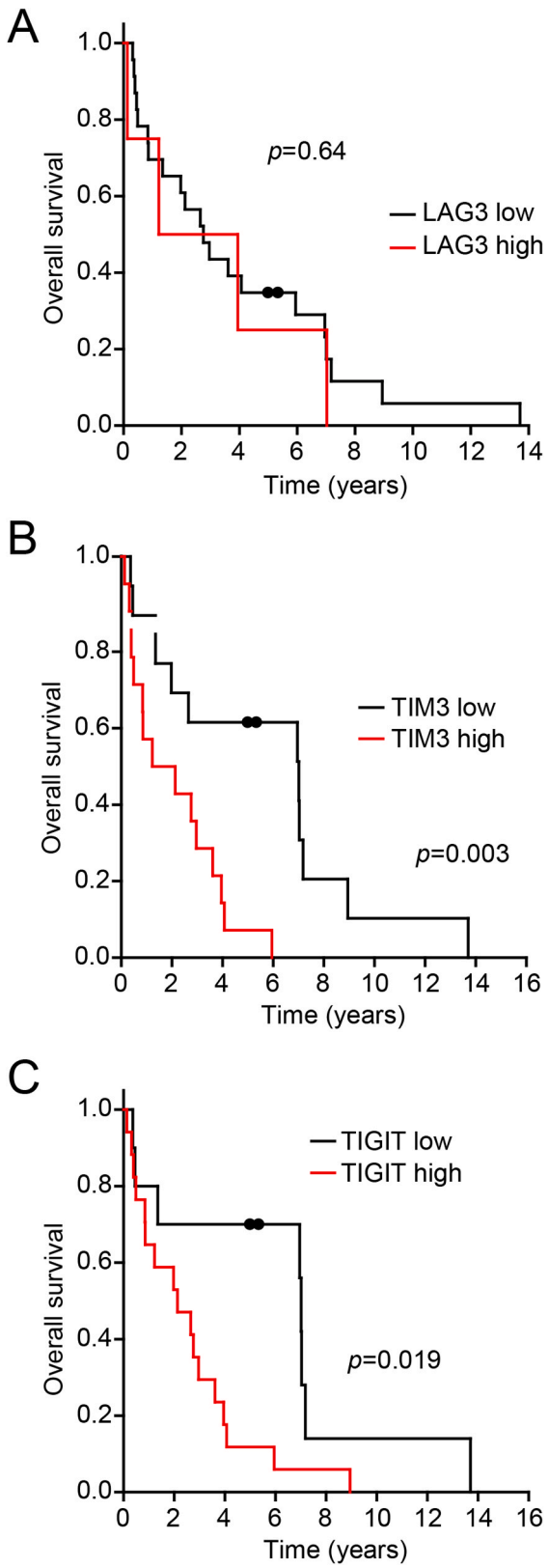

Fig. 3. Infiltration of sinonasal mucosal melanoma (SNMM) with tumor infiltrating lymphocytes (TILs) expressing TIM-3 or TIGIT is associated with reduced overall survival. SNMM patients were stratified according to the degree of infiltration with LAG-3+ (A; low: n = 23; high: n = 4), TIM-3+ (B; low: n = 13; high: n = 14) and TIGIT+ (C; low: n = 10; high: n = 17) TILs and overall survival was plotted for each group using the Kaplan-Meier method. Differences between groups were tested for statistical significance with the log-rank test.

the bladder, kidney, cervix, esophagus, liver and pancreas [40]. High LAG-3 expression was also associated with an impaired prognosis in patients with CM [41]. In 2022, the FDA approved a combination therapy of PD-L1 and LAG-3 inhibitors for the treatment of advanced CM after this therapy was shown to significantly improve progression-free survival [42]. First case reports have also reported promising results of an anti-PD-L1/anti-LAG-3 therapy in SNMM [3,43]. In our patient cohort, the percentage of SNMMs with a high LAG-3+ score was too small to draw statistically meaningful conclusions. Further studies are needed to determine the prognostic value of LAG-3 and its potential as a novel ICI target in SNMM.

The co-inhibitory receptor TIM-3 is expressed by T cells, NK cells, mast cells, B cells and macrophages [17,44]. Immunoregulatory functions mediated via TIM-3 promote immune tolerance in the healthy organism and prevent autoimmunity [45]. In cancer, upregulation of TIM-3 has been implicated in T cell exhaustion and an insufficient antitumor immune response [46,47]. Accordingly, studies have shown that strong TIM-3 expression is associated with a poorer prognosis in thyroid, lung, colon and gastric cancer [48–51]. In CM, TIM-3 expression correlated with poor clinical outcome and TIM-3 blockade led to restored cytotoxicity of antitumor NK cells [52]. In our study, we found that SNMMs frequently exhibit strong TIM-3+ TIL infiltrates. Consistent with the results from other tumor entities, strong TIM-3 expression was associated with a higher tumor stage and impaired overall survival. These data suggest that TIM-3 can be used to predict outcome in SNMM and may be a promising target for novel immunotherapies in SNMM patients [18].

TIGIT, a co-inhibitory receptor of the immunoglobulin superfamily, is expressed by T cells and NK cells and found at high levels in the TME of various malignant tumors [53,54]. Upon ligand binding, TIGIT exerts inhibitory effects on both innate and acquired immune responses by preventing T cell activation and inhibiting NK cell functions [55,56]. An increase in TIGIT+ TILs was observed in several malignancies, and high TIGIT+ TILs are associated with worse prognosis in CM, non-small cell lung cancer (NSCLC) and follicular lymphoma [57–59]. Currently, clinical studies are underway to investigate the effects of TIGIT blockade on patient outcomes in various types of cancer. While preliminary results had demonstrated significantly improved overall response rates and survival times with anti-TIGIT therapy [17,60], other studies did not find therapeutic benefits [17]. The reasons for these discrepancies in treatment success across different tumor types remain unclear [17,38]. Here, we report dense TIGIT+ TILs in 63 % of the analyzed SNMM cohort. Consistent with findings in other tumor types, high TIGIT expression in TILs correlated with reduced overall survival and an advanced tumor stage suggesting that TIGIT inhibitors may be promising therapeutic candidates in SNMM.

A limitation of our study is the comparatively small number of cases of this rare disease, which prevented multivariate analysis of the data. Nevertheless, our findings contribute to a more comprehensive characterization of the next generation of immune checkpoints in SNMM. We identified TIM-3+ and TIGIT+ TILs as strong negative prognostic indicators of survival that could guide personalized treatment approaches. In addition, our results suggest that TIM-3 and TIGIT are involved in tumor-mediated immunosuppression in SNMM and are promising targets of novel ICI therapies. Further multicenter studies are warranted to confirm our findings.

5. Conclusions

Our results indicate that high densities of TIM-3+ and TIGIT+ TILs are strong prognostic factors for impaired survival in SNMM and act as critical immune checkpoints in tumor-mediated immunosuppression. TIM-3 and TIGIT could serve as prospective targets for next-generation ICI therapies in SNMM, potentially helping to reduce side effects and overcome therapy resistance in this rare malignancy.

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CRediT authorship contribution statement

Georg J. Ledderose: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Supervision, Writing – review & editing. **Carola Ledderose:** Data curation, Formal analysis, Investigation, Resources, Validation, Writing – review & editing. **Stephan Ledderose:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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References

[1] A. Ganti, et al., Treatment modalities in sinonasal mucosal melanoma: a national cancer database analysis, Laryngoscope 130 (2) (2020) 275–282.
[2] R.J. Sohal, et al., Mucosal melanoma: a rare entity and review of the literature, Cureus 12 (7) (2020) e9483.
[3] A. Scherzad, et al., Multimodal treatment and immune checkpoint inhibition in sinonasal mucosal melanoma: real-world data of a retrospective, single-center study, Eur. Arch. Otorhinolaryngol. 280 (9) (2023) 4215–4223.
[4] D.G. Pfister, et al., Head and neck cancers, version 2.2020, NCCN clinical practice guidelines in oncology, J. Natl. Compr. Canc. Netw. 18 (7) (2020) 873–898.
[5] B. Lian, et al., The natural history and patterns of metastases from mucosal melanoma: an analysis of 706 prospectively-followed patients, Ann. Oncol. 28 (4) (2017) 868–873.
[6] V.J. Lund, Sinonasal malignant melanoma, Adv. Otorhinolaryngol. 84 (2020) 185–196.
[7] G.V. Long, et al., Cutaneous melanoma, Lancet 402 (10400) (2023) 485–502.
[8] A. Maurer, et al., Sinonasal mucosal melanoma treatment response assessment to immune checkpoint inhibitors using hybrid positron emission tomography imaging, Sci. Rep. 13 (1) (2023) 18847.
[9] S.J. Furney, et al., Genome sequencing of mucosal melanomas reveals that they are driven by distinct mechanisms from cutaneous melanoma, J. Pathol. 230 (3) (2013) 261–269.
[10] S.P. D'Angelo, et al., Efficacy and safety of nivolumab alone or in combination with ipilimumab in patients with mucosal melanoma: a pooled analysis, J. Clin. Oncol. 35 (2) (2017) 226–235.
[11] O. Hamid, et al., Antitumour activity of pembrolizumab in advanced mucosal melanoma: a post-hoc analysis of KEYNOTE-001, 002, 006, Br. J. Cancer 119 (6) (2018) 670–674.
[12] A.N. Shoushtari, et al., The efficacy of anti-PD-1 agents in acral and mucosal melanoma, Cancer 122 (21) (2016) 3354–3362.
[13] T.F. Nishijima, et al., Safety and tolerability of PD-1/PD-L1 inhibitors compared with chemotherapy in patients with advanced cancer: a meta-analysis, Oncologist 22 (4) (2017) 470–479.
[14] F.Y. Kreidieh, H.A. Tawbi, The introduction of LAG-3 checkpoint blockade in melanoma: immunotherapy landscape beyond PD-1 and CTLA-4 inhibition, Ther. Adv. Med. Oncol. 15 (2023), p. 17588359231186027.
[15] P. Teterycz, et al., Multimodal treatment of advanced mucosal melanoma in the era of modern immunotherapy, Cancers (Basel) 12 (11) (2020).
[16] S. Ledderose, et al., Characterization of the tumor-infiltrating lymphocyte landscape in sinonasal mucosal melanoma, Pathol. Res. Pract. 241 (2023) 154289.
[17] N. Joller, A.C. Anderson, V.K. Kuchroo, LAG-3, TIM-3, and TIGIT: distinct functions in immune regulation, Immunity 57 (2) (2024) 206–222.
[18] N. Sauer, et al., TIM-3 as a promising target for cancer immunotherapy in a wide range of tumors, Cancer Immunol. Immunother. 72 (11) (2023) 3405–3425.
[19] W. Tang, et al., TIGIT, a novel immune checkpoint therapy for melanoma, Cell Death Dis. 14 (7) (2023) 466.
[20] G. Cazzato, et al., T cell immunoglobulin and mucin domain 3 (TIM-3) in cutaneous melanoma: a narrative review, Cancers (Basel) 15 (6) (2023).
[21] L. Cai, et al., Targeting LAG-3, TIM-3, and TIGIT for cancer immunotherapy, J. Hematol. Oncol. 16 (1) (2023) 101.
[22] S. Ledderose, et al., Prognostic value of tumor-infiltrating lymphocytes in sinonasal mucosal melanoma, Laryngoscope 132 (7) (2022) 1334–1339.
[23] M.B. Amin, E.S, F. Greene, et al., AJCC Cancer Staging Manual, Springer International Publishing, 2016.
[24] C.K. Park, S.K. Kim, Clinicopathological significance of intratumoral and peritumoral lymphocytes and lymphocyte score based on the histologic subtypes of cutaneous melanoma, Oncotarget 8 (9) (2017) 14759–14769.
[25] C. Pilard, et al., Cancer immunotherapy: it's time to better predict patients' response, Br. J. Cancer 125 (7) (2021) 927–938.
[26] P.G. Coulie, et al., Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy, Nat. Rev. Cancer 14 (2) (2014) 135–146.
[27] W.H. Fridman, et al., The immune contexture in cancer prognosis and treatment, Nat. Rev. Clin. Oncol. 14 (12) (2017) 717–734.
[28] J.J. Havel, D. Chowell, T.A. Chan, The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy, Nat. Rev. Cancer 19 (3) (2019) 133–150.
[29] S. Bagchi, R. Yuan, E.G. Engleman, Immune checkpoint inhibitors for the treatment of cancer: clinical impact and mechanisms of response and resistance, Annu. Rev. Pathol. 16 (2021) 223–249.
[30] R.D. Paucek, D. Baltimore, G. Li, The cellular immunotherapy revolution: arming the immune system for precision therapy, Trends Immunol. 40 (4) (2019) 292–309.
[31] L. Mazzarella, et al., The evolving landscape of 'next-generation' immune checkpoint inhibitors: a review, Eur. J. Cancer 117 (2019) 14–31.
[32] S. Jin, et al., Immune Co-inhibitory receptors CTLA-4, PD-1, TIGIT, LAG-3, and TIM-3 in upper tract urothelial carcinomas: a large cohort study, J. Immunother. 46 (4) (2023) 154–159.
[33] V. Aggarwal, C.J. Workman, D.A.A. Vignali, LAG-3 as the third checkpoint inhibitor, Nat. Immunol. 24 (9) (2023) 1415–1422.
[34] C.T. Huang, et al., Role of LAG-3 in regulatory T cells, Immunity 21 (4) (2004) 503–513.
[35] F. Triebel, et al., LAG-3, a novel lymphocyte activation gene closely related to CD4, J. Exp. Med 171 (5) (1990) 1393–1405.
[36] S. Hannier, et al., CD3/TCR complex-associated lymphocyte activation gene-3 molecules inhibit CD3/TCR signaling, J. Immunol. 161 (8) (1998) 4058–4065.
[37] P. Hemon, et al., MHC class II engagement by its ligand LAG-3 (CD223) contributes to melanoma resistance to apoptosis, J. Immunol. 186 (9) (2011) 5173–5183.
[38] Z.Z. Yang, et al., Expression of LAG-3 defines exhaustion of intratumoral PD-1(+) T cells and correlates with poor outcome in follicular lymphoma. Oncotarget 8 (37) (2017) 61425–61439.
[39] Y. He, et al., LAG-3 Protein expression in non-small cell lung cancer and its relationship with PD-1/PD-L1 and tumor-infiltrating lymphocytes, J. Thorac. Oncol. 12 (5) (2017) 814–823.
[40] R. Li, et al., Prognostic significance of Lymphocyte-activation gene 3 (LAG3) in patients with solid tumors: a systematic review, meta-analysis and pan-cancer analysis, Cancer Cell Int. 23 (1) (2023) 306.
[41] Y.J. Kim, et al., Correlation between tumor-associated macrophage and immune checkpoint molecule expression and its prognostic significance in cutaneous melanoma, J. Clin. Med. 9 (8) (2020).
[42] H.A. Tawbi, et al., Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma, N. Engl. J. Med. 386 (1) (2022) 24–34.
[43] D. Cerbon, et al., Abscopal effect observed in visceral and osseous metastases after liver SBRT in combination with nivolumab and relatlimab for sinonasal mucosal melanoma-a case report, Front. Oncol. 13 (2023) 1143335.
[44] Y. Wolf, A.C. Anderson, V.K. Kuchroo, TIM3 comes of age as an inhibitory receptor, Nat. Rev. Immunol. 20 (3) (2020) 173–185.
[45] L. Monney, et al., Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease, Nature 415 (6871) (2002) 536–541.
[46] J. Fourcade, et al., Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients, J. Exp. Med. 207 (10) (2010) 2175–2186.
[47] K. Sakuishi, et al., Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity, J. Exp. Med. 207 (10) (2010) 2187–2194.
[48] X. Shi, et al., Immune Co-inhibitory receptors PD-1, CTLA-4, TIM-3, LAG-3, and TIGIT in medullary thyroid cancers: a large cohort study, J. Clin. Endocrinol. Metab. 106 (1) (2021) 120–132.
[49] Y. Zhang, et al., Co-expression of TIM-3 and CEACAM1 promotes T cell exhaustion in colorectal cancer patients, Int. Immunopharmacol. 43 (2017) 210–218.
[50] K. Zang, et al., TIM-3 as a prognostic marker and a potential immunotherapy target in human malignant tumors: a meta-analysis and bioinformatics validation, Front. Oncol. 11 (2021) 579351.
[51] J. Jiang, et al., Decreased galectin-9 and increased Tim-3 expression are related to poor prognosis in gastric cancer, PLoS One 8 (12) (2013) e81799.
[52] I.P. da Silva, et al., Reversal of NK-cell exhaustion in advanced melanoma by Tim-3 blockade, Cancer Immunol. Res. 2 (5) (2014) 410–422.
[53] J.M. Chauvin, H.M. Zarour, TIGIT in cancer immunotherapy, J. Immunother. Cancer 8 (2) (2020).
[54] H. Harjunpaa, C. Guillerey, TIGIT as an emerging immune checkpoint, Clin. Exp. Immunol. 200 (2) (2020) 108–119.
[55] N. Stanietsky, et al., The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity, Proc. Natl. Acad. Sci. USA 106 (42) (2009) 17858–17863.
[56] N. Joller, et al., Cutting edge: TIGIT has T cell-intrinsic inhibitory functions, J. Immunol. 186 (3) (2011) 1338–1342.
[57] P. Kaminska, et al., Circulating melanoma cell numbers correlate with TIGIT-positive cytotoxic T cell counts in advanced-stage melanoma patients, Cells 12 (6) (2023).

[58] Y. Sun, et al., Combined evaluation of the expression status of CD155 and TIGIT plays an important role in the prognosis of LUAD (lung adenocarcinoma), Int. Immunopharmacol. 80 (2020) 106198.
[59] Z.Z. Yang, et al., TIGIT expression is associated with T-cell suppression and exhaustion and predicts clinical outcome and Anti-PD-1 response in follicular lymphoma, Clin. Cancer Res. 26 (19) (2020) 5217–5231.
[60] B.C. Cho, et al., Tiragolumab plus atezolizumab versus placebo plus atezolizumab as a first-line treatment for PD-L1-selected non-small-cell lung cancer (CITYSCAPE): primary and follow-up analyses of a randomised, double-blind, phase 2 study, Lancet Oncol. 23 (6) (2022) 781–792.