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# Anti-LAG-3: A change in the paradigm of advanced melanoma treatment

*Anti-LAG-3: uma mudança de paradigma no tratamento do melanoma avançado*

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**ABSTRACT**

Melanoma is the deadliest skin cancer globally. It arises from the malignant transformation of melanocytes through multiple complex pathways. While cutaneous melanoma, originating from the epidermis, is the most common form, other rarer subtypes also exist. The complex physiopathology of melanoma, characterized by a high mutational load and metastatic potential, when correlated with the hallmarks of cancer, clearly demonstrates its predisposition to immunotherapy.

Immunotherapy has significantly improved clinical outcomes, with the median survival of patients with advanced melanoma increasing from 6 months to 6 years following treatment with immune checkpoint inhibitors. However, the high risk of toxicity, particularly with the current standard combination therapies, and problems related to relapse or resistance to treatment, remain a major concern, with some mechanisms yet to be fully elucidated. Therefore, the discovery of new molecules targeting alternative immune checkpoints (ICs) has become one of the leading approaches.

This review, focusing on lymphocyte-activation gene 3 (LAG-3), characterizes this novel IC at the structural and molecular level, as well as describes its mechanism of action. Moreover, it highlights key clinical trials involving anti-LAG-3 molecules, including the landmark RELATIVITY-047 study, which led to the approval of relatlimab, the first anti-LAG-3 monoclonal antibody, in combination with nivolumab, as a first-line treatment for advanced melanoma. By reporting these promising clinical results, which demonstrate that this novel combination therapy is safer and as effective as other first-line therapies, this review points to a paradigm shift in the treatment of advanced melanoma.

**Keywords:** melanoma, immunotherapy, anti-LAG-3, relatlimab, clinical trials.

**RESUMO**

O melanoma é o cancro de pele mais mortal do mundo. Este resulta da transformação maligna dos melanócitos através de múltiplas vias de sinalização complexas. Embora o melanoma cutâneo, com origem na epiderme, seja a forma mais comum, existem outros subtipos mais raros. A complexa fisiopatologia do melanoma, caracterizada por uma elevada carga mutacional e potencial metastático, quando correlacionada com os *hallmarks d*o cancro, transparece de forma evidente a sua predisposição para o tratamento com imunoterapia. A imunoterapia melhorou significativamente o prognóstico clínico, com a sobrevivência média dos doentes com melanoma avançado a aumentar de 6 meses para 6 anos, após o tratamento com inibidores dos *checkpoints* imunológicos. No entanto, o elevado risco de toxicidade, em particular com as atuais terapias combinadas padrão, e os problemas relacionados com recaídas ou com a resistência ao tratamento, continuam a ser um grande desafio, havendo alguns mecanismos ainda por elucidar. Por conseguinte, a descoberta de novas moléculas que visam *checkpoints* imunológicos alternativos tornou-se uma das principais abordagens. Este artigo, centrado no gene de ativação de linfócitos 3 (LAG-3), caracteriza este inovador *checkpoint* imunológico a nível estrutural e molecular, descrevendo também o seu mecanismo de ação. Além disso, destaca os principais ensaios clínicos que envolvem moléculas anti-LAG-3, incluindo o estudo de referência RELATIVITY-047, que levou à aprovação do relatlimab, o primeiro anticorpo monoclonal anti-LAG-3, em combinação com o nivolumab, como tratamento de primeira linha para o melanoma avançado. Ao destacar estes resultados clínicos promissores, que demonstram que esta nova terapêutica combinada é mais segura e tão eficaz como outras terapêuticas de primeira linha, esta revisão aponta para uma mudança de paradigma no tratamento do melanoma avançado.

**Palavras-chave:** melanoma, imunoterapia, anti-LAG-3, relatlimab, ensaios clínicos.

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## INTRODUCTION

Immunotherapy represents one of the most promising and effective strategies in melanoma treatment[1]. By harnessing the patient's immune system, this approach strengthens the ability to recognize, target and destroy cancer cells, leading to notable improvements in clinical outcomes[2,3].

Advanced melanoma, whether unresectable or metastatic, is among the most immunogenic tumors, owing to its high mutational burden[4]. This characteristic and its significant metastatic potential and resistance to conventional therapies make it a perfect candidate for immunotherapy treatment[5].

Immunotherapy, particularly through immune checkpoint inhibitors (ICIs), has revolutionized the treatment of advanced melanoma, and has shown efficacy in managing a wide range of other tumors as well[6]. The importance of ICIs within the clinical paradigm was further underscored by the awarding of the 2018 Nobel Prize in Physiology or Medicine to James P. Allison and Tasuku Honjo, for their groundbreaking discovery of cancer therapy via inhibition of negative IC regulation[4].

This review aims to provide a comprehensive description of melanoma, by linking its physiopathology with the principal hallmarks of cancer, and to present an overview of ICIs immunotherapy in the treatment of this disease, emphasizing the benefits of the emerging anti-lymphocyte-activation gene 3 (LAG-3) therapy, as evidenced in the latest clinical trials.

To this end, a search was carried out on search engines such as PubMed® and ScienceDirect®, using the following terms, individually or in combination: "melanoma", "classification" "physiopathology", "hallmarks of cancer", "immune checkpoint inhibitors", "anti-CTLA-4", "anti-PD-1", "anti-LAG-3" and "clinical trials".

### Melanoma

*Characterization and Epidemiology*

Melanoma is a malignant tumor characterized by the uncontrolled proliferation of melanocytes, melanin-producing cells that play a vital role in the pathogenesis of this skin cancer[7]. Depending on its location, different types of melanomas can be identified. Cutaneous, mucosal and ocular (uveal) are the most important[8].

Cutaneous melanoma, which originates in the basal layer of the epidermis, is the most common type of melanoma[9]. Studies conducted by the International Agency for Research on Cancer (IARC) predict that the number of new cases of cutaneous melanoma per year will increase by more than 50% between 2020 and 2040. Consequently, it is anticipated that annual new cases will exceed 500.000, with mortality rates rising by more than two-thirds, reaching nearly 100.000 deaths per year[10].

Conversely, mucosal and ocular melanomas are less common but generally have a worse clinical prognosis[11]. Following cutaneous melanoma, ocular melanoma, particularly that which originates in the uvea (ocular choroid, ciliary body and iris) is the second most common type[12,13]. It represents 3-5% of all melanomas and is considered the most common primary intraocular malignancy[14].

Mucosal melanoma arises from melanocytes present in the mucous membranes of the respiratory, gastrointestinal and

genitourinary tracts, and this rare condition accounts for approximately 1% of melanoma cases[15].

Despite melanoma representing only 1% of all skin cancers, it is the deadliest skin cancer worldwide[16]. According to GLOBOCAN, the web database of the IARC, around 330.000 new cases of melanoma were diagnosed globally, and nearly 60.000 people died from the disease in 2022[17]. It is expected that these figures will continue to rise, as the incidence of melanoma is growing rapidly, faster than other cancers, making it a major public health problem[18].

The European scenario mirrors this trend. Based on the latest 2022 data from the European Cancer Information System (ECIS), melanoma is the 6th most frequently occurring cancer in Europe[19]. With incidence rates being higher in men than in women, melanoma is also one of the most common cancers among young adults[20,21].

In Portugal, there are approximately 1.500 new cases of melanoma every year[22]. The age-standardized incidence and mortality rates are 10.2/100000 individuals and 2.5/100000 individuals, respectively, making it the 18$^{th}$ most common and the 20$^{th}$ deadliest cancer[19].

*Physiopathology of Melanoma*

The physiopathology of melanoma is complex and characterized by intricate molecular dynamics. It arises due to multiple genetic changes, with ultraviolet (UV) being considered the primary mutagenic risk factor. Beyond UV exposure, other risk factors, including the number of nevi, genetic susceptibility and family history of melanoma have been linked to melanoma progression[23].

Regarding melanocytic neoplasms, two different types can be considered: a benign one, termed melanocytic nevi, and a malignant one, referred to as melanoma[24].

The progression from benign to malignant is not linear but can be conceptualized into five stages: melanocytic nevi, dysplastic nevi, melanoma *in situ*, invasive melanoma and metastatic melanoma[25]. This progression between stages involves an interaction of both genetic factors and UV-induced damage[5].

*Melanocytic Nevi*

Melanocytic nevi are benign proliferations of melanocytes[25]. Depending on whether they develop at or after birth, melanocytic nevi can be congenital or acquired, respectively[26].

Despite being considered the first stage of melanoma progression, not all melanomas pass through this phase. As a matter of fact, only about 30% of melanomas derive from a pre-existing benign precursor melanocytic nevus[27]. These skin lesions are, therefore, quite stable and are more likely to regress than to progress to melanoma[25]. However, there seems to be a relationship between the number and size of melanocytic nevi and the risk of developing melanoma[28].

Concerning their genesis, these benign neoplasms originate mainly from mutations in the v-Raf murine sarcoma viral oncogene homolog B1 *(BRAF)*[27]. This genetic modification, which occurs in around 50% of melanomas and 70% of benign nevi, constitutively activates the mitogen-activated protein kinase (MAPK) pathway, causing oncogenic proliferation[29,30]. *BRAF$^{V600E}$* mutation is the most common and involves the su-

bstitution of valine (V) for glutamic acid (E) at codon 600[31].

However, $BRAF^{V600E}$ mutation alone, in most cases, is not enough to evolve into malignant stages[32]. This can be explained by a phenomenon called oncogene-induced senescence (OIS). OIS is a powerful tumor-suppressive mechanism that can be enabled after oncogenic-activating genomic modifications, such as $BRAF^{V600E}$ mutations[33]. It leads to the overexpression of certain tumor suppressor proteins, including p16, which control the cell division process[33]. Therefore, melanoma progression from a benign stage to a malignant one does not occur frequently. Nevertheless, some melanocytic nevi can develop into intermediate lesions, known as dysplastic nevi[34].

*Dysplastic Nevi*

Dysplastic nevi are pigmented lesions with benign and malignant histopathological features[25]. These intermediate neoplasms were first reported by Clark and colleagues in 1978. Since then, there has been a debate about its definition and importance in the clinical evaluation of melanoma[35].

Although in most cases they are stable lesions that tend to regress over time, it remains a melanoma risk factor[35]. In addition, dysplastic nevi are known to have a higher mutational load than benign lesions and a lower mutational load than malignant lesions[25]. Indeed, it has different mutations from the previous stage. Besides the mutations involving the MAPK pathway, such as *BRAF* or neuroblastoma RAS viral oncogene homolog *(NRAS)* mutations, genetic modifications in the telomerase reverse transcriptase *(TERT)* promoter and hemizygous alterations of cyclin-dependent kinase inhibitor 2A *(CDKN2A)* gene have also been identified[25].

These genetic alterations are critical for melanoma progression. *NRAS* mutations disrupt the MAPK pathway, leading to sustained cell proliferation[8]. Mutations in the *TERT* promoter induce the transcription of telomerase, allowing cells to avoid senescence[36]. *CDKN2A* encodes the p14 and p16 proteins, both of which play key roles in the regulation of cell division[37].

*Melanoma in situ*

The term "melanoma *in situ*" (MIS) is used to describe a horizontal (radial) growth phase of melanocytes entirely within the epidermis[25]. These lesions are staged as Tis, according to the tumor, node, metastasis (TNM) system developed by the American Joint Committee on Cancer (AJCC). Briefly, this system is based on three key points. The first concerns the primary tumor (T) and assesses its thickness and the presence or absence of ulcerations. The second key point is related to the lymph node (N) involvement and describes how many lymph nodes contain melanoma cells. Lastly, the metastasis (M) category is used to classify melanoma according to whether it has metastasized[38].

Once melanoma has been classified according to the TNM system, it can be further categorized into five stages (0, I, II, III, IV). Therefore, melanoma *in situ* can also be referred to as stage 0 melanoma[39].

As expected, MIS has a higher frequency of mutations compared to intermediate lesions. Several studies using assays

with greater sensitivity have detected a higher frequency of mutations affecting the MAPK signaling pathway, predominantly in the *BRAF*, neurofibromin 1 *(NF1)* and *NRAS* genes[25]. Besides this, mutations in the *TERT* promoter also play an important role in the pathogenesis of MIS[40].

These lesions can persist for years before progressing to invasive melanoma, suggesting that additional mutations are required, along with the ability to evade the immune system[25].

*Invasive Melanoma*

Unlike MIS, which remains confined to the epidermis, melanoma becomes invasive when tumor cells spread to other tissues, such as the dermis or submucosa[25]. Invasive melanoma typically grows in a vertical pattern and most often originates from MIS. Consequently, it has a significant probability of metastasizing[40].

Invasive melanoma is characterized by a high mutational load, with copy-number alterations being prevalent[41]. One of the most critical mutations is the biallelic inactivation of *CDKN2A*, as it marks the transition to invasive melanomas[40].

Moreover, other genetic alterations, such as deletion of phosphatase and tensin homolog *(PTEN)* tumor suppressor gene and *BRAF* mutations are detected. Amplifications of the murine double minute 2 *(MDM2)*, *TERT* and yes-associated protein 1 *(YAP1)* genes, along with mutations in the switch/sucrose non-fermentable (SWI/SNF) chromatin remodeling complex can also be detected and exert a significant impact at this stage[25,41]. Indeed, the *MDM2* gene encodes the *MDM2* protein, an important regulator of p53 activity[42]. *YAP1*, encoded by the *YAP1* gene, controls cell growth and proliferation[43]. Mutations within the SWI/SNF complex, specifically AT-rich interaction domain 2 *(ARID2)* and AT-rich interaction domain 1A *(ARID1A)*, impair its tumor suppressor activity[25].

*Metastatic Melanoma*

According to the National Cancer Institute, a cancer is considered metastatic when cancer cells spread from the site of the original (primary) tumor and form a new tumor in other organs or tissues of the body[44].

Metastasis can be categorized into three types: locoregional, regional, and distant. Locoregional metastasis is classified as satellites or intransit metastasis; regional metastasis involves the lymph nodes; and distant metastasis can affect skin, lung, brain and other sites[41]. Interestingly, the primary sites where melanoma metastasis commonly occurs include lymph nodes, lungs, liver, bones, and brain[34].

Undoubtedly, genetic changes persist, with metastatic melanoma showing a higher rate of mutations and chromosomal aberrations. This increased mutational burden includes a significant incidence of *BRAF* and *NRAS* activating mutations[41].

Additionally, mutations have been identified in the *CDKN2A* and EPH receptor A3 *(EPHA3)* genes, as well as in tumor suppressor genes, including tumor protein p53 *(TP53)* and retinoblastoma, although rare and *PTEN*. Point out that these mutations are strongly related to exposure to UV radiation[41].

*Pathways to Melanoma*

Not all types of melanomas undergo every stage of progression. Some may derive from a melanocytic or dysplastic nevi, while others may arise *de novo*[9]. Moreover, melanoma may manifest as melanoma *in situ* or progress to invasive forms. Its etiology is also diverse, as not all cases are attributed to intense UV exposure, and the genetic mutations underlying melanoma development vary.

Since cutaneous melanoma is the most common form, melanoma is often classified in the literature into four primary types: superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, and acral lentiginous melanoma. However, this classification oversimplifies the complexity of melanoma.

To address this complexity, in 2018, the World Health Organization (WHO) classified nine different evolutionary pathways for the genesis of cutaneous, mucosal and uveal melanomas. This classification was based on UV radiation exposure, expressed by cumulative sun damage (CSD), genetic alterations and clinical and pathological characteristics of the precursor lesions[28,45].

Considering the main etiological factor (CSD), melanomas can be clustered into two groups[23]:

- Melanomas typically associated with CSD (low or high-CSD);
- Melanomas not consistently associated with CSD (no CSD).

Table 1 provides a summary of the differences among the nine pathways. Note that nodular cutaneous melanoma occupies a special place in this classification because all or most of these pathways refer to it.

**Table 1.** Classification of melanoma according to its pathway (Adapted from[8,23])

| UV Exposure | | Pathway | Melanoma Subtype | Common Mutations | |
|---|---|---|---|---|---|
| Melanomas typically associated with CSD | Low-CSD | I | Superficial Spreading Melanoma | *BRAFV600* [b]<br>*CDKN2A* [a]<br>*NRAS* [b] | *TERT* [d]<br>*PTEN* [a]<br>*TP53* [a] |
| | High-CSD | II | Lentigo Maligna Melanoma | *NRAS* [b]<br>*BRAF non-V600E* [b]<br>*KIT* [b]<br>*TERT* [d] | *NF1* [a]<br>*CDKN2A* [a]<br>*PTEN* [a]<br>*TP53* [a]<br>*RAC1* [b] |
| | | III | Desmoplastic Melanoma | *NFI* [a]<br>*NFKBIE* [d] | *NRAS* [b]<br>*PIK3CA* [b] |

**Table 1.** Classification of melanoma according to its pathway (Adapted from[8,23]) (cont.)

| | UV Exposure | Pathway | Melanoma Subtype | Common Mutations | |
|---|---|---|---|---|---|
| Melanomas not consistently associated with CSD | | IV | Spitz Melanoma | HRAS [b]<br>ALK [e]<br>CDKN2A [a] | NTRK1 [e]<br>NTRK3 [e] |
| | | V | Acral Melanoma | KIT [b]<br>NRAS [b]<br>BRAF [b]<br>HRAS [b]<br>KRAS [b] | NTRK3 [e]<br>ALK [e]<br>NF1[a]<br>CDKN2A [a]<br>TERT [f]<br>CCND1 [d] |
| | | VI | Mucosal Melanoma | KIT [b]<br>NRAS [b]<br>KRAS [b]<br>BRAF [b]<br>NF1 [b]<br>CDKN2A [a] | SF3B1 [a]<br>CCND1 [d]<br>CDK4 [d]<br>MDM2 [d] |
| | | VII | Melanomas arising in congenital nevi | NRAS [b] | BRAFV600E [b] |
| | | VIII | Melanomas arising in blue nevi | GNAQ [b]<br>GNA11 [b]<br>CYSLTR2 [b] | BAP1 [a]<br>EIF1AX [c]<br>SF3B1[c] |
| | | IX | Uveal Melanoma | GNA11[b]<br>GNAQ [b]<br>CYSLTR2 [b]<br>PLCB4 [b] | BAP1 [a]<br>EIFAX [c]<br>SF3B1 [c] |

Abbreviations: CSD - Cumulative Sun Damage; UV - Ultraviolet.
[a] Loss-of-Function Mutation; [b] Gain-of-Function Mutation; [c] Change-of-Function Mutation; [d] Amplification; [e] Rearrangement [f] Promoter Mutation.

*Hallmarks of Melanoma*

Hallmarks of cancer refer to a series of essential functional abilities that human cells acquire as they progress from a normal state to a neoplastic growth state. These capabilities are crucial for enabling cells to develop into malignant tumors. As such, it is a key tool for unraveling the vast complexity of cancer phenotypes and genotypes into a provisional set of underlying principles[46].

According to the most recent literature, 14 hallmarks of cancer are now recognized, as illustrated in Figure 1[46]. This chapter explores the most significant hallmarks of cancer in melanoma development.


**Figure 1**. Hallmarks of Cancer (With permission from[46]).

*Genomic Instability and Mutation*

This enabling characteristic plays a significant role in tumor progression, as it is responsible for the phenotypic variation in melanoma[47]. Genomic instability increases the mutation rate, potentially acting as the main trigger for other hallmarks of cancer[47,48]. As previously analyzed, the genesis of several melanoma subtypes of melanoma is positively related to exposure to UV radiation, making it one of the main factors leading to this genomic instability[48].

The mutations resulting from these genetic alterations are diverse but will be included under other cancer hallmarks, considering their molecular characteristics.

*Evading Growth Suppressors*

To grow uncontrollably, tumor cells must evade anti-growth signals. These signals are mediated by tumor suppressor proteins, such as the p53 - the "guardian of the genome" - and the retinoblastoma protein (pRB)[48]. In melanoma, *CDKN2A* is the most inactivated tumor suppressor gene. As mentioned, it encodes the tumor suppressor proteins p16 and p14[37].

The primary role of the p16 protein is to bind and inhibit the cyclin-dependent kinases CDK4 and CDK6. This action prevents these kinases from phosphorylating the pRB, resulting in cell cycle arrest at the G1/S checkpoint and promoting cell senescence. When the *CDK-2NA* gene is mutated, p16 loses its function. As a result, CDK4 and CDK6 are not inhibited and phosphorylate pRB, allowing the cell cycle to progress from G1 to S phase. This mutation, thus, supports melanoma progression, by promoting melanocytes proliferation, delaying senescence and initiating invasion[37,48].

On the other hand, the p14 protein antagonizes MDM2-mediated ubiquitination, thereby preventing p53 degradation[37]. MDM2 negatively regulates p53, by promoting its ubiquitination and subsequent proteasome-mediated degradation[49]. If the *CDK2NA* gene is mutated, p14 protein cannot inhibit the activity of MDM2, leading to p53 inactivation. Consequently, cells evade apoptosis and proliferate indefinitely, sustaining the development of melanoma.

Despite the importance of both proteins as tumor growth suppressors, most *CDKN2A* mutations are p16-dependent, so p16 appears to be more prominent that p14[37].

*Sustaining Proliferative Signaling*

The most important trait of cancer cells is their ability to maintain chronic proliferation. This is achieved by constantly activating signaling pathways that promote cell growth and division[50].

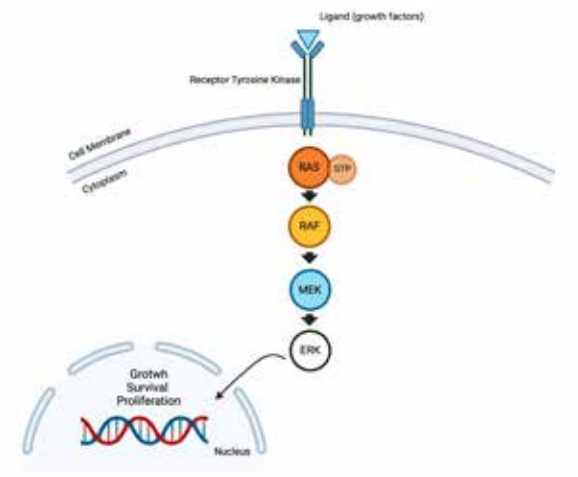
Sustaining proliferative signaling in melanoma is primarily achieved by a *BRAF* mutation. Other driver mutations, such as *NRAS* mutation can also be present (15%-20% of melanomas), making it the second most frequent mutation type. Both mutations interfere with the MAPK pathway[30,51].

*MAPK Pathway*

The MAPK pathway regulates vital cellular functions, namely proliferation, growth, survival and apoptosis[8,30].

This signaling pathway is active under normal conditions but is overactivated in melanoma[8]. It comprises cytoplasmic serine/threonine and tyrosine kinases, including rat sarcoma virus (RAS), rapidly accelerated fibrosarcoma (RAF), mitogen-activated protein kinase (MEK), and extracellular signal-regulated kinase (ERK). RAS is a GTPase with three isoforms encoded by the Harvey rat sarcoma viral oncogene homolog *(HRAS)*, *NRAS*, or Kirsten rat sarcoma viral oncogene homolog *(KRAS)*, while RAF is a protein kinase that includes three isoforms encoded by the A-Raf proto-oncogene, serine/threonine kinase *(ARAF)*, *BRAF* and C-Raf proto-oncogene, serine/threonine kinase *(CRAF)*[30]. MEK is a dual specificity kinase that activates ERK, a serine/threonine protein kinase that transmits mitogen signals and, upon activation, translocates to the nucleus and regulates transcription factors activity and gene expression[52].

Typically, the activation process begins when growth factors bind to tyrosine kinase receptors. This event triggers the activation of Ras family proteins, specifically the monomeric G proteins. Consequently, this initiates a cascade that activates RAF serine/threonine kinases, which in turn activate MEK. Active MEK then dually phosphorylates ERK, enabling its translocation to the nucleus, where it activates transcription factors, thereby intensifying the transcription of genes involved in cell growth, proliferation, survival and migration[8,53]. The MAPK pathway and its activation cascade is represented in Figure 2.




Abbreviations: ERK - Extracellular Signal-Regulated Kinase; GTP - Guanosine Triphosphate; MEK - Mitogen-Activated Protein Kinase; RAF - Rapidly Accelerated Fibrosarcoma; RAS - Rat Sarcoma Virus

Figure 2. MAPK Pathway (Created in BioRender).

*BRAF Mutation*

BRAF mutation, especially $BRAF^{V600E}$, is the most prominent driver of constitutive activation of the MAPK pathway in melanoma.

The primary cause of $BRAF^{V600E}$ mutation remains a topic of debate, notably because it does not exhibit a UV signature mutation. It is characterized by a T to A transversion, atypical of the UV radiation-induced DNA damage, which generally causes C to T or CC to TT transversions at dipyrimidine sites. Several theories have been proposed to

explain the emergence of the *BRAF*$^{V600E}$ mutation. These include its possible role as a minor direct byproduct of UV radiation, errors in DNA polymerases following UV exposure or indirect UV damage mediated by reactive oxygen species (ROS)[25,27].

However, it is known that *BRAF* mutation increases its biochemical activity. Thus, phosphorylation and activation of MEK will occur more frequently, which in turn activates ERK, leading to excessive cell proliferation[8].

*NRAS Mutation*

*NRAS* mutation is the most common mutation in the RAS genes in melanoma but it occurs less frequently than in other solid tumors[8]. Q61R mutation, which results from the substitution of glutamine (Q) for arginine (R) at position 61, is the most frequent NRAS mutation in this skin cancer[54].

Unlike *BRAF* mutations, which exclusively activate the MAPK signaling pathway, some evidence proposes that *NRAS* mutations can initiate both the MAPK and phosphatidylinositol 3 kinase (PI3K) pathways. Furthermore, *NRAS* and *BRAF* mutations are seldom found together, suggesting that a mutation in either gene is sufficient to activate the MAPK pathway[8].

As expected, this genetic change leads to constitutive activation of the downstream RAF-MEK-ERK signaling pathway, which promotes cell proliferation and survival.

*Enabling Replicative Immortality*

Cancer cells have the unique ability to replicate indefinitely, which is essential for tumor development. Unlike normal cells, which can only divide a limited number of times before stopping due to the shortening of telomeres (Hayflick Limit), cancer cells can bypass this restriction. Normally, when telomeres, the protective DNA sequences at the ends of chromosomes, become too short, cells cease dividing and either enter senescence or die[50].

However, melanoma is associated with the upregulation of the *TERT* gene. The telomerase complex includes a catalytic subunit, the *TERT*, and the telomerase RNA component (TERC)[48]. Normally, telomerase is silent in most human somatic cells, being expressed only during development[55].Nevertheless, mutations in the promotor of the *TERT* gene upregulate its transcriptional activity and expression, resulting in increased telomerase activity. Thereby, this upregulation maintains telomere length and overcomes the telomere-induced senescence barrier, granting cancer cells the ability to proliferate indefinitely, which promotes cell immortalization and the development of melanoma[36].

*Activating Invasion and Metastatis*

Metastasis allows cancer cells to spread throughout the body, resulting in a poor prognosis. Indeed, metastatic melanoma is the deadliest form of the disease.

Successful metastasis is accomplished through five steps: invasion, intravasation, circulation, extravasation, and colonization at secondary tumor sites[56].

To metastasize, cancer cells must undergo numerous changes. Among these, the epithelial-to-mesenchymal transition (EMT) is particularly important. This genetic and epigenetic-based process is critical for cell invasion and metasta-

sis and is described as a phenomenon in which epithelial tumor cells acquire mesenchymal phenotypic properties[57].
However, melanoma is not an epithelial tumor, since melanocytes arise from the neural crest. Therefore, according to accumulating evidence, melanoma experiences a complex phenomenon called phenotype switching, which results in an EMT-like process[56]. During this process, melanoma cells lose adhesive factors, switching from a proliferative, differentiated and stationary, epithelial-like state to an invasive, dedifferentiated and metastatic, mesenchymal-like state[58-59].
Normally, melanocytes are connected to basal keratinocytes by adhesion molecules, such as E-cadherins. However, during this transition, E-cadherins are replaced by N-cadherins. This modification contributes to the migration of melanoma cells, as N-cadherins support their survival and increase contact with other cells, promoting migration and proliferation[34-60].
Notably, this transition is not permanent. Some evidence has demonstrated that cells can switch back to a mesenchymal state after undergoing EMT, in a process called mesenchymal-to-epithelial transition (MET). Melanoma cells, through their ability to switch phenotypes, undergo a MET-like process that promotes the spread of cancer to other organs, as the cells return to a more proliferative state[57].

*Avoiding Immune Destruction*

The immune system is essential for survival, defending the host against various pathogens and cancer cells. Notwithstanding, cancer cells develop ways to evade detection and destruction by manipulating multiple immune pathways.

*T Cells Dysfunction*

T cells are an essential component of the acquired immune response. Based on the type of glycoprotein expressed on their membrane surface, two types of T cells can be discerned: CD4+ T cells, commonly referred to as helper T cells (Th), and CD8+ T cells, also known as cytotoxic T cells (Tc)[61]. Both play an important role in preventing melanoma progression. While CD4+ T cells release cytokines to induce or suppress other immune cells, CD8+ T cells kill infected and tumor cells[62].
Melanoma cells disrupt the effective immunological activity of T cells to evade immune system control. One of the most prominent immune pathways affected involves the abnormal expression of IC molecules. ICs are proteins expressed on the surface of immune cells, such as T cells, that recognize and bind to partner proteins expressed on other cells, including antigen-presenting cells (APCs) and tumor cells. This interaction sends an "off" signal to the T cells, suppressing their activation and hindering their immune and effector functions[63].
Under normal conditions and immunological homeostasis, this transmission of inhibitory signals occurs to prevent overreaction and autoimmunity[64]. Nevertheless, in melanoma, the immunological homeostasis is disturbed, and the overexpression of IC receptors and their ligands on immune and tumor cells, results in immune inhibition rather than tumor cell death. Numerous ICs have been studied and others are currently under investigation. LAG-3 is the focus of the latest ICI therapy.
Regarding immune suppression, regulatory T cells (Tregs) are fundamental. Tregs are a subtype of CD4+ T cells

whose primary function is to regulate the immune system by suppressing other immune cells[65]. Tregs, like IC, are critical for maintaining self-tolerance.
In melanoma, these immunosuppressive T cells are increased in the TME[65]. Tregs release immunosuppressive cytokines, such as interleukin 10 (IL-10), IL-35, and transforming growth factor-beta (TGF-β), that suppress immune cell activity and release cell membrane soluble mediators, such as granzymes and perforin, which induce T cell apoptosis[65,66]. Melanoma cells can also increase the expression of IC receptors on the surface of Tregs, once again leading to the escape of the immune system. On the other hand, Tregs interfere with metabolic pathways within the TME, by depleting ATP. This reduces the proliferation of effector T cells and suppresses the activity of dendritic and myeloid cells[65].

*Dendritic Cells*

Antigen processing and presentation is a critical step for T cell activation and proliferation, making it a key process during an immune response. It is mediated by professional APCs, such as dendritic cells (DCs), B cells and macrophages. These cells recognize, process and present antigens to T cells through the major histocompatibility complex (MHC). CD4+ T cells recognize the antigen in complex with MHC class II (MHC II) and CD8+ T cells recognize the antigen only when it is presented via MHC class I (MHC I).
During melanoma progression, the proper function, maturation and migration of DCs are significantly disrupted. Accordingly, tumor and associated immune cells produce vascular endothelial growth factor (VEGF), which suppresses DCs maturation and migration via the signal transducer and activator of transcription 3 (STAT-3) pathway[67,68].
Additionally, Tregs and tumor cells release high levels of TGF-β, and IL-10[68]. These immunosuppressive cytokines interfere with the maturation and migration of DCs to the lymph nodes by negatively regulating the expression of MHC II and the co-stimulatory B7 molecules (CD80/CD86)[66,68]. This results in defective antigen presentation, which is crucial for T cell activation[66].
Moreover, antigen presentation by melanoma cells is also disturbed. Melanoma cells can present their antigens to active CD8+ T cells via MHC I, located on the surface of their membrane. As a result of epigenetic changes, melanoma cells downregulate and mask MHC I, making them less recognizable to the immune system[66,67].

*Myeloid-Derived Suppressor Cells*

Myeloid-derived suppressor cells (MDSCs) are a subset of myeloid cells with immunosuppressive functions. Due to their ability to inhibit an adequate immune response and promote cancer metastasis, several reports have shown a negative correlation between high levels of MDSCs and survival[69].
MDSCs express negative IC molecules and produce cytokines, namely TGF-β and IL-10, as well as prostaglandin E2 (PGE2), and exosomes. These elements contribute to the dysfunction of T and natural killer (NK) cells, while also promoting the induction of Tregs. Additionally, MDSCs facilitate the dissemination of cancer cells by promoting angiogenesis and supporting the processes of EMT and MET[70].

Another immune escape mechanism orchestrated by MDSCs involves their differentiation into tumor-associated macrophages (TAMs) and the subsequent oscillation between M1 or M2 macrophages[66]. The M1 phenotype, mediated by Th1 polarization of TAMs, has anti-tumor properties. M1 macrophages exert their anti-tumor properties by secreting pro-inflammatory cytokines, ROS, and nitric oxide (NO). In addition, they act as effective APCs, thereby enhancing the adaptive anti-tumor immune response. On the other hand, M2 polarization, which is induced by Th2 and predominates in melanoma patients, inhibits T cell activity by activating Tregs and promotes melanoma progression[66,69]. This switch from M1 to M2 macrophages occurs as melanoma progresses to more advanced stages, supporting tumor immune invasion[66].

*Immune Checkpoint Inhibitors*

Over the past decade, there have been major advances in the treatment of melanoma, largely due to the growing development of immunotherapy. The introduction of the first ICI in 2011, as illustrated in Figure 3, changed the therapeutic paradigm and gave patients renewed hope. ICIs have proven effective in increasing both progression-free survival (PFS) and overall survival (OS) in several patients, establishing them as a key option in melanoma treatment[71]. As a matter of fact, according to the American Society of Clinical Oncology (ASCO) guidelines, ICIs are the mainstay of first-line treatment for advanced melanoma[72]. The recent approval of the first LAG-3 blocking antibody by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), in 2022 (Figure 3), has brought a breath of fresh air to advanced melanoma immunotherapy, and made the decision for first-line ICI therapy, which previously relied on cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 receptor (PD-1) inhibitors, more challenging[73].

This chapter will cover each of the ICI immunotherapies used in advanced stages of melanoma, with a particular focus on anti-LAG-3 immunotherapy.

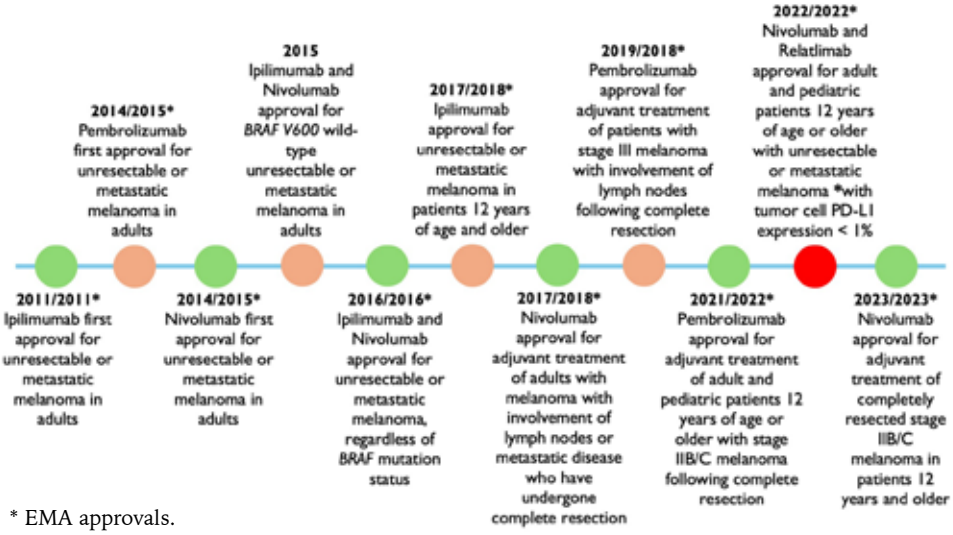



Figure 3. Timeline of FDA and EMA approvals of ICI-based immunotherapies for the treatment of melanoma[74-94].

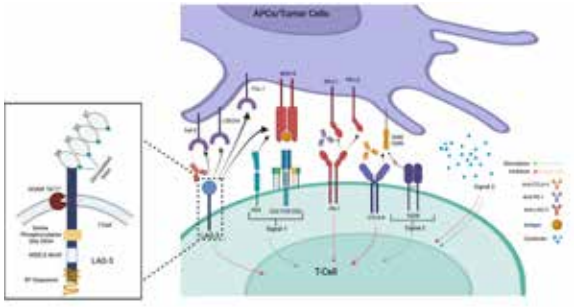
*Anti-CTLA-4*

CTLA-4 is a cell surface receptor expressed on T cells that plays a crucial role in immune homeostasis. It is constitutively activated in Tregs, whereas conventional T cells express high levels of this receptor only upon activation[95].

T cell activation depends on three major signals: antigen presentation and recognition, co-stimulation, and cytokines. The initial signal involves the binding of the T cell receptor (TCR) to the antigen-MHC complex presented by APCs and the interaction between CD4 co-receptor and MHC. This interaction, which upregulates CTLA-4 on T cells, is not sufficient for full T cell activation and a second signal is required. This second signal is mediated by the co-stimulatory interaction between CD28, expressed on T cells, and B7 molecules on APCs, and it is precisely in this process that CTLA-4 interferes. Cytokines, in turn, influence the polarization of naive T cells into different subtypes.

CTLA-4 acts as a competitive antagonist of the CD28 receptor, by inhibiting its co-stimulatory signal with its B7 ligands. Although both receptors bind to B7 molecules expressed on the surface of APCs, CTLA-4 has a higher affinity. Thus, by mediating a co-inhibitory signal through its interaction, CTLA-4 initiates a signaling cascade that results in the suppression of T cells activity[71].

Anti-CTLA-4 monoclonal antibodies block the interaction between CTLA-4 and B7 molecules, allowing CD28 co-stimulation, which subsequently activates T cells and enhances the immune response against cancer cells[96]. Figure 4 illustrates this mechanism.



Abbreviations: ADAM 10 - A Disintegrin and Metalloproteinase 10; ADAM 17 - A Disintegrin and Metalloproteinase 17; CTLA-4 - Cytotoxic T-Lymphocyte-Associated Protein 4; FGL-1 - Fibrinogen-Like Protein 1; Gal-3 - Galectin-3; LAG-3 - Lymphocyte-Activation Gene 3; LSECtin - Lymph Node Sinusoidal Endothelial Cell C-Type Lectin; MHC II - Major Histocompatibility Complex II; PD-1 - Programmed Cell Death *Protein 1 Receptor; PD-L1 - Programmed Death-Ligand 1; PD-L2 - Programmed Death-Ligand 2; TCR - T Cell Receptor.*

Figure 4. Overview of the mechanism of action of LAG-3, CTLA-4 and PD-1 with a focus on LAG-3 structure (Created in BioRender).

Ipilimumab, a fully human anti-CTLA-4 monoclonal antibody, was the first ICI approved by the FDA and the EMA in 2011. This approval was a game changer in melanoma therapy, marking a revolutionary breakthrough in cancer treatment. Since then, research into ICIs has continued, and immunotherapy is increasingly at the forefront of the treatment of many cancers.

*Anti-PD-1*

The receptor PD-1 is expressed on various immune cells, namely T cells, B cells and NK cells[96]. PD-1 mediates a co-inhibitory signal by binding to its ligands, programmed death-ligand 1 (PD-L1) and programmed death-ligand 2 (PD-L2), leading to T cell inactivation[71]. PD-L1 and PD-L2 are widely expressed. While PD-L1 is expressed on T cells, B cells, DCs, and macrophages, as well as in non-lymphoid tissues among tumor cells or stromal elements in the TME,

PD-L2 is mainly expressed on DCs and monocytes but can also be expressed in other immune and non-immune cells[71,96]. Melanoma cells can express both PD-1 ligands, which allow them to escape immune destruction[97].

In 2014, the FDA approved pembrolizumab, a humanized anti-PD-1 monoclonal antibody, for the treatment of advanced melanoma. In the same year, nivolumab, a fully human anti-PD-1 monoclonal antibody, was approved for the same therapeutic indications (Figure 3). Both antibodies work by binding to the PD-1 receptor, thereby blocking its inhibitory signaling and reactivating T cell function, as presented in the Figure 4[98].

To achieve better clinical outcomes, the combination of anti-CTLA-4 and anti-PD-1 monoclonal antibodies is widely employed. Indeed, the association of ipilimumab and nivolumab is one of the first-line treatments in advanced melanoma, as multiple studies have shown greater clinical outcomes, traduced by longer PFS and higher objective response rate (ORR), compared to monotherapy[99,100].In terms of median survival, the combination therapy showed striking results, extending patient survival from a mere 6 months to approximately 6 years[3].

Remarkably, CheckMate 067, a phase III study that compared the safety and efficacy of nivolumab alone, nivolumab and ipilimumab dual therapy, or ipilimumab monotherapy, in previously untreated patients with unresectable stage III or IV melanoma, was the cornerstone for the extended approval of nivolumab and ipilimumab for the treatment of advanced melanoma regardless of *BRAF* mutation status[101].

However, safety remains a major concern, as several immune-related adverse events (irAEs) have been reported among patients, many of which have led to premature discontinuation of treatment[99,102]. These side effects, along with treatment tolerance and resistance, underscored the urgent need to develop new ICIs that target alternative pathways[99]. Anti-LAG-3 has emerged as one of the most promising candidates[103].

*Anti-LAG-3*

First described in 1990 by Frédéric Triebel and colleagues, LAG-3 is an IC receptor expressed by activated T cells, NK cells, B cells, and DCs[103]. LAG-3 is formed by 498 amino acids and is encoded by a gene located on human chromosome 12, adjacent and homologous to CD4[104]. Structurally, it consists of 3 regions: transmembrane region, extracellular region, and cytoplasmic region. In the transmembrane-cytoplasmic region, LAG-3 is cleaved from the cell membrane by the action of A disintegrin and metalloproteinases 10 and 17 (ADAM10 and ADAM17). The extracellular region comprises four immunoglobulin superfamily domains (D1, D2, D3 and D4), which are essential for binding to ligands. Finally, the cytoplasmatic region can be divided into 3 parts: the serine phosphorylation site S454, the KIEELE motif, and the glutamate-proline dipeptide repeat motif, known as EP sequence[103,104]. Figure 4 illustrates the structure of LAG-3.

LAG-3 expression on T cells is upregulated by TCR stimulation or by cytokines, such as IL-2, IL-7 and IL-12[105,106]. Regarding its ligands, LAG-3 interacts with MHC II, galectin-3 (Gal-3), lymph node sinusoidal endothelial cell C-type

lectin (LSECtin), fibrinogen-like protein 1 (FGL-1) and α-synuclein fibrils (α-syn), to dampen T cell functions[107].

MHC II on APCs is the canonical ligand of LAG-3. Given the similarity between LAG-3 and CD4, and the fact that LAG-3 has a greater affinity for MHC II, it would be expected that one of the main inhibitory mechanisms of LAG-3 would be its competition with CD4. However, this remains controversial, since some studies revealed that LAG-3 does not block the interaction between MHC II and CD4[103,108]. Rather, it is more likely that LAG-3, once combined with MHC II through its D1 domain, exerts its inhibitory effects via its cytoplasmic domain[103]. Even though the exact mechanisms of this signaling are not fully understood, as a result of this negative regulation occurs a downregulation of T cell cytokine and granzyme production, CD4+ and CD8+ T cell activation and expansion are inhibited and Treg differentiation is increased[104,108,109].

Gal-3 was the second LAG-3 ligand to be described[106]. It is expressed in melanoma cells and other cells within the TME, and its interaction with LAG-3, according to *in vitro* experiments, inhibits the secretion of interferon-gamma (IFN-γ) by CD8+ T cells, thus affecting an adequate immune response[103]. Another potential LAG-3 ligand is LSECtin. LSECtin is expressed in melanoma cells, and by binding to the four glycosylated sites expressed in the extracellular region of LAG-3, suppresses T cell response and sustains tumor growth[103,110]. FGL-1 is also an important ligand of LAG-3, and it is overexpressed in melanoma[106]. Through its interaction with the D1 and D2 domains of LAG-3, FGL-1 mediates its immunosuppressive activity by inhibiting the activation of antigen-specific T cells[103,110]. Besides, LAG-3 appears to interact with α-syn in the central nervous system, suggesting that LAG-3 may also play a role outside the immune system[103].

As far as melanoma is concerned, it is characterized by aberrant expression of LAG-3, with tumor-infiltrating lymphocytes (TILs) in the TME expressing high levels of this IC. This high expression enhances immune escape and melanoma growth[109]. Therefore and considering all that have been discussed about the functions of LAG-3, it is expected that LAG-3 inhibition will restore the immunological functions of several immune cells and halt the progression of melanoma. Reflecting this, relatlimab, the first human LAG-3 blocking monoclonal antibody, was approved by the FDA and the EMA in 2022 for the treatment of unresectable or metastatic melanoma. Relatlimab binds to LAG-3 and inhibits its interaction with MHC II and other ligands, thereby not only enhancing T cell immune activity and cytokine release but also inhibiting Treg activity[103,104].

Interestingly, increasing evidence suggests that LAG-3 and PD-1 can be co-expressed in CD4+ and CD8+ TILs and synergistically mediate their immunosuppressive functions. In preclinical murine melanoma models, dual genetic knockout of both LAG-3 and PD-1 led to delayed tumor growth and increased survival rates[73,110]. These data highlight that simultaneous targeting of both ICs may provide therapeutic benefits, potentially overcoming the resistance observed with single agent treatments[73]. In fact, relatlimab was approved in combination with nivolumab under the trade name Opdualag™, following a phase II/III clinical trial[83].

*Clinical Trials*

The approval of Opdualag™ was based on the RELATIVITY-047 clinical trial. Since then, numerous studies have been conducted to assess the efficacy and safety of this innovative dual immunotherapy, as well as to explore new molecules targeting LAG-3.

*RELATIVITY-047*

RELATIVITY-047 is a II/III phase, double-blind and randomized trial comparing the efficacy and safety of the combination of relatlimab and nivolumab *versus* nivolumab monotherapy, in 714 patients with previously untreated metastatic or unresectable melanoma[111]. After a median follow-up of 13.2 months, the results turned out to be very promising, which supported its approval as a new first-line treatment option for advanced melanoma[111-112]. In fact, the study successfully met its primary endpoint, with the combination of relatlimab and nivolumab more than doubling the median PFS compared to nivolumab alone (10.1 months *versus* 4.6 months) and reducing the risk of melanoma progression by 25%. Notably, among the study subgroups, the combination therapy also reached longer PFS[111].

Regarding safety, the dual therapy was associated with a higher rate of treatment-related adverse events (TRAEs) of any grade, with these occurring in 81.1% of patients, compared to 69.9% of those treated with nivolumab alone. Grade 3-4 TRAEs were observed in 18.9% of patients receiving the combination *versus* 9.7% in the monotherapy group, with more patients discontinuing treatment due to these adverse events in the combination group (8.5% vs 3.1%). The most common irAEs were hypothyroidism or thyroiditis, affecting 18.0% of patients treated with relatlimab and nivolumab and 13.9% of patients after nivolumab treatment[111].

Overall, adverse events were more frequent in the relatlimab and nivolumab group, nevertheless, no new or unexpected safety signals were associated with this novel combination treatment[111].

The secondary endpoints remained blinded, because the difference in OS did not reach statistical significance[111]. Therefore, an updated report with a median follow-up of 19.3 months was conducted[113]. From this second follow-up, it was evident that OS rates at 12, 24, and 36 months were consistently better with combination therapy compared to monotherapy. Specifically, OS rates were 77.0% *versus* 71.6% at 12 months, 63.7% *versus* 58.3% at 24 months, and 55.8% *versus* 48.8% at 36 months, respectively. On the other hand, ORR was higher with the dual immunotherapy. In the updated analysis, ORR was 43.1% for relatlimab plus nivolumab group and 32.6% for the nivolumab therapy. Complete and partial responses were achieved in a greater percentage of patients with combination therapy. It should be noted that relatlimab combined with nivolumab continued to demonstrate a durable PFS advantage. This ongoing PFS benefit was observed alongside a stable safety profile compared to the initial report[112,113].

RELATIVITY-047 also assessed the health-related quality of life (HRQoL) of patients. The results showed that, although more adverse events were re-

ported with the combination therapy, overall tolerability was similar in both arms, supporting the dual PD-1 and LAG-3 inhibition as a viable first-line treatment[114].

Lastly, it is important to highlight that these findings suggest a favorable safety profile and comparable efficacy when compared to reports from clinical trials involving dual checkpoint inhibition with CTLA-4 and PD-L1 inhibitors. However, as cross-trial comparisons should be made with caution, a head-to-head clinical trial comparing both therapeutic combinations in different key subgroups may be of interest to gather more data and allow a clearer choice between the first lines of treatment. Moreover, such a trial could also play a decisive role in identifying and validating additional predictive biomarkers for these subgroups undergoing therapy. This is particularly significant given that these therapies are the most widely used in the treatment of advanced melanoma and there is currently a lack of reliable biomarkers in melanoma[102-115]. An indirect comparative analysis between the RELATIVITY-047 and the CheckMate 067 clinical trials is presented in Table 2 below.

**Table 2.** Comparison of the efficacy and safety data of RELATIVITY-047 and CheckMate 067 clinical trials[101,111,113,116]

| Results | RELATIVITY-047 | | CheckMate 067 | | |
|---|---|---|---|---|---|
| | Relatlimab/ Nivolumab | Nivolumab | Ipilimumab/ Nivolumab | Nivolumab | Ipilimumab |
| **Median PFS (months)** | 10.2 | 4.6 | 11.5 | 6.9 | 2.9 |
| **Median OS (months)** | NR | 34.1 | NR | 37.6 | 19.9 |
| **ORR (%)** | 43.1 | 32.6 | 57.6 | 43.7 | 19.0 |
| **2-year PFS (%)** | 38.5 | 29.0 | 43.0 | 37.0 | 12.0 |
| **2-year OS (%)** | 63.7 | 58.3 | 64.0 | 59.0 | 45.0 |
| **TRAEs of any grade (%)** | 81.1 (14.6*) | 69.9 (6.7*) | 95.5 (36.4*) | 82.1 (7.7*) | 86.2 (14.8*) |
| **Grade 3-4 (%)** | 18.9 (8.5*) | 9.7 (3.1*) | 55.0 (29.4*) | 16.3 (5.1*) | 27.3 (13.2*) |

Abbreviations: NR - Not Reached; ORR - Objective Response Rate; OS - Overall Survival; PFS - Progression Free Survival; TRAEs - Treatment-Related Adverse Events.
*Led do treatment discontinuation.

*RELATIVITY-020*

Despite therapeutic innovation in advanced melanoma, approximately 40% of patients continue to have no significant response, even with first-line immunotherapies. Thus, novel therapies, such as relatlimab and nivolumab, are crucial to improving outcomes and preventing disease progression[117].

RELATIVITY-020, an open-label phase I/IIa, dose escalation and cohort expansion trial, assessed the efficacy and safety of the combination of relatlimab and nivolumab in 518 patients with advanced melanoma who had documented progression after immunotherapy. Two study cohorts, D1 and D2, were conducted. Each cohort was established according to the prior treatment received by the participants and followed distinct dosing regimens. Cohort D1 included only patients with one prior line of anti-PD-1 therapy, either alone or in combination with anti-CTLA4. In contrast, D2 allowed multiple lines of treatment, including patients who had received prior adjuvant or neoadjuvant anti-PD-1 therapies[112,117].

In terms of efficacy, the ORR observed was 12% and 9.2% in the D1 and D2 pooled, respectively. The median PFS was lower in cohort D1 compared to D2 (2.1 vs 3.2 months). Furthermore, the median duration of response (DOR), although not achieved in D1 group, was 12.8 months in D2 cohort. Finally, the median OS was 14.7 months in D1 group and 17.1 months in D2[117].

Safety was measured by the rate of adverse events and was similar in both groups. As a matter of fact, the incidence of TRAEs of any grade was 67.5% in D1 cohort and 68.9% in D2 cohort. Of these, 15% and 12.8% corresponded to grade 3-4 reactions in groups D1 and D2, respectively. Rash (7.3%) was the most frequent irAEs in D1 and D2 groups[117].

Considering all these data, the combination therapy of relatlimab and nivolumab provided promising results in terms of efficacy and safety, regardless of the cohorts. Therefore, for patients who are refractory to previous PD-(L)1 inhibitor-containing regimens, the new dual therapy could be a very viable option. Nevertheless, the single-arm design of this study is a limitation, and further studies, like phase III trials, are needed to confirm the use of this new combination in later lines of therapy[112,117]. In parallel, better predictive biomarkers should also be investigated, in order to ensure a significant clinical response among the subgroups.

*Other Studies*

An example is a study involving 30 patients with resectable clinical stage III or oligometastatic stage IV melanoma without prior ICI therapy, that evaluated the safety and efficacy of relatlimab and nivolumab as neoadjuvant and adjuvant therapy[118]. This study followed an earlier trial comparing nivolumab and ipilimumab with nivolumab alone, also in the neoadjuvant and adjuvant settings[119].

Remarkably, the neoadjuvant combination of nivolumab and relatlimab demonstrated positive results. A complete pathological response (pCR) was observed in more than half of the patients (57%), with 70% of patients experiencing some clinical response[118]. In contrast, pCR rates were significantly

lower with nivolumab plus ipilimumab and nivolumab monotherapy (45% and 25%, respectively)[119]. Moreover, recurrence-free survival (RFS) rates were 97% and 82% in the first and second year, respectively, after treatment with the novel dual therapy[118].

Regarding safety, no grade 3-4 irAEs were reported in the neoadjuvant setting. Contrarily, 26% of patients experienced irAES in the adjuvant setting, which raises the question of whether adjuvant therapy is necessary following an effective neoadjuvant regimen[118]. When comparing these results with both arms of the previous trial, the difference in safety is striking. Indeed, 27% of combination patients had their surgery postponed due to immunotherapy toxicity and 73% reported grade 3 TRAEs[119].

Despite the small sample size and the need for further studies, these data are very encouraging, as neoadjuvant therapy with LAG-3 and PD-1 blockade shows lower toxicity and a higher efficacy and tolerability than previous regimens.

Beyond relatlimab, other anti-LAG-3 therapies are currently under investigation, including fianlimab, an anti-LAG-3 human monoclonal antibody. Interestingly, fianlimab in combination with cemiplimab, an anti-PD-1 monoclonal antibody, has shown promising results in preclinical studies. This new dual immunotherapy is undergoing several phase III clinical trials[112].

Eftilagimod alfa (IMP321), bi-specific antibodies targeting LAG-3 and CTLA-4 (XmAb22841) or PD-1 and LAG-3 (RO7247669 and INCA32459), and other innovative anti-LAG-3 molecules, such as INCAGN02385, are also being evaluated[102,112]. Table 3, lists the ongoing clinical trials targeting anti-LAG-3 molecules.

**Table 3.** Ongoing clinical trials investigating relatlimab and other anti-LAG-3 molecules in melanoma[120-136]

| Agent | NCT Number | Condition | Focus | Phase | Status |
|---|---|---|---|---|---|
| Relatlimab | NCT05704647 | Active melanoma brain metastases | Evaluate the efficacy and safety of nivolumab and relatlimab combination | II | Recruiting |
| | NCT03743766 | Unresectable or metastatic melanoma without prior treatment | Evaluate the antitumor activity of relatlimab and nivolumab | II | Active, not recruiting |
| | NCT05625399 | Untreated metastatic or unresectable melanoma | Compare nivolumab and relatlimab subcutaneous fixed-dose combinations with intravenous administration | III | Recruiting |
| | NCT04552223 | Metastatic uveal melanoma without prior treatment | Evaluate the efficacy (ORR) of nivolumab and relatlimab combination | II | Active, not recruiting |
| | NCT06295159 | Locoregionally advanced melanoma | Compare nivolumab plus relatlimab, nivolumab and ipilimumab in neoadjuvant therapy | I | Recruiting |

**Table 3.** Ongoing clinical trials investigating relatlimab and other anti-LAG-3 molecules in melanoma[120-136] (cont)

| Agent | NCT Number | Condition | Focus | Phase | Status |
|---|---|---|---|---|---|
| Relatlimab | NCT05002569 | Completely resected stage III-IV melanoma | Compare adjuvant therapy of nivolumab plus relatlimab with nivolumab monotherapy | III | Active, not recruiting |
| | NCT05418972 | High risk, clinical stage II cutaneous melanoma | Evaluate the efficacy of nivolumab and relatlimab in the neoadjuvant setting | II | Recruiting |
| | NCT05704933 | Surgically resectable melanoma brain metastases | Evaluate the safety and feasibility of nivolumab with ipilimumab or relatlimab | I | Active, not recruiting |
| | NCT05077280 | Metastatic uveal melanoma | Evaluate the safety and tolerability of nivolumab and relatlimab with radiotherapy | II | Recruiting |
| Fianlimab | NCT05352672 | Untreated unresectable locally advanced melanoma or metastatic melanoma | Compare the efficacy of fianlimab and cemiplimab combination against pembrolizumab | III | Recruiting |
| | NCT06246916 | Unresectable or metastatic melanoma | Compare the efficacy and safety of fianlimab and cemiplimab with nivolumab and relatlimab combination | III | Recruiting |
| | NCT05608291 | Completely resected high-risk melanoma | Compare the efficacy and safety of fianlimab combined with cemiplimab against pembrolizumab | III | Recruiting |
| | NCT06190951 | Resectable stage III and IV melanoma | Compare the efficacy of fianlimab and cemiplimab combination against pembrolizumab | II/III | Not yet recruiting |
| XmAb22841 | NCT05695898 | Metastatic melanoma refractory to prior ICI immunotherapy with and without central nervous system disease | Evaluate the safety and tolerability of XmAb22841 (CTLA-4 X LAG-3) in combination with XmAb23104 (PD-1 X ICOS) | Ib/II | Active, not recruiting |

**Table 3.** Ongoing clinical trials investigating relatlimab and other anti-LAG-3 molecules in melanoma[120-136] (cont)

| Agent | NCT Number | Condition | Focus | Phase | Status |
|---|---|---|---|---|---|
| **INCAGN02385** | NCT04370704 | Advanced melanoma | Evaluate the safety and tolerability of INCAGN02385 (Anti-LAG-3) + INCAGN02390 (Anti-TIM-3) and INCAGN02385 + INCAGN02390 + INCMGA00012 (Anti-PD-1) combinations | I/II | Active, not recruiting |
| **RO7247669** | NCT04140500 | Metastatic melanoma | Evaluate the safety and tolerability of RO7247669 (Anti PD-1 and LAG-3) | I/II | Recruiting |
| **INCA32459** | NCT05577182 | Unresectable or metastatic melanoma | Evaluate the safety and tolerability of INCA32459 (Anti PD-1 and LAG-3) | I | Recruiting |

Abbreviations: CTLA-4 - Cytotoxic T-Lymphocyte-Associated Protein 4; ICOS - Inducible T Cell Costimulatory; LAG-3 - Lymphocyte-Activation Gene 3; ORR - Objective Response Rate; PD-1 - Programmed Cell Death Protein 1 Receptor; TIM-3 - Mucin Domain-Containing Protein 3.

## Conclusion and Future Perspectives

Melanoma is a complex and deadly skin cancer, characterized by several pathways of development and a high mutational load. In its most advanced stages, melanoma has a severe prognosis. Progress in immunotherapy, particularly ICIs, have therefore become extremely important, making this therapy the cornerstone of the treatment of advanced melanoma.

Relatlimab, the first approved anti-LAG-3 antibody, in combination with nivolumab, has shown promising clinical results in the treatment of untreated and refractory advanced melanoma, as well as in the adjuvant and neoadjuvant settings. However, multiple critical questions remain unanswered.

As discussed in this review, the precise mechanism of action of LAG-3 is still unclear. Since LAG-3 plays an important role in the immunoregulation of melanoma and other human cancers, further work is needed to elucidate its mechanism of action, as this knowledge is undoubtedly essential for the optimal use of any anti-LAG-3 immunotherapy and for future research into new molecules targeting this IC.

Regarding the combination therapy of relatlimab and nivolumab, this review has highlighted its clinical results, with safety results standing out compared to

other first-line therapies. In fact, this was a key factor in its approval, as ICI immunotherapy is associated with many adverse events, many of which are serious and lead to treatment discontinuation. Nevertheless, additional studies are required to evaluate the efficacy and safety of this combination in patient subgroups beyond those included in the aforementioned clinical trials, such as brain metastases or rare melanoma subtypes.

Additionally, given the recent approval of this novel dual therapy, long-term data on PFS and OS are still needed, along with the identification of predictive biomarkers. Immunotherapy is an expensive treatment and is associated with resistance and tolerability, so the existence of accurate biomarkers would ensure a rigorous selection of patients who would actually benefit clinically from this therapy, sparing them from unwanted side effects and treatment failure.

Apart from LAG-3, other ICIs, such as T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) and T cell immunoglobulin and ITIM domain (TIGIT) also hold the potential to revolutionize melanoma treatment. The emergence of these and other novel molecules, alongside the several anti-LAG-3 agents currently undergoing clinical trials, points to a promising future for therapeutic strategies in advanced melanoma.

Through sustained innovation and rigorous pursuit of unanswered questions, immunotherapy with anti-LAG-3 is expected to maintain its position at the forefront of advanced melanoma treatment. Continued progress in this field could therefore, unquestionably, lead to further improvements in the efficacy and safety of ICIs therapies, ultimately enhancing both survival outcomes and quality of life for patients.

**BIBLIOGRAPHY**

1. Ralli M, Botticelli A, Visconti IC, Angeletti D, Fiore M, Marchetti P, et al. Immunotherapy in the Treatment of Metastatic Melanoma: Current Knowledge and Future Directions. J Immunol Res [Internet]. 28 de Junho de 2020;2020:1–12. Disponível em: https://www.hindawi.com/journals/jir/2020/9235638/

2. Kumar AR, Devan AR, Nair B, Vinod BS, Nath LR. Harnessing the immune system against cancer: current immunotherapy approaches and therapeutic targets. Mol Biol Rep. 20 de Dezembro de 2021;48(12):8075–95.

3. Knight A, Karapetyan L, Kirkwood JM. Immunotherapy in Melanoma: Recent Advances and Future Directions. Cancers (Basel). 9 de Fevereiro de 2023;15(4):1106.

4. Marzagalli M, Ebelt ND, Manuel ER. Unraveling the crosstalk between melanoma and immune cells in the tumor microenvironment. Semin Cancer Biol [Internet]. Dezembro de 2019;59:236–50. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S1044579X19301087

5. Kim HJ, Kim YH. Molecular Frontiers in Melanoma: Pathogenesis, Diagnosis, and Therapeutic Advances. Int J Mol Sci [Internet]. 4 de Março de 2024;25(5):2984. Disponível em: https://www.mdpi.com/1422-0067/25/5/2984

6. Naimi A, Mohammed RN, Raji A, Chupradit S, Yumashev AV, Suksatan

W, et al. Tumor immunotherapies by immune checkpoint inhibitors (ICIs); the pros and cons. Cell Communication and Signaling. 7 de Abril de 2022;20(1):44.

7. Wagstaff W, Mwamba RN, Grullon K, Armstrong M, Zhao P, Hendren-Santiago B, et al. Melanoma: Molecular genetics, metastasis, targeted therapies, immunotherapies, and therapeutic resistance. Vol. 9, Genes and Diseases. Chongqing University; 2022. p. 1608–23.

8. Strashilov S, Yordanov A. Aetiology and Pathogenesis of Cutaneous Melanoma: Current Concepts and Advances. Int J Mol Sci [Internet]. 15 de Junho de 2021;22(12):6395. Disponível em: https://www.mdpi.com/1422-0067/22/12/6395

9. Lisa C. Zaba JYW and SMS. Dermatology. 4th Edition 2018. 2018.

10. Arnold M, Singh D, Laversanne M, Vignat J, Vaccarella S, Meheus F, et al. Global Burden of Cutaneous Melanoma in 2020 and Projections to 2040. JAMA Dermatol. 1 de Maio de 2022;158(5):495.

11. Kuk D, Shoushtari AN, Barker CA, Panageas KS, Munhoz RR, Momtaz P, et al. Prognosis of Mucosal, Uveal, Acral, Nonacral Cutaneous, and Unknown Primary Melanoma From the Time of First Metastasis. Oncologist. 1 de Julho de 2016;21(7):848–54.

12. Chattopadhyay C, Kim DW, Gombos DS, Oba J, Qin Y, Williams MD, et al. Uveal melanoma: From diagnosis to treatment and the science in between. Cancer. 15 de Agosto de 2016;122(15):2299–312.

13. van Poppelen NM, de Bruyn DP, Bicer T, Verdijk R, Naus N, Mensink H, et al. Genetics of Ocular Melanoma: Insights into Genetics, Inheritance and Testing. Int J Mol Sci. 30 de Dezembro de 2020;22(1):336.

14. Krantz BA, Dave N, Komatsubara KM, Marr BP, Carvajal RD. Uveal melanoma: epidemiology, etiology, and treatment of primary disease. Clinical Ophthalmology [Internet]. 31 de Janeiro de 2017;Volume 11:279–89. Disponível em: https://www.dovepress.com/uveal-melanoma-epidemiology-etiology-and-treatment-of-primary-disease-peer-reviewed-article-OPTH

15. Sergi MC, Filoni E, Triggiano G, Cazzato G, Internò V, Porta C, et al. Mucosal Melanoma: Epidemiology, Clinical Features, and Treatment. Curr Oncol Rep [Internet]. 29 de Novembro de 2023;25(11):1247–58. Disponível em: https://link.springer.com/10.1007/s11912-023-01453-x

16. Saginala K, Barsouk A, Aluru JS, Rawla P, Barsouk A. Epidemiology of Melanoma. Medical Sciences [Internet]. 20 de Outubro de 2021;9(4):63. Disponível em: https://www.mdpi.com/2076-3271/9/4/63

17. International Agency for Research on Cancer- Global Cancer Observatory. Melanoma of Skin [Internet]. 2022 [citado 8 de Maio de 2024]. Disponível em: https://gco.iarc.who.int/media/globocan/factsheets/cancers/16-melanoma-of-skin-fact-sheet.pdf

18. NURLA LA, FORSEA AM. Melanoma epidemiology in Europe: what is new? Italian Journal of Dermatology and Venereology. Abril de 2024;159(2).

19. ECIS - European Cancer Information System. Estimates of Cancer Incidence and Mortality in 2022 [Internet].

[citado 8 de Maio de 2024]. Disponível em: https://ecis.jrc.ec.europa.eu/explorer.php

20. International Agency for Research on Cancer. Skin Cancer [Internet]. [citado 8 de Maio de 2024]. Disponível em: https://www.iarc.who.int/cancer-type/skin-cancer/

21. American Cancer Society. Key Statistics for Melanoma Skin Cancer [Internet]. [citado 8 de Maio de 2024]. Disponível em: https://www.cancer.org/cancer/types/melanoma-skin-cancer/about/key-statistics.html

22. Liga Portuguesa Contra o Cancro. Melanoma [Internet]. [citado 8 de Maio de 2024]. Disponível em: https://www.ligacontracancro.pt/melanoma/

23. Teixido C, Castillo P, Martinez-Vila C, Arance A, Alos L. Molecular Markers and Targets in Melanoma. Cells [Internet]. 5 de Setembro de 2021;10(9):2320. Disponível em: https://www.mdpi.com/2073-4409/10/9/2320

24. Urso C. Melanocytic Skin Tumors: Genetic Aberrations and Clinicopathological Classification. Dermatol Pract Concept. 31 de Dezembro de 2019;e2020005.

25. Shain AH, Bastian BC. From melanocytes to melanomas. Vol. 16, Nature Reviews Cancer. Nature Publishing Group; 2016. p. 345–58.

26. Huang JM, Chikeka I, Hornyak TJ. Melanocytic Nevi and the Genetic and Epigenetic Control of Oncogene-Induced Senescence. Dermatol Clin [Internet]. 1 de Janeiro de 2017;35(1):85–93. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S073386351630081X

27. Damsky WE, Bosenberg M. Melanocytic nevi and melanoma: unraveling a complex relationship. Oncogene. 19 de Outubro de 2017;36(42):5771–92.

28. Shreberk-Hassidim R, Ostrowski SM, Fisher DE. The Complex Interplay between Nevi and Melanoma: Risk Factors and Precursors. Int J Mol Sci [Internet]. 10 de Fevereiro de 2023;24(4):3541. Disponível em: https://www.mdpi.com/1422-0067/24/4/3541

29. Grigore L, Ungureanu L, Bejinariu N, Seceac C, Vasilovici A, Senila S, et al. Complete regression of primary melanoma associated with nevi involution under BRAF inhibitors: A case report and review of the literature. Oncol Lett. 19 de Novembro de 2018;

30. Shan KS, Rehman TU, Ivanov S, Domingo G, Raez LE. Molecular Targeting of the BRAF Proto-Oncogene/Mitogen-Activated Protein Kinase (MAPK) Pathway across Cancers. Int J Mol Sci. 3 de Janeiro de 2024;25(1):624.

31. Castellani G, Buccarelli M, Arasi MB, Rossi S, Pisanu ME, Bellenghi M, et al. BRAF Mutations in Melanoma: Biological Aspects, Therapeutic Implications, and Circulating Biomarkers. Cancers (Basel). 8 de Agosto de 2023;15(16):4026.

32. Cheng L, Lopez-Beltran A, Massari F, MacLennan GT, Montironi R. Molecular testing for BRAF mutations to inform melanoma treatment decisions: a move toward precision medicine. Modern Pathology. Janeiro de 2018;31(1):24–38.

33. Zhu H, Blake S, Kusuma FK, Pearson RB, Kang J, Chan KT. Oncogene-induced senescence: From biology to therapy. Mech Ageing Dev [Internet]. Abril de 2020;187:111229. Disponível em: https://linkinghub.elsevier.com/re-

trieve/pii/S0047637420300245

34. Eddy K, Shah R, Chen S. Decoding Melanoma Development and Progression: Identification of Therapeutic Vulnerabilities. Front Oncol. 4 de Fevereiro de 2021;10.

35. Dessinioti C, Befon A, Stratigos AJ. The Association of Nevus-Associated Melanoma with Common or Dysplastic Melanocytic Nevus: A Systematic Review and Meta-Analysis. Cancers (Basel). 30 de Janeiro de 2023;15(3):856.

36. Guo Y, Chen Y, Zhang L, Ma L, Jiang K, Yao G, et al. TERT Promoter Mutations and Telomerase in Melanoma. Li X, editor. J Oncol [Internet]. 19 de Julho de 2022;2022:1–11. Disponível em: https://www.hindawi.com/journals/jo/2022/6300329/

37. Kreuger IZM, Slieker RC, van Groningen T, van Doorn R. Therapeutic Strategies for Targeting CDKN2A Loss in Melanoma. Journal of Investigative Dermatology [Internet]. Janeiro de 2023;143(1):18-25.e1. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S0022202X22017699

38. Keung EZ, Gershenwald JE. The eighth edition American Joint Committee on Cancer (AJCC) melanoma staging system: implications for melanoma treatment and care. Expert Rev Anticancer Ther. 3 de Agosto de 2018;18(8):775–84.

39. Melanoma Research Alliance. Understanding Melanoma Staging [Internet]. [citado 9 de Junho de 2024]. Disponível em: https://www.curemelanoma.org/about-melanoma/melanoma-staging/understanding-melanoma-staging

40. Elder DE, Bastian BC, Cree IA, Massi D, Scolyer RA. The 2018 World Health Organization Classification of Cutaneous, Mucosal, and Uveal Melanoma: Detailed Analysis of 9 Distinct Subtypes Defined by Their Evolutionary Pathway. Arch Pathol Lab Med [Internet]. 1 de Abril de 2020;144(4):500–22. Disponível em: http://meridian.allenpress.com/aplm/article/144/4/500/427497/The-2018-World-Health-Organization-Classification

41. Elder DE. Melanoma progression. Pathology. Fevereiro de 2016;48(2):147–54.

42. Hou H, Sun D, Zhang X. The role of MDM2 amplification and overexpression in therapeutic resistance of malignant tumors. Cancer Cell Int. 22 de Dezembro de 2019;19(1):216.

43. Xiao Y, Zhou L, Andl T, Zhang Y. YAP1 controls the N-cadherin-mediated tumor-stroma interaction in melanoma progression. Oncogene. 15 de Março de 2024;43(12):884–98.

44. National Cancer Institute. Metastasis [Internet]. [citado 9 de Junho de 2024]. Disponível em: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/metastasis

45. Ferrara G, Argenziano G. The WHO 2018 Classification of Cutaneous Melanocytic Neoplasms: Suggestions From Routine Practice. Front Oncol [Internet]. 2 de Julho de 2021;11. Disponível em: https://www.frontiersin.org/articles/10.3389/fonc.2021.675296/full

46. Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discov [Internet]. 1 de Janeiro de 2022;12(1):31–46. Disponível em: https://aacrjournals.org/cancerdiscovery/article/12/1/31/675608/Hall-

marks-of-Cancer-New-DimensionsHallmarks-of

47. Mahumud RA, Shahjalal Md. The Emerging Burden of Genetic Instability and Mutation in Melanoma: Role of Molecular Mechanisms. Cancers (Basel). 15 de Dezembro de 2022;14(24):6202.

48. Broertjes J. UNAV Journal for Medical Students The Ten Hallmarks of Cancer in Cutaneous Malignant Melanoma. 2015;

49. Kung CP, Weber JD. It's Getting Complicated—A Fresh Look at p53-MDM2-ARF Triangle in Tumorigenesis and Cancer Therapy. Front Cell Dev Biol [Internet]. 26 de Janeiro de 2022;10. Disponível em: https://www.frontiersin.org/articles/10.3389/fcell.2022.818744/full

50. Rockland Immunochemicals. Hallmarks Of Cancer [Internet]. [citado 17 de Junho de 2024]. Disponível em: https://www.rockland.com/resources/hallmarks-of-cancer/

51. van Not OJ, Blokx WAM, van den Eertwegh AJM, de Meza MM, Haanen JB, Blank CU, et al. BRAF and NRAS Mutation Status and Response to Checkpoint Inhibition in Advanced Melanoma. JCO Precis Oncol. Setembro de 2022;(6).

52. Guo Y, Pan W, Liu S, Shen Z, Xu Y, Hu L. ERK/MAPK signalling pathway and tumorigenesis (Review). Exp Ther Med [Internet]. 15 de Janeiro de 2020; Disponível em: http://www.spandidos-publications.com/10.3892/etm.2020.8454

53. Barbosa R, Acevedo LA, Marmorstein R. The MEK/ERK Network as a Therapeutic Target in Human Cancer. Molecular Cancer Research. 1 de Março de 2021;19(3):361–74.

54. Randic T, Kozar I, Margue C, Utikal J, Kreis S. NRAS mutant melanoma: Towards better therapies. Cancer Treat Rev [Internet]. Setembro de 2021;99:102238. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S0305737221000864

55. Delyon J, Vallet A, Bernard-Cacciarella M, Kuzniak I, Reger de Moura C, Louveau B, et al. TERT Expression Induces Resistance to BRAF and MEK Inhibitors in BRAF-Mutated Melanoma In Vitro. Cancers (Basel). 24 de Maio de 2023;15(11):2888.

56. Vandyck HH, Hillen LM, Bosisio FM, van den Oord J, zur Hausen A, Winnepenninckx V. Rethinking the biology of metastatic melanoma: a holistic approach. Cancer and Metastasis Reviews [Internet]. 19 de Junho de 2021;40(2):603–24. Disponível em: https://link.springer.com/10.1007/s10555-021-09960-8

57. Pedri D, Karras P, Landeloos E, Marine J, Rambow F. Epithelial-to-mesenchymal-like transition events in melanoma. FEBS J. 30 de Março de 2022;289(5):1352–68.

58. Shirley CA, Chhabra G, Amiri D, Chang H, Ahmad N. Immune escape and metastasis mechanisms in melanoma: breaking down the dichotomy. Front Immunol [Internet]. 14 de Fevereiro de 2024;15. Disponível em: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1336023/full

59. Zhou Q, Wang J, Zhang Z, Wuethrich A, Lobb RJ, Trau M. Tracking the EMT-like phenotype switching during targeted therapy in melanoma by analyzing extracellular vesicle phenotypes. Biosens Bioelectron. Janeiro de

2024;244:115819.

60. Loh CY, Chai J, Tang T, Wong W, Sethi G, Shanmugam M, et al. The E-Cadherin and N-Cadherin Switch in Epithelial-to-Mesenchymal Transition: Signaling, Therapeutic Implications, and Challenges. Cells. 20 de Setembro de 2019;8(10):1118.

61. Mahmoud F, Shields B, Makhoul I, Avaritt N, Wong HK, Hutchins LF, et al. Immune surveillance in melanoma: From immune attack to melanoma escape and even counterattack. Cancer Biol Ther. 3 de Julho de 2017;18(7):451–69.

62. Tay RE, Richardson EK, Toh HC. Revisiting the role of CD4+ T cells in cancer immunotherapy—new insights into old paradigms. Cancer Gene Ther. 27 de Fevereiro de 2021;28(1–2):5–17.

63. National Cancer Institute. Immune Checkpoint Inhibitors [Internet]. [citado 20 de Julho de 2024]. Disponível em: https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/checkpoint-inhibitors

64. Guo Z, Zhang R, Yang AG, Zheng G. Diversity of immune checkpoints in cancer immunotherapy. Front Immunol. 7 de Março de 2023;14.

65. Huang L, Guo Y, Liu S, Wang H, Zhu J, Ou L, et al. Targeting regulatory T cells for immunotherapy in melanoma. Molecular Biomedicine [Internet]. 19 de Dezembro de 2021;2(1):11. Disponível em: https://link.springer.com/10.1186/s43556-021-00038-z

66. Eddy K, Chen S. Overcoming Immune Evasion in Melanoma. Int J Mol Sci. 26 de Novembro de 2020;21(23):8984.

67. Tucci M, Passarelli A, Mannavola F, Felici C, Stucci LS, Cives M, et al. Immune System Evasion as Hallmark of Melanoma Progression: The Role of Dendritic Cells. Front Oncol [Internet]. 5 de Novembro de 2019;9. Disponível em: https://www.frontiersin.org/article/10.3389/fonc.2019.01148/full

68. Mahmoud F, Shields B, Makhoul I, Avaritt N, Wong HK, Hutchins LF, et al. Immune surveillance in melanoma: From immune attack to melanoma escape and even counterattack. Cancer Biol Ther [Internet]. 3 de Julho de 2017;18(7):451–69. Disponível em: https://www.tandfonline.com/doi/full/10.1080/15384047.2017.1323596

69. Ozbay Kurt FG, Lasser S, Arkhypov I, Utikal J, Umansky V. Enhancing immunotherapy response in melanoma: myeloid-derived suppressor cells as a therapeutic target. Journal of Clinical Investigation. 3 de Julho de 2023;133(13).

70. Tomela K, Pietrzak B, Galus Ł, Mackiewicz J, Schmidt M, Mackiewicz AA, et al. Myeloid-Derived Suppressor Cells (MDSC) in Melanoma Patients Treated with Anti-PD-1 Immunotherapy. Cells. 2 de Março de 2023;12(5):789.

71. Sabbatino F, Liguori L, Pepe S, Ferrone S. Immune checkpoint inhibitors for the treatment of melanoma. Expert Opin Biol Ther [Internet]. 4 de Maio de 2022;22(5):563–76. Disponível em: https://www.tandfonline.com/doi/full/10.1080/14712598.2022.2038132

72. Seth R, Agarwala SS, Messersmith H, Alluri KC, Ascierto PA, Atkins MB, et al. Systemic Therapy for Melanoma: ASCO Guideline Update. Journal of Clinical Oncology. 20 de Outubro de 2023;41(30):4794–820.

73. Kreidieh FY, Tawbi HA. The introduction of LAG-3 checkpoint blockade in melanoma: immunotherapy

landscape beyond PD-1 and CTLA-4 inhibition. Ther Adv Med Oncol [Internet]. 17 de Janeiro de 2023;15. Disponível em: http://journals.sagepub.com/doi/10.1177/17588359231186027

74. Bristol-Myers Squibb. FDA Approves YERVOYTM (ipilimumab) for the Treatment of Patients with Newly Diagnosed or Previously-Treated Unresectable or Metastatic Melanoma, the Deadliest Form of Skin Cancer [Internet]. 2011 [citado 22 de Julho de 2024]. Disponível em: https://news.bms.com/news/details/2011/FDA-Approves-YERVOY-ipilimumab-for-the-Treatment-of-Patients-with-Newly-Diagnosed-or-Previously-Treated-Unresectable-or-Metastatic-Melanoma-the-Deadliest-Form-of-Skin-Cancer/default.aspx

75. Merck. Merck Receives Accelerated Approval of KEYTRUDA® (pembrolizumab), the First FDA-Approved Anti-PD-1 Therapy [Internet]. 2014 [citado 22 de Julho de 2024]. Disponível em: Merck Receives Accelerated Approval of KEYTRUDA® (pembrolizumab), the First FDA-Approved Anti-PD-1 Therapy

76. Bristol-Myers Squibb. Bristol-Myers Squibb Receives Accelerated Approval of Opdivo (nivolumab) from the U.S. Food and Drug Administration [Internet]. 2014 [citado 22 de Julho de 2024]. Disponível em: https://news.bms.com/news/details/2014/Bristol-Myers-Squibb-Receives-Accelerated-Approval-of-Opdivo-nivolumab-from-the-US-Food-and-Drug-Administration/default.aspx

77. Bristol-Myers Squibb. Bristol-Myers Squibb Receives Approval from the U.S. Food and Drug Administration for the Opdivo (nivolumab) + Yervoy (ipilimumab) Regimen in BRAF V600 Wild-Type Unresectable or Metastatic Melanoma [Internet]. 2015 [citado 22 de Julho de 2024]. Disponível em: https://news.bms.com/news/details/2015/Bristol-Myers-Squibb-Receives-Appro-val-from-the-US-Food-and-Drug-Administration-for-the-Opdivo-nivolumab-Yervoy-ipilimumab-Regimen-in-BRAF-V600-Wild-Type-Unresectable-or-Metastatic-Melanoma/default.aspx

78. Bristol-Myers Squibb. Bristol-Myers Squibb's Opdivo (nivolumab) + Yervoy (ipilimumab) Regimen Receives Expanded FDA Approval in Unresectable or Metastatic Melanoma Across BRAF Status1 [Internet]. 2016 [citado 22 de Julho de 2024]. Disponível em: https://news.bms.com/news/details/2016/Bristol-Myers-Squibbs-Opdivo-nivolumab-Yervoy-ipilimumab-Regimen-Receives-Expanded-FDA-Approval-in-Unresectable-or-Metastatic-Melanoma-Across-BRAF-Status1/default.aspx

79. Bristol-Myers Squibb. U.S. Food and Drug Administration Expands Approval of Yervoy® (ipilimumab) to Include Pediatric Patients 12 Years and Older with Unresectable or Metastatic Melanoma [Internet]. 2017 [citado 22 de Julho de 2024]. Disponível em:https://news.bms.com/news/details/2017/US-Food-and-Drug-Administration-Expands-Approval-of-Yervoy-ipilimumab-to-Include-Pediatric-Patients-12-Years-and-Older-with-Unresectable-or-Metastatic-Melanoma/default.aspx

80. Bristol-Myers Squibb. Bristol-Myers Squibb Receives FDA Approval for Opdivo (nivolumab) as Adjuvant Therapy in Patients with Completely Resected Melanoma with Lymph Node Involvement or Metastatic Disease [Internet]. 2017 [citado 22 de Julho de 2024]. Disponível em: https://news.bms.com/news/details/2017/Bristol-Myers-Squibb-Receives-FDA-Approval-for-Opdivo-nivolumab-as-Adjuvant-Therapy-in-Patients-with-Completely-Resected-Melanoma-with-Lymph-Node-Involvement-or-Metastatic-Disease/default.aspx

81. Food and Drug Administration. FDA approves pembrolizumab for adjuvant treatment of melanoma [Internet]. 2019 [citado 22 de Julho de 2024]. Disponível em: https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-pembrolizumab-adjuvant-treatment-melanoma

82. Food and Drug Administration. FDA approves pembrolizumab for adjuvant treatment of Stage IIB or IIC melanoma [Internet]. 2021 [citado 22 de Julho de 2024]. Disponível em: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-pembrolizumab-adjuvant-treatment-stage-iib-or-iic-melanoma

83. Food and Drug Administration. FDA approves Opdualag for unresectable or metastatic melanoma [Internet]. 2022 [citado 22 de Julho de 2024]. Disponível em: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-opdualag-unresectable-or-metastatic-melanoma

84. Food and Drug Administration. FDA approves nivolumab for adjuvant treatment of Stage IIB/C melanoma [Internet]. 2023 [citado 22 de Julho de 2024]. Disponível em: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-nivolumab-adjuvant-treatment-stage-iibc-melanoma

85. European Medicines Agency. Yervoy [Internet]. 2011 [citado 21 de Julho de 2024]. Disponível em: https://www.ema.europa.eu/en/medicines/human/EPAR/yervoy

86. Merck. European Commission Approves Merck's Anti-PD-1 Therapy, KEYTRUDA® (pembrolizumab), for Both First-line and Previously-treated Patients with Advanced Melanoma [Internet]. 2015 [citado 21 de Julho de 2024]. Disponível em: https://www.merck.com/news/european-commission-approves-mercks-anti-pd-1-therapy-keytruda-pembrolizumab-for-both-first-line-and-previously-treated-patients-with-advanced-melanoma/

87. Bristol-Myers Squibb. European Commission Approves Bristol-Myers Squibb's Opdivo (nivolumab), the First and Only PD-1 Checkpoint Inhibitor Approved in Europe, for Both First-Line and Previously-Treated Advanced Melanoma Patients [Internet]. 2015 [citado 21 de Julho de 2024]. Disponível em: https://news.bms.com/news/details/2015/European-Commission-Approves-Bristol-Myers-Squibbs-Opdivo-nivolumab-the-First-and-Only-PD-1-Checkpoint-Inhibitor-Approved-in-Europe-for-Both-First-Line-and-Previously-Treated-Advanced-Melanoma-Patients/default.aspx

88. Bristol-Myers Squibb. European Commission Approves the First and Only Immuno-Oncology Combination, Bristol-Myers Squibb's Opdivo® (nivolumab) + Yervoy® (ipilimumab) Regimen, for Treatment of Advanced Melanoma [Internet]. 2016 [citado 21 de Julho de 2024]. Disponível em: https://news.bms.com/news/details/2016/European-Commission-Approves-the-First-and-Only-Immuno-Oncology-Combination-Bristol-Myers-Squibbs-Opdivo-nivolumab-Yervoy-ipilimumab-Regimen-for-Treatment-of-Advanced-Melanoma/default.aspx

89. Bristol-Myers Squibb. European Commission Approves Bristol-Myers Squibb's Yervoy (ipilimumab) for Treatment of Pediatric Patients 12 Years and Older with Unresectable or Metastatic Melanoma [Internet]. 2018 [citado 21 de Julho de 2024]. Disponível em: https://news.bms.com/news/corporate-financial/2018/European-Commission-Approves-Bristol-Myers-Squibbs-Yervoy-ipilimumab-for-Treatment-of-Pediatric-Patients-12-Years-and-Older-with-Unresectable-or-Metastatic-Melanoma/default.aspx

90. Bristol-Myers Squibb. European Commission Approves Bristol-Myers Squibb's Opdivo (nivolumab) for the Adjuvant Treatment of Adult Patients with Melanoma with Involvement of Lymph Nodes or Metastatic Disease Who Have Undergone Complete Resection [Internet]. 2018 [citado 21 de Julho de 2024]. Disponível em: https://news.bms.com/news/corporate-financial/2018/European-Commission-Approves-Bristol-Myers-Squibbs-Opdivo-nivolumab-for-the-Adjuvant-Treatment-of-Adult-Patients-with-Melanoma-with-Involvement-of-Lymph-Nodes-or-Metastatic-Disease-Who-Have-Undergone-Complete-Resection/default.aspx

91. Merck. European Commission Approves Merck's KEYTRUDA® (pembrolizumab) as Adjuvant Therapy for Adults with Resected Stage III Melanoma [Internet]. 2018 [citado 21 de Julho de 2024]. Disponível em: https://www.merck.com/news/european-commission-approves-mercks-keytruda-pembrolizumab-as-adjuvant-therapy-for-adults-with-resected-stage-iii-melanoma/

92. Merck. European Commission Approves KEYTRUDA® (pembrolizumab) as Adjuvant Treatment for Adult and Adolescent Patients (≥12 Years of Age) With Stage IIB or IIC Melanoma Following Complete Resection [Internet]. 2022 [citado 21 de Julho de 2024]. Disponível em: https://www.merck.com/news/european-commission-approves-keytruda-pembrolizumab-as-adjuvant-treatment-for-adult-and-adolescent-patients-%E2%89%A512-years-of-age-with-stage-iib-or-iic-melanoma-following-complete-resecti/

93. Bristol-Myers Squibb. Bristol Myers Squibb Receives European Commission Approval for LAG-3-Blocking Antibody Combination, Opdualag (nivolumab and relatlimab), for the Treatment of Unresectable or Metastatic Melanoma with Tumor Cell PD-L1 Expression < 1% [Internet]. 2022 [citado 21 de Julho de 2024]. Disponível em: https://news.bms.com/news/corporate-financial/2022/Bristol-Myers-Squibb-Receives-European-Commission-Approval-for-LAG-3-Blo- cking-Antibody-Com-

bination-Opdualag-nivolumab-and-relatlimab-for-the-Treatment-of-Unresectable-or-Metastatic-Melanoma-with-Tumor-Cell-PD-L1-Expression-1/default.aspx

94. Bristol-Myers Squibb. Bristol Myers Squibb Receives European Commission Approval for Opdivo (nivolumab) as an Adjuvant Treatment for Patients with Completely Resected Stage IIB or IIC Melanoma [Internet]. 2023 [citado 21 de Julho de 2024]. Disponível em: https://news.bms.com/news/details/2023/Bristol-Myers-Squibb-Receives-European-Commission-Approval-for-Opdivo-nivolumab-as-an-Adjuvant-Treatment-for-Patients-with-Completely-Resected-Stage-IIB-or-IIC-Melanoma/default.aspx

95. Oyewole-Said D, Konduri V, Vazquez-Perez J, Weldon SA, Levitt JM, Decker WK. Beyond T-Cells: Functional Characterization of CTLA-4 Expression in Immune and Non-Immune Cell Types. Front Immunol. 15 de Dezembro de 2020;11.

96. Willsmore ZN, Coumbe BGT, Crescioli S, Reci S, Gupta A, Harris RJ, et al. Combined anti-PD-1 and anti-CTLA-4 checkpoint blockade: Treatment of melanoma and immune mechanisms of action. Eur J Immunol. 23 de Março de 2021;51(3):544–56.

97. Obeid JM, Erdag G, Smolkin ME, Deacon DH, Patterson JW, Chen L, et al. PD-L1, PD-L2 and PD-1 expression in metastatic melanoma: Correlation with tumor-infiltrating immune cells and clinical outcome. Oncoimmunology. 20 de Novembro de 2016;5(11):e1235107.

98. Borst J, Busselaar J, Bosma DMT, Ossendorp F. Mechanism of action of PD-1 receptor/ligand targeted cancer immunotherapy. Eur J Immunol. 21 de Agosto de 2021;51(8):1911–20.

99. Vázquez-Montero L, de la Gala M del CÁ, de la Cruz-Merino L. Nivolumab plus ipilimumab in metastatic melanoma: a critical appraisal focused on specific subpopulations. Front Oncol. 19 de Junho de 2023;13.

100. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Lao CD, et al. Long-Term Outcomes With Nivolumab Plus Ipilimumab or Nivolumab Alone *Versus* Ipilimumab in Patients With Advanced Melanoma. Journal of Clinical Oncology. 10 de Janeiro de 2022;40(2):127–37.

101. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. New England Journal of Medicine. 2 de Julho de 2015;373(1):23–34.

102. Albrecht LJ, Livingstone E, Zimmer L, Schadendorf D. The Latest Option: Nivolumab and Relatlimab in Advanced Melanoma. Curr Oncol Rep. 1 de Junho de 2023;25(6):647–57.

103. Huo JL, Wang YT, Fu WJ, Lu N, Liu ZS. The promising immune checkpoint LAG-3 in cancer immunotherapy: from basic research to clinical application. Front Immunol. 26 de Julho de 2022;13.

104. Su J, Fu Y, Cui Z, Abidin Z, Yuan J, Zhang X, et al. Relatlimab: a novel drug targeting immune checkpoint LAG-3 in melanoma therapy. Front Pharmacol [Internet]. 10 de Janeiro de 2024;14. Disponível em: https://www.frontiersin.org/articles/10.3389/fphar.2023.1349081/full

105. Sauer N, Szlasa W, Jonderko L, O-lizło M, Kunachowicz D, Kulbacka J, et al. LAG-3 as a Potent Target for Novel Anticancer Therapies of a Wide Range of Tumors. Int J Mol Sci. 1 de Setembro de 2022;23(17):9958.

106. Chocarro L, Blanco E, Zuazo M, Arasanz H, Bocanegra A, Fernández-Rubio L, et al. Understanding LAG-3 Signaling. Int J Mol Sci. 17 de Maio de 2021;22(10):5282.

107. Gide TN, Paver EC, Yaseen Z, Maher N, Adegoke N, Menzies AM, et al. Lag-3 expression and clinical outcomes in metastatic melanoma patients treated with combination anti-lag-3 + anti-PD-1-based immunotherapies. Oncoimmunology [Internet]. 31 de Dezembro de 2023;12(1). Disponível em: https://www.tandfonline.com/doi/full/10.1080/2162402X.2023.2261248

108. Graydon CG, Mohideen S, Fowke KR. LAG3's Enigmatic Mechanism of Action. Front Immunol [Internet]. 8 de Janeiro de 2021;11. Disponível em: https://www.frontiersin.org/articles/10.3389/fimmu.2020.615317/full

109. Marin-Acevedo JA, Kimbrough EO, Lou Y. Next generation of immune checkpoint inhibitors and beyond. J Hematol Oncol. 19 de Março de 2021;14(1):45.

110. Shan C, Li X, Zhang J. Progress of immune checkpoint LAG 3 in immunotherapy (Review). Oncol Lett. 8 de Setembro de 2020;20(5):1–1.

111. Tawbi HA, Schadendorf D, Lipson EJ, Ascierto PA, Matamala L, Castillo Gutiérrez E, et al. Relatlimab and Nivolumab versus Nivolumab in Untreated Advanced Melanoma. New England Journal of Medicine [Internet]. 6 de Janeiro de 2022;386(1):24–34. Disponível em: http://www.nejm.org/doi/10.1056/NEJMoa2109970

112. Terlecka AM, Rutkowski P, Sobczuk P. LAG-3 as a therapeutic target in melanoma. Oncology in Clinical Practice. 8 de Abril de 2024;

113. Long G V., Stephen Hodi F, Lipson EJ, Schadendorf D, Ascierto PA, Matamala L, et al. Overall Survival and Response with Nivolumab and Relatlimab in Advanced Melanoma. NEJM Evidence [Internet]. 28 de Março de 2023;2(4). Disponível em: https://evidence.nejm.org/doi/10.1056/EVIDoa2200239

114. Schadendorf D, Tawbi H, Lipson EJ, Stephen Hodi F, Rutkowski P, Gogas H, et al. Health-related quality of life with nivolumab plus relatlimab versus nivolumab monotherapy in patients with previously untreated unresectable or metastatic melanoma: RELATIVITY-047 trial. Eur J Cancer. Julho de 2023;187:164–73.

115. Ouwerkerk W, van den Berg M, van der Niet S, Limpens J, Luiten RM. Biomarkers, measured during therapy, for response of melanoma patients to immune checkpoint inhibitors: a systematic review. Melanoma Res [Internet]. Outubro de 2019;29(5):453–64. Disponível em: https://journals.lww.com/10.1097/CMR.0000000000000589

116. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, et al. Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. New England Journal of Medicine. 5 de Outubro de 2017;377(14):1345–56.

117. Antonio Ascierto P, Lipson EJ, Dummer R, Larkin J, Long G V, Sanborn

RE, et al. Nivolumab and Relatlimab in Patients With Advanced Melanoma That Had Progressed on Anti-Programmed Death-1/Programmed Death Ligand 1 Therapy: Results From the Phase I/IIa RELATIVITY-020 Trial. J Clin Oncol [Internet]. 2023;41:2724–35. Disponível em: https://doi.

118. Amaria RN, Postow M, Burton EM, Tetzlaff MT, Ross MI, Torres-Cabala C, et al. Neoadjuvant relatlimab and nivolumab in resectable melanoma. Nature [Internet]. 3 de Novembro de 2022;611(7934):155–60. Disponível em: https://www.nature.com/articles/s41586-022-05368-8

119. Amaria RN, Reddy SM, Tawbi HA, Davies MA, Ross MI, Glitza IC, et al. Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. Nat Med [Internet]. 8 de Novembro de 2018;24(11):1649–54. Disponível em: https://www.nature.com/articles/s41591-018-0197-1

120. M.D. Anderson Cancer Center. Phase II Study of Nivolumab in Combination With Relatlimab in Patients With Active Melanoma Brain Metastases [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT05704647

121. John Kirkwood U of P. Nivolumab, BMS-936558 in Combination With Relatlimab, BMS-986016 in Patients With Metastatic Melanoma Naïve to Prior Immunotherapy in the Metastatic Setting [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT03743766

122. Bristol-Myers Squibb. A Study of Subcutaneous Nivolumab + Relatlimab Fixed-dose Combination (FDC) in Previously Untreated Metastatic or Unresectable Melanoma (RELATIVITY-127) [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT05625399

123. Jose Lutzky MU of M. Nivolumab Plus Relatlimab in Patients With Metastatic Uveal Melanoma [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT04552223

124. H. Lee Moffitt Cancer Center and Research Institute. Neoadjuvant and Adjuvant Anti-PD1 or Combinations for Locoregionally Advanced Melanoma [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT06295159

125. Bristol-Myers Squibb. A Study to Assess Adjuvant Immunotherapy With Nivolumab Plus Relatlimab Versus Nivolumab Alone After Complete Resection of Stage III-IV Melanoma (RELATIVITY-098) [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT05002569

126. Melanoma Institute Australia. A Phase 2 Clinical Trial of Neoadjuvant Relatlimab and Nivolumab in High Risk, Clinical Stage II Cutaneous Melanoma (Neo ReNi II) [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT05418972

127. H. Lee Moffitt Cancer Center and Research Institute. Pilot Study of Nivolumab w/Ipilimumab or Relatlimab in Surgically Resectable Melanoma Brain Metastases [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT05704933

128. California Pacific Medical Center Research Institute. A Study of Con-

current Stereotactic Body Radiotherapy With Opdualag in Metastatic Uveal Melanoma [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT05077280

129. Regeneron Pharmaceuticals. Clinical Study of Fianlimab in Combination With Cemiplimab Versus Pembrolizumab in Adolescent and Adult Patients With Previously Untreated Unresectable Locally Advanced or Metastatic Melanoma [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT05352672

130. Regeneron Pharmaceuticals. A Study With Combinations of Anti-LAG-3 and Anti-PD-1 Antibodies in Adult Participants With Advanced or Metastatic Melanoma [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT06246916

131. Regeneron Pharmaceuticals. A Trial to See if the Combination of Fianlimab With Cemiplimab Works Better Than Pembrolizumab for Preventing or Delaying Melanoma From Coming Back After it Has Been Removed With Surgery [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT05608291

132. Regeneron Pharmaceuticals. A Trial to Learn if Fianlimab and Cemiplimab Are Safe and Work Better Than Anti-PD1 Alone in Adult Participants With Resectable Stage 3 or 4 Melanoma [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT06190951

133. Adil Daud U of CSF. XmAb23104 (PD1 X ICOS) and XmAb22841 (CTLA-4 X LAG3) in Treating Melanoma Prior Immune Checkpoint Inhibitor Therapy [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT05695898

134. Incyte Corporation. Study of Combination Therapy With INCMGA00012 (Anti-PD-1), INCAGN02385 (Anti-LAG-3), and INCAGN02390 (Anti-TIM-3) in Participants With Select Advanced Malignancies [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT04370704

135. Hoffmann-La Roche. Dose Escalation Study of a PD1-LAG3 Bispecific Antibody in Patients With Advanced and/or Metastatic Solid Tumors [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT04140500

136. Incyte Corporation. Study of INCA32459 a LAG-3 and PD-1 Bispecific Antibody in Participants With Select Advanced Malignancies [Internet]. [citado 30 de Julho de 2024]. Disponível em: https://clinicaltrials.gov/study/NCT05577182