

LAG-3 as a therapeutic target in melanoma

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Abstract

The advent of immune checkpoint inhibitors (ICIs) marked a paradigm shift in melanoma therapy, particularly in advanced settings. Although anti-PD-1 and anti-CTLA-4 antibodies have already established their efficacy and safety profiles through extensive trials, the recognition of lymphocyte activation gene 3 (LAG-3, CD223) around three decades ago, introduced a promising alternative in improving the antitumor immune response. As a potent reactive checkpoint expressed in various populations of immune cells, including CD4+, CD8+, and regulatory T-cells, LAG-3 has attracted attention across multiple tumor types, including melanoma. Preliminary findings and emerging phase III evidence already highlight the advantage of combining anti-PD-1 with anti-LAG-3 agents over anti-PD-1 monotherapy. This review investigates the current landscape of anti-LAG-3 therapies, encapsulating recent breakthroughs and pivotal trials, including the landmark RELATIVITY-047 study on the combination of nivolumab and relatlimab. In addition to comprehensively evaluating the latest findings, this analysis describes the ongoing phase I–III trials exploring novel agents, shedding light on the most promising data and perspectives. By synthesizing the latest advances in anti-LAG-3 treatment modalities, this article could serve as a timely and insightful resource for clinicians and researchers, navigating the evolving landscape of melanoma immunotherapy.

Keywords: melanoma, immune checkpoint inhibitors, immunotherapy, LAG-3, relatlimab, fiantlimab

Introduction

Immune checkpoint inhibitors (ICIs), now considered a standard of care in advanced melanoma [1], offer a more robust antitumor response and allow T-cell activation, thus significantly improving clinical outcomes [2]. Exploring the significance of cell surface proteins in T-cell responses transformed the treatment of metastatic and unresectable melanoma [3] and encouraged the wide availability of anti-programmed cell death protein 1 (anti-PD-1) and anti-CTLA-4 agents, such as nivolumab, pembrolizumab, and ipilimumab [1]. Although the aforementioned ICIs offer efficient therapeutic options for many, responses remain lacking in approximately half of patients

with metastatic melanoma [4]. With the presence of the lymphocyte activation gene 3 molecule (CD223, LAG-3) in large populations of tumor-infiltrating lymphocytes (TILs) and its unique role in the regulation of cytokine secretion and T-cell exhaustion, many active clinical trials intend to explore the antitumor activity of anti-LAG-3 agents in various tumors, including melanoma [5]. After preliminary research confirmed significant up-regulation of *LAG-3* in melanoma, the RELATIVITY-047 phase II/III trial validated the advantage of the combination of nivolumab and relatlimab over nivolumab alone in patients with advanced melanoma [6]. Promising results led to the inclusion of a relatlimab-nivolumab combination in the latest melanoma treatment guidelines and approvals from the Food and Drug Administration (FDA) and the European Commission [7]. Bispecific antibodies are also emerging to offer synergistic anti-LAG-3 and anti-PD1 antitumor activity [4].

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LAG-3 in immunology

LAG-3 (CD223) is an inhibitory transmembrane receptor involved in tumor-mediated immune suppression [8]. LAG-3 expression is increased on activated CD4+, CD8+, and regulatory T-cells, as well as plasmacytoid dendritic cells and natural killer (NK) cells [5, 9]. The CD223 molecule plays a significant immunoregulatory role, as it uses its CD4-like structure to interact with the major histocompatibility complex II (MHCII), thus negatively regulating antigen-specific T-cell activation [10, 11]. The binding of LAG-3 and MHCII is not universal, and CD223 only recognizes protein-MHCII complexes (pMHCII) [12]. However, LAG-3 can also independently limit the activation of CD4+ and CD8+ T-cells by binding to complexes of T-cell receptors-CD3 (TCR-CD3) and stopping signal transduction [13]. It results in T-cell exhaustion due to constant antigen stimulation as well as weak T-cell responses [14]. LAG-3 exhibits an affinity for a variety of ligands present in various types of cells, including galectin-3 (GAL-3) in vulvar squamous cell carcinoma [15], fibrinogen-like protein 1 (FGL1) in non-small cell lung cancers [16, 17], α -synuclein preformed fibrils (PFF) in Parkinson's disease [18], and cell surface lectin (LSEctin) in melanoma cells [19]. The function of specific CD8+ tumor-infiltrating cells (TILs), including IFN- γ /TNF α production, is debilitated by the co-expression of PD-1 and LAG-3 [20]. Dual immunohistochemistry staining for PD-1 and LAG-3 showed a high prevalence of LAG-3 in tumor-infiltrating lymphocytes (TILs) in various types of solid tumors, with a diverse concomitant presence of PD-1 [21]. Therefore, the anti-LAG-3 and anti-PD1 combination proved efficient in preclinical and clinical studies, overcoming resistance to a single antibody through their synergistic effect [6, 8].

LAG-3 molecule in melanoma

In melanoma, increased LAG-3 expression in T-cells aids in tumor escape tactics [22]. Furthermore, the MHC-II-LAG-3 interaction in melanoma cells inhibits their apoptosis [23]. Anti-LAG-3 antibodies show promise in their potential involvement with other checkpoint inhibitors, reducing suppression of immune responses by Treg cells and facilitating signaling through TCR [22]. Furthermore, LAG-3 T-cell expression influences other outcomes of ICI therapy – it is associated with increased resistance to the combination of ipilimumab and nivolumab in patients with melanoma. The aforementioned resistance to anti-PD1 is correlated with an increased presence of LAG-3+ T-cells in melanoma metastases [24]. Through interaction with its ligand, cell surface lectin (LSEctin), LAG-3 plays a co-regulatory role

in lowering IFN- γ secretion and participates in tumor escape mechanisms by debilitating an immune response in melanoma cells [19, 25]. Elevated expression of LAG-3 and its ligands, MHCII and Gal-3, was found in high-risk uveal melanoma [26]. Another significant factor is the methylation of the LAG-3 promoter, which may be a potential predictive marker in melanoma, as it has proven significant in terms of CD223 expression, IFN- γ secretion, and immune cell populations infiltrating the tumor [25].

Clinical trials with agents targeting LAG-3

The most widespread anti-LAG-3 antibodies, including relatlimab and fianlimab, are now being investigated in clinical trials enrolling patients with melanoma (Tab. 1). Furthermore, the soluble LAG-3 protein efitagimod alpha (IMP321) is also investigated in this population (Tab. 1). With already available, encouraging, and revolutionary results on the efficacy and safety of anti-LAG-3 treatment [27], more results are highly anticipated, as most anti-LAG-3 studies are still ongoing (Tab. 1).

Relatlimab

Relatlimab is an IgG4 humanized monoclonal antibody. It is the first anti-LAG-3 agent approved for melanoma treatment by the FDA in 2022, in combination with nivolumab [28]. Relatlimab reinstates the exhausted T-cells' effector functions [29] and blocks MHCII binding through its strong interactions with LAG-3 receptors [30]. Research involving patients with leukemia who received relatlimab demonstrated leukemic cells' depletion *ex vivo*, potential blockage of anti-apoptotic signaling, and an increase in T-cell and NK-cell levels, with an enhancement of cytokine production [31]. Presented below are the most relevant relatlimab trials and outcomes in melanoma patients.

Relatlimab and nivolumab in the first line of treatment for advanced melanoma

RELATIVITY-047 is a phase II/III trial comparing the efficacy of the combination of nivolumab-relatlimab (NIVO-RELA) with nivolumab monotherapy (NIVO) in the treatment of patients with advanced melanoma, who did not receive prior therapy [27]. Eligibility criteria included unresectable stage III/IV melanoma without active central nervous system (CNS) metastases, no previous treatment, or recurrence at least 6 months after the completion of adjuvant anti-PD-1, anti-CTLA-4, or BRAF/MEK inhibitor therapy. Patients received an infusion of fixed-dose combination (FDC) nivolumab and relatlimab (480/160 mg) or nivolumab (480 mg) only every 4 weeks. Overall, 714 patients (355 in the NIVO-RELA arm and 359 in the NIVO arm) were enrolled [27].

Table 1. Completed and ongoing clinical trials with relatlimab, fianlimab, and eftilagimod alpha in patients with melanoma

Agent	Responsible party/trial name	Population	Phase	Main focus	Trial status	Results
Relatlimab	Bristol-Myers Squibb, RELATIVITY-047 (NCT03470922)	Previously untreated unresectable or metastatic melanoma	II/III	NIVO-RELA vs. NIVO efficacy and safety comparison in previously untreated pts	Active, not recruiting	Published
	Bristol-Myers Squibb, RELATIVITY-020 (NCT01968109)	Progression during anti-PD-1 treatment	I/IIa	NIVO-RELA preliminary efficacy and safety in patients with progression on systemic treatment including anti-PD-1	Active, not recruiting	Published
	John Kirkwood, University of Pittsburgh (NCT03743766)	Stage IIIb–IV, unresectable or metastatic melanoma	II	NIVO-RELA or RELA/NIVO monotherapy, followed by combination treatment — antitumor activity assessment	Recruiting	Published for 22/42 pts
	M. D. Anderson Center (NCT05704647)	Brain metastases with no prior irradiation	II	NIVO-RELA administered for up to 25 cycles – assessment of intracranial response rate and clinical benefit	Recruiting	–
	H. Lee Moffitt Cancer Center and Research Institute (NCT05704933)	Brain metastases resectable 7–10 days post-treatment	Pilot study	NIVO-RELA vs. NIVO-IPI — evaluation of immune cell populations	Recruiting	–
	Jose Lutzky, MD, University of Miami (NCT04552223)	Metastatic uveal melanoma with no prior treatment	II	NIVO-RELA administration for up to 24 months — ORR analysis	Active, not recruiting	–
	M. D. Anderson Center (NCT02519322)	Stage IIIb–IV resectable melanoma	II	Neoadjuvant: NIVO monotherapy vs. NIVO-RELA vs. NIVO-IPI — assessment of pathologic responses	Completed	Posted
	Melanoma Institute Australia, Neo Reni II (NCT05418972)	Stage II high-risk cutaneous melanoma	II	Neoadjuvant FDC NIVO-RELA with/without adjuvant NIVO-RELA	Recruiting	–
Fianlimab	Regeneron Pharmaceuticals (NCT05608291)	Already resected high-risk melanoma	III	Fianlimab-cemiplimab vs. pembrolizumab only — RFS comparison	Recruiting	–
	Regeneron Pharmaceuticals (NCT05352672)	Unresectable stage III/IV melanoma	III	Fianlimab-cemiplimab vs. pembrolizumab only — PFS comparison	Recruiting	–
	Regeneron Pharmaceuticals (NCT03005782)	Malignancies with proven progression and no other treatment options	I	Fianlimab-cemiplimab vs. fianlimab monotherapy — ORR and safety	Active, not recruiting	Posted
Eftilagimod alpha (IMP321)	TACTI-mel (NCT02676869)	Metastatic or unresectable melanoma	I	IMP321 with pembrolizumab — dose escalation	Completed	Posted
	Prof. Serge Leyvraz, Centre Hospitalier Universitaire Vaudois (NCT00324623)	Stage IV melanoma	I	IMP321 vaccine as an adjuvant to PBMCs transfer and chemotherapy	Completed	Posted
	Prof Olivier Michielin, M.D., Ph.D., Centre Hospitalier Universitaire Vaudois (NCT01308294)	Stage II–IV melanoma in HLA-A2 positive pts	I/IIa	Vaccine with IMP321, Montanide, and tumor antigenic peptides — evaluating tumor responses	Completed (terminated with low enrolment)	Posted

FDC — fixed-dose combination; IPI — ipilimumab; NIVO — nivolumab; ORR — objective response rate; PBMCs — peripheral blood mononuclear cells; PD-1 — programmed cell death protein 1; PFS — progression-free survival; pts — patients; RELA — relatlimab; RFS — recurrence-free survival

Progression-free survival (PFS) showed the superiority of combination treatment over nivolumab monotherapy [27, 32]. PFS at 12 months was 47.7% for NIVO-RELA, and 36.0% for nivolumab alone, and the median PFS was 10.2 and 4.6 months, respectively [hazard ratio (HR) = 0.81; 95% confidence interval (CI) 0.67–0.97] [32, 33]. For patients with a 1% or higher expression of programmed death ligand 1 (PD-L1), the median PFS was not much different between the two treatment options with 15.7 months for NIVO-RELA and 14.7 months for nivolumab alone (HR = 0.95; 95% CI 0.68–1.33). On the contrary, for patients with 1% or lower expression, the benefit gained from the addition of relatlimab was much higher, with median PFS of 6.4 months, compared to 2.9 months for NIVO (HR = 0.66; 95% CI 0.51–0.84). In general, the advantage of NIVO-RELA treatment was observed regardless of PD-L1 expression, *BRAF* mutation status, or stage of the disease. Therefore, in patients with unfavorable pre-existing characteristics, there was still a benefit of combination therapy [27]. Secondary endpoints were the overall response rate (ORR) and overall survival (OS), which also demonstrated the higher efficacy of dual therapy [32]. The ORR was 43.7% for the combination of NIVO-RELA and 33.7% for nivolumab. In the NIVO-RELA group, the median OS was not reached [95% CI 31.5–not reached (NR)], and for nivolumab only, it was 33.2 months (HR = 0.82; 95% CI 0.67–1.02) [33]. Table 2 lists the PFS, ORR, and OS values for both groups, showcasing the NIVO-RELA efficacy.

The safety profile differed between combination treatment and monotherapy with treatment-related adverse events (AEs) of any grade observed in 81.1% of NIVO-RELA patients and 69.9% of NIVO patients [27]. During combination treatment, grade 3/4 AEs developed in 21.1% of the patients, and 11% in

the nivolumab monotherapy arm. However, discontinuation caused by AEs was slightly more common in the monotherapy group (17.2% vs. 15.2%) [32]. The most common immune-related adverse effects after combination treatment, in 18% of patients, were thyroiditis or hypothyroidism. Although myocarditis events in the NIVO-RELA group were fully treatable, they were more prevalent in 1.7% of the patients, compared to 0.6% in the nivolumab arm. In general, AEs were more frequent in the NIVO-RELA arm, however, there were no new safety signals [27]. Real-life evidence points to the importance of monitoring cardiac function in NIVO-RELA patients. Despite the low prevalence of myocarditis events (< 1%), they can be severe and potentially more frequent as new phase III/IV data suggest [34].

Peripheral blood analysis from patients treated in the RELATIVITY-047 trial showed that levels of NK cells, Tregs, CD8+ T-cells, and CD4+ T-cells, in particular, were higher after NIVO-RELA treatment than after NIVO monotherapy. The results point to modulation of CD4+ T-cells as a crucial component of RELA activity. Furthermore, lower CD8+ T-cell levels in pre-treatment tumor tissue resulted in a larger PFS benefit for the NIVO-RELA vs. NIVO group. However, for patients with elevated LAG-3+ CD8+ T-cells, an advantage was still visible in the combination therapy group. These data confirm how biomarkers in NIVO-RELA treatment are interconnected, and hence require a detailed definition [35]. Other baseline characteristics were also analyzed to determine which patients benefit the most from the novel combination. For patients with baseline liver metastases, the median PFS was 6.5 months in the NIVO-RELA group and much lower, 3.6 months, in the NIVO group (HR = 0.81; 95% CI 0.53–1.23). The NIVO-RELA advantage was also hugely significant in patients with lung metastases, as the median PFS was 12.2 months vs. 4.8 months for NIVO alone (HR = 0.77; 95% CI 0.57–1.05) [36]. Furthermore, NIVO-RELA decreased the frequency of new CNS metastases diagnosed during therapy (5% vs. 9%) and extended the median time to developing CNS metastases from 6.6 to 11.1 months. These results could support the idea of exploring NIVO-RELA efficacy in CNS metastatic patients specifically [37]. A phase II trial by M.D. Anderson Cancer Center on NIVO-RELA administered to patients with melanoma and active CNS metastases [38, 39] and a pilot study on single-dose NIVO-RELA before brain metastases surgery [40] are ongoing (Tab. 1).

In RELATIVITY-047, the health-related quality of life (HRQoL) was measured using several scales, including the Functional Assessment of Cancer Therapy-Melanoma (FACT-M) [41]. In most cases and in both treatment regimens, HRQoL initially declined, with consequent stabilization. These results,

Table 2. Results of the efficacy of nivolumab and relatlimab from the RELATIVITY-047 trial (adapted from: Tawbi et al. [27, 33], Long et al. [32])

	Nivolumab- relatlimab	Nivolumab
PFS		
Median	10.2 months	4.6 months
12-month	47.7%	36.0%
OS		
Median	NR	33.2 months
12-month	77.0%	71.6%
24-month	61.8%	58.3%
36-month	54.1%	48.4%
48-month	51.5%	42.5%
ORR	43.7%	33.7%

NR — not reached; ORR — overall response rate; OS — overall survival; PFS — progression-free survival

which showed a relatively good tolerability of combination therapy, were true for all subscales used. Interestingly, despite the aforementioned increase in AE in the NIVO-RELA group, the number of patients who declared being affected by treatment-related adverse events (TRAE) was low (< 6%) in both groups. These conclusions could further confirm the use of NIVO-RELA as a viable first-line treatment option [41].

The RELATIVITY-047 results support the consideration of a combination of anti-LAG-3 and anti-PD-1 agents as a new treatment option in patients with advanced melanoma without prior treatment [27, 32, 41]. Furthermore, they confirm the clinical significance of LAG-3 blockage in melanoma and support the advantage of combining two checkpoint inhibitors over monotherapy [27, 32]. Updated in October 2023, the American Society of Oncology (ASCO) guidelines for systemic therapy of melanoma include a combination of nivolumab and relatlimab for patients diagnosed with metastatic or unresectable cutaneous melanoma, regardless of the status of the *BRAF* mutation [7]. Importantly, the European Medicines Agency and the European Commission approved the NIVO-RELA combination only for patients at least 12 years of age with advanced or unresectable melanoma, with PD-L1 expression of < 1%.

The NIVO-RELA combination has not been formally compared with the combination of nivolumab and ipilimumab (NIVO-IPI), which is considered the preferred regimen in first-line settings. Only indirect comparisons have been made based on patient-level data [42–44]. The comparison shows further promise, as there could be an earlier PFS benefit in the inhibition of LAG-3 and PD-1 than in the inhibition of CTLA-4 and PD-1 [42]. However, a comparison of efficacy requires further research, as another network meta-analysis did not show differences in the ORR and PFS rate [44]. In terms of safety, NIVO-RELA may be tolerated better than NIVO-IPI, with fewer treatment-related grade 3/4 AEs [42–44]. Furthermore, the inverse probability of treatment weighting allowed for reliable efficacy data comparisons (OS, ORR, PFS), and demonstrated comparable effectiveness of both investigated combinations. The median PFS was 12 months for NIVO-RELA and 11.2 months for NIVO-IPI (HR = 1.07; 95% CI 0.87–1.31). The ORR values were 48% and 50%, respectively (HR = 0.93; 95% CI 0.74–1.17). However, in some subgroups, including patients with elevated lactate dehydrogenase or a *BRAF* mutation, NIVO-IPI may have had an advantage [43].

Interesting insights into NIVO-RELA activity can be obtained from another phase II trial that compared NIVO-RELA with NIVO monotherapy (NCT03743766) with an early assessment of pathological responses in a biopsy on treatment. In the

lead-in phase, patients received one cycle of NIVO or RELA in monotherapy, or the two combined, and then were all treated with NIVO-RELA infusions [45]. After the initial phase, immune cell populations in blood and tumor samples were investigated for subsequent comparison with the effectiveness of treatment after receiving combined infusions [46]. Although not all patients were enrolled yet, 20 samples were already evaluated [47]. The immune-related pathological response (irPR) was examined after the initial phase, at 4 weeks. At that point, the major pathological response was 25% and the highest after lead-in with NIVO-RELA, providing a valuable, preliminary confirmation of the superiority of combination treatment. Although it did not show a connection with the radiological response, the major pathological response at the 4-week biopsy could serve as an early indicator of the effectiveness of the treatment [47]. Full analysis of all endpoints can be expected after trial completion in 2027 [45, 47].

Relatlimab and nivolumab in the pre-treated patients with melanoma

The RELATIVITY-020 phase I/IIa trial aimed to determine the effectiveness of therapy with the combination of nivolumab and relatlimab in patients already pretreated in advanced settings [48]. Primary endpoints included safety and efficacy based on the objective response rate. The safety of single-agent vial (SAV) versus FDC infusions was compared. Two studied cohorts, D1 and D2, differed in previously received treatment. Cohort D1 included patients with only one prior line of treatment containing anti-PD-1 agents with or without an anti-CTLA4 antibody, while in the D2 cohort, several lines of treatment were allowed, including previous adjuvant and neoadjuvant treatment. Patients in the D1 cohort received a combination of nivolumab and relatlimab, either 240/80 mg every 2 weeks (SAV) or 460/160 mg every 4 weeks (SAV or FDC). The D2 cohort was treated with 460/160 mg of the same combination every 4 weeks (SAV) [48].

The efficacy comparison showed a modest efficacy and response advantage in patients with D1 (only one prior treatment). The median PFS was 2.1 months (95% CI 1.9–3.5) and 3.2 months (95% CI 1.9–3.6) for the D1 and D2 groups, respectively. In D1, the median duration of response (DOR) was not reached (NR; 95% CI 12.9–NR), and in D2, it was 12.8 months (95% CI 6.9–12.9). Furthermore, patients with LAG-3 expression of $\geq 1\%$ were much better responders, with an ORR of 14.1% (95% CI 9.6–19.8), compared to 5.4% (95% CI 1.8–12.2) in the < 1% expression group [48]. However, there was a shared finding in both groups — patients with variable and unfavorable characteristics still responded, including those

with < 1% LAG-3 or PD-1 expression, CNS metastases, elevated lactate dehydrogenase (LDH), and prior BRAF/MEK inhibitor therapy, or anti-CTLA4 ICIs. Therefore, although better responses are observed in patients with higher expression of LAG-3 or PD-1, this factor should not be considered in isolation, and further studies are needed to define predictive markers and the efficacy of NIVO-RELA as a subsequent therapy option. Regarding the safety profile, treatment-related AEs of any grade were observed in 68% of patients, the most common being rash (7.7%), thyroiditis or hypothyroidism (5.4%), and colitis or diarrhea (4.4%). In total, 14.3% of the patients experienced grade 3 or 4 treatment-related AEs, and there were no treatment-related deaths [48].

The most significant takeaway from RELATIVITY-020 is that the combination of nivolumab and relatlimab shows clinically significant immunogenicity in patients who previously progressed in systemic treatment, including anti-PD-(L)1 agents. However, due to the single-arm phase I/II design of this study, further phase III trials with patients who received anti-LAG-3-anti-PD-1 treatment after prior systemic therapy are needed [48]. Moreover, a large proportion of patients do not benefit from NIVO-RELA after progression on previous immunotherapy, and better biomarkers of efficacy are needed. Further trials, including a phase I/IIa study on RELA-IPI combination in patients who progressed to anti-PD1 treatment [49] and a trial with memory-like NK cells with NIVO-RELA after prior treatment, are ongoing [50].

Relatlimab and nivolumab in neoadjuvant treatment

In the breakthrough randomized phase II SWOG S1801 trial with patients with resectable stage III/IV melanoma, the neoadjuvant-adjuvant pembrolizumab treatment regimen had much higher efficacy than the adjuvant-only scheme [2]. Consequently, this treatment strategy is now being explored with the use of anti-LAG-3 antibodies. A randomized trial involving relatlimab enrolled patients with stage IIIb–IV resectable melanoma, without prior ICI therapy. It aimed to compare nivolumab neoadjuvant and adjuvant treatment (arm A), NIVO-IPI neoadjuvant treatment with further adjuvant therapy of nivolumab (arm B), and NIVO-RELA neoadjuvant treatment with further adjuvant therapy of NIVO-RELA (arm C) [51, 52]. In arm C, the 1- and 2-year recurrence-free survival (RFS) rates were 97% and 82%, respectively. There was a high rate of complete pathological responses (pCR) in 57% of patients, with some response in 70% of patients. Arms A and B showed much lower pCR rates — 25% and 45%, respectively. In arm C, for those with any pathologic response, the 1-year and 2-year RFS rates were 100% and 92%,

respectively, and in patients without pathologic response, they were much lower at 92% and 69%, respectively. In post-treatment samples from responders, there was an increase in CD4+ and CD8+ T-cell infiltration and immune cell numbers in general. In NIVO-RELA combination treatment, there were no delays in surgeries caused by immunotherapy toxicity [52] compared to arm B (NIVO-IPI), where 27% of patients had their surgery postponed [53]. Although in the adjuvant phase, 26% of arm-C patients reported grade 3/4 AEs, the authors suggest that it could distort the promising neoadjuvant outcomes (no grade 3/4 AEs), making it an important issue for further trials. In conclusion, despite the small sample size, this trial shows that the neoadjuvant NIVO-RELA combination has a satisfactory safety profile and high clinical activity [52].

Another ongoing, one-arm phase II study Neo ReNi II indicates that the synergism of nivolumab and relatlimab will allow pathological response after 2 doses of combined neoadjuvant treatment [54]. After these infusions, patients will undergo surgery and, optionally, receive adjuvant treatment with the same combination, determined by the pathological response. Besides this factor, the study will mainly focus on recruitment feasibility, with a further analysis of event-free survival (EFS), OS, RFS, safety profile, QoL, biomarkers, and microbiome. Although only 20 patients will be enrolled, Neo ReNi II may offer ample data for further phase III studies of neoadjuvant anti-LAG-3 treatment [54].

Fianlimab

Fianlimab, another anti-LAG-3 IgG4 human monoclonal antibody, exhibits a high affinity to LAG-3 molecules and is hinge-stabilized [55–57]. Similarly to relatlimab, it impedes MHCII binding and thereby facilitates T-cell activation and tumor cell depletion by cytotoxic T-cells. Fianlimab substantially increases cytokine production through tumor-specific T-cells, usually when combined with cemiplimab [57]. Consequently, the majority of clinical trials are dedicated to assessing the combined efficacy of these two agents.

A phase I trial enrolled patients with various advanced malignancies [58], including 98 patients with advanced (unresectable or metastatic) melanoma with or without prior treatment, who were treated with cemiplimab and fianlimab [59]. For all patients with melanoma, the ORR was 61.2%, and the estimated median PFS was 15.3 months (95% CI 9.4–NR). In patients with prior adjuvant anti-PD1 treatment, the ORR was 61.5% and the estimated median PFS was 11.8 months, suggesting a potentially significant activity of cemiplimab-fianlimab after adjuvant anti-PD1 treatment. Immune-related AEs emerged in 65.3% of all patients, similar to anti-PD1 results, except for a significant rate (12.2%)

of adrenal insufficiency of any grade [59]. Furthermore, the study showed that cemiplimab-fianlimab combination has good clinical activity in patients with baseline poor prognosis factors (high LDH levels, M1c staging), compared to other ICIs [60]. These promising results laid the ground for two phase III trials (Tab. 1). An ongoing phase III international study aims to determine the efficacy of cemiplimab-fianlimab treatment versus pembrolizumab monotherapy (anti-PD-1) in patients with high-risk resected melanoma (stage IIc–IV) as an adjuvant strategy [56, 61]. A similar phase III trial will provide a comparison of the same treatment strategies with progression-free survival (PFS) as the primary endpoint in patients with locally advanced or metastatic unresectable melanoma (stage III–IV) [62].

Soluble anti-LAG-3 agent — efitlagimod alpha

Efitlagimod alpha (efti, IMP321, sLag) is a soluble LAG-3 fusion protein [63]. While anti-LAG-3 monoclonal antibodies act on activated T-cells, efti directly alters APC and hence regulates T-cells, dendritic cells, NK cells, and monocytes. Efti significantly promotes dendritic cell activation, stimulates IL-12 and TNF- α , and promotes the activity of tumor-antigen-specific CD8⁺ T-cells. Moreover, *in vitro* measurements of TNF- α and IFN- γ levels proved that the combination of efti with an anti-PD-1 has a powerful advantage over monotherapy [64]. A phase I, dose-escalation study TACTI-MEL explored the combination of efti and pembrolizumab in terms of tolerability and dosage [65] in patients with metastatic or unresectable melanoma [66]. In part A, efti was added to pembrolizumab as 1 mg, 6 mg, or 30 mg injections, and in part B 30 mg of efti was used [64].

In terms of safety, in part A, there were no efti-related serious? Adverse events, and 50% of the patients had no AEs possibly related to treatment, including rash or fatigue (both at 11.1%). In part B, only one serious? Adverse event (anaphylactic reaction) occurred, and in this group, one patient stopped treatment due to AEs. Most (83.3%) patients in the 30-mg cohort reported some AEs possibly related to efti, and the most common were related to injection sites (up to 66%). In terms of efficacy, in part A, there was a 33% immune-related ORR (irORR), a 66% immune-related disease control rate (irDCR), and the median PFS was 4.7 months. For part B, there was a much higher irORR rate at 50%, a similar irDCR at 67%, and mPFS was not reached. Data were pooled from both parts, and the best overall response was plotted, with an exploratory irORR of 54% and an irDCR of 75% [64]. These results must be interpreted with caution since only 24 patients (18 in part A and 6 in part B) were treated in the trial.

In conclusion, the results suggest a potential clinical and immunogenic benefit of efitlagimod alpha pembrolizumab combination and underline its manageable safety profile [64, 65]. The recommended dose was also confirmed in trials with the efiti-avelumab combination [63]. A phase II TACTI-002 trial is ongoing to evaluate the effectiveness and safety profile of the efiti-pembro combination; however, it will not include patients with melanoma [67], and considering the aforementioned phase I results, such a trial for these participants is much anticipated.

Novel anti-LAG-3 molecules

As mentioned above, dual immune checkpoint blockade provides increased T-cell responses [8]. Therefore, bispecific molecules are a promising concept, now being examined in melanoma treatment. These include XmAb[®]22841 (Xmab), which provides simultaneous inhibition of CTLA-4 and LAG-3 [4]. It is investigated in a multicentre phase Ib/II study (NCT05695898) in patients with advanced or metastatic melanoma [4, 68] resistant to available ICI therapies. Patients with advanced solid tumors (including melanoma) refractory to standard ICIs were also enrolled in another phase I XmAb trial, DUET-4 (NCT03849469), where it is administered in monotherapy or with pembrolizumab [69]. RO7247669 is a bispecific antibody targeting PD-1 and LAG-3, and it is now being investigated in a phase I study (NCT04140500) in patients with stage III/IV unresectable melanoma after prior anti-PD-1 treatment [70]. Other anti-LAG-3 molecules include INCAGN02385. Patients with melanoma are enrolled in an ongoing phase I/II trial where it is administered in combination with anti-TIM-3 and anti-PD-1 antibodies (NCT04370704) [71]. Twenty-one patients with previously treated advanced solid tumors were already enrolled (8 with melanoma). The current results indicate that the safety profile of the evaluated combination is similar to that of monotherapy, and the doses of specific agents will be determined after the completion of the trial [71, 72]. LBL-007 is another anti-LAG-3 agent, which stimulates the release of IL-2. Previous studies showcased its adequate safety profile and potential antitumor activity in solid tumors [73]. In a phase I clinical trial, patients with advanced melanoma (metastatic or unresectable) were treated with LBL-007, toripalimab (anti-PD-1), and/or axitinib [74]. Overall response rate values and prevalence of AEs suggest a manageable safety profile and the potential effectiveness of the LBL-007-toripalimab combination in advanced melanoma naïve to previous treatment. The same combination with axitinib added is worth exploring in mucosal melanoma, as it also shows potential efficacy and good tolerability [75]. INCA32459, an

anti-LAG-3-anti-PD-1 bispecific agent, is now being examined in a phase I trial (NCT05577182), to determine its dosage, potential efficacy, and safety profile [76]. Other novel anti-LAG-3 agents include IBI-110 [77–79] and BI 754111 [80–85], both of which showed potentially promising efficacy and a good safety profile in the treatment of advanced solid tumors and now require more trials specifically addressing patients with melanoma.

Summary

LAG-3 is a unique, immunomodulatory, CD4-like molecule, expressed in various populations of immune cells, including tumor-infiltrating lymphocytes. Anti-LAG-3 agents, including relatlimab and fianlimab, offer activation of novel pathways in suppression of tumor evasion mechanisms. Although many trials are still in the enrolment stage, phase III results that confirm the superiority of PD-1-LAG-3 inhibition over anti-PD-1 monotherapy allowed the addition of the NIVO-RELA combination to the treatment guidelines for advanced melanoma (Tab. 1). This discovery of strong synergism has also motivated the introduction of bispecific anti-PD-1-anti-LAG-3 and anti-CTLA-4-anti-LAG-3 antibodies, now examined in phase I/II trials. Furthermore, the soluble LAG-3 protein, efitlagimod alpha, also offers promising results and may be used in vaccines with tumor-antigenic peptides. Challenging subpopulations of patients, including those with uveal melanoma and CNS metastases, are also enrolled in dedicated ongoing trials to further verify treatment effectiveness and confirm greater use of anti-LAG-3 therapies in advanced cases. Importantly, nivolumab and relatlimab combinations have not been directly compared with nivolumab and ipilimumab to clearly guide selection of first-line therapy. Anti-LAG-3 and anti-PD-1 combination could potentially surpass anti-PD-1 and anti-CTLA4 therapy, especially with a favorable toxicity profile, but better biomarkers are needed to distinguish subgroups of best responders.

Article Information and Declarations

Author contributions

A.T.: literature review, preparation of the original version of the manuscript; P.R.: preparation of the final version of the manuscript, supervision of the team; P.S.: preparation of the work concept, literature review, preparation of the final version of the manuscript, supervision of the team.

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Conflict of interest

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Supplementary material

None.

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