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Analysis of clinical and histopathological features in patients affected by advanced cutaneous squamous cell carcinoma: a prospective study to investigate possible correlations with response to cemiplimab

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1. INTRODUCTION

1.1 Cutaneous Squamous Cell Carcinoma: Clinical Relevance and Epidemiology

Cutaneous squamous cell carcinoma (cSCC) accounts for approximately 20–25% of all cutaneous malignancies and represents the second most common form of keratinocyte skin cancer worldwide, after basal cell carcinoma.[1–4] Despite its high prevalence, the true global incidence of cSCC remains difficult to define accurately. Epidemiologic data are often incomplete, as non-melanoma skin cancers (NMSC) are not systematically recorded in many national cancer registries and are frequently aggregated with mucosal squamous cell carcinomas.[2,5] The National Comprehensive Cancer Network (NCCN) highlights that this underreporting contributes to an underestimation of the overall disease burden, particularly in elderly and immunosuppressed populations.[1]

At a global level, incidence rates of cSCC have increased steadily over recent decades, particularly in fair-skinned populations, reflecting cumulative ultraviolet (UV) radiation exposure, population aging, and the growing number of immunosuppressed individuals.[2–4]

The highest incidence rates have been reported in Australia, where intense UV exposure and a predominantly fair-skinned population contribute to a substantial disease burden.[6] In 2002, the overall incidence of cSCC in Australia was estimated at 387 cases per 100,000 individuals, with a marked increase observed since the mid-1980s.

In the United States, cSCC shows a steadily increasing incidence. Population-based data from Olmsted County, Minnesota, demonstrated an approximately 263% increase in cSCC incidence between the periods 1976–1984 and 2000–2010.[7] Accurate national incidence and mortality rates remain difficult to define, as non-melanoma skin cancers are not systematically included in U.S. cancer registries, and available epidemiologic data are largely derived from regional studies and indirect analyses.

Additional insight into the disease burden is provided by nationwide claims-based analyses. Using administrative data, Rogers et al. estimated that in 2012 approximately 5.4 million non-melanoma skin cancers were treated in the United States, affecting about 3.3 million individuals. Within the Medicare fee-for-service population, comprising predominantly individuals aged 65 years or older, age-adjusted treatment rates for squamous cell carcinoma were comparable to those for basal cell carcinoma, underscoring the substantial burden of cSCC among elderly patients.[8]

Across Europe, epidemiologic data indicate substantial geographic variability in the incidence of cutaneous squamous cell carcinoma. A multicenter analysis of white European populations reported the highest incidence rates in Northern and Western regions, including South Wales (31.7 per 100,000 person-years) and Switzerland (28.9 per 100,000 person-years), with lower rates observed in Southern and Eastern Europe, such as Croatia (8.9 per 100,000 person-years).[9]

This heterogeneity is likely influenced not only by differences in ultraviolet radiation exposure and demographic characteristics, but also by variability in national cancer registration practices and reporting systems. European reviews emphasize that non-melanoma skin cancers, including cSCC, remain inconsistently captured across countries, leading to underestimation of true incidence and limiting cross-country comparisons.[5]

Within this international context, epidemiologic data from Italy remain limited due to the absence of a unified national registry for non-melanoma skin cancers. Estimates derived from regional sources provide partial insight into disease burden. Data from the Cutaneous Cancer Registry of the Trentino region reported an incidence of 29 cases per 100,000 inhabitants between 1993 and 1998.[10] More recent data from the Italian Association of Cancer Registries (AIRTUM) reported an annual incidence of 19.46 cases per 100,000 for SCC of the head and neck region, including cutaneous variants.[11] Furthermore, Italian cancer

registry estimates suggested that approximately 19,000 new cases of cutaneous squamous cell carcinoma were diagnosed in 2018, with a marked male predominance, higher incidence after the age of 65 years, and a characteristic north–south geographic gradient.

Despite being traditionally regarded as a malignancy with relatively low per-case mortality, cutaneous squamous cell carcinoma is associated with a clinically meaningful risk of disease-specific death at a population level. Analyses from the United States estimated that in 2012 between 4,000 and 9,000 patients died from cSCC, with mortality rates in certain geographic regions approaching those of melanoma and other solid malignancies.[12] International clinical guidelines emphasize that this apparent paradox reflects the high incidence of cSCC: while the majority of tumors follow an indolent clinical course, a clinically relevant subset exhibits aggressive behavior, with a significant risk of local recurrence, regional nodal metastasis, and disease-specific mortality.[1]

Overall, cSCC predominantly affects elderly individuals, shows a consistent male predominance, and continues to increase in incidence worldwide. Although disease-specific mortality remains lower than that of melanoma on a per-case basis, the absolute number of cSCC-related deaths and the growing proportion of advanced and high-risk tumors underscore the increasing clinical and public health relevance of this malignancy, as highlighted by both epidemiologic studies and international clinical guidelines.

1.2 Risk factors for the development of cutaneous squamous cell carcinoma

The development of cutaneous squamous cell carcinoma is strongly associated with a combination of environmental, host-related, and tumor-specific factors. Among environmental exposures, chronic ultraviolet (UV) radiation represents the most important and well-established risk factor, particularly cumulative lifetime exposure rather than intermittent intense exposure.[2,4,7] Tumors typically arise on chronically sun-exposed areas,

such as the head and neck, scalp, and dorsal surfaces of the hands, supporting the role of long-term UV-induced DNA damage.

Advanced age is a major risk factor for cSCC, reflecting cumulative UV exposure and age-related decline in immune surveillance. The disease predominantly affects individuals older than 60-65 years and shows a consistent male predominance, which has been attributed to both behavioral and biological factors.

Immunosuppression represents one of the strongest host-related risk factors for cSCC. Solid organ transplant recipients and patients receiving long-term immunosuppressive therapy exhibit a markedly increased incidence of cSCC, with more aggressive tumor behavior, higher recurrence rates, and increased risk of nodal and distant metastasis.[1,2,7] International clinical guidelines emphasize that cSCC arising in immunocompromised patients should be considered intrinsically high risk.

Additional recognized risk factors include fair skin phototypes, a history of actinic keratoses, chronic inflammatory or scarred skin, and exposure to ionizing radiation or chemical carcinogens, such as arsenic. The concept of field cancerization, characterized by widespread UV-induced genetic alterations in chronically sun-damaged skin, provides a biological explanation for the frequent occurrence of multiple primary cSCCs in the same patient.[7]

Certain viral infections, particularly cutaneous human papillomavirus (HPV), have been implicated as potential co-factors in cSCC development, especially in immunosuppressed individuals; however, their exact role in carcinogenesis remains incompletely defined.[2,7]

1.3 Molecular basis for cutaneous squamous cell carcinoma development

Cutaneous squamous cell carcinoma arises through the cumulative acquisition of UV-induced genetic and epigenetic alterations that progressively disrupt keratinocyte differentiation, cell-cycle control, and genomic integrity.[13]

Chronic UVB exposure leads to the formation of cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts, generating a characteristic *UV signature* mutational pattern marked by C→T and CC→TT transitions. These alterations are prominently observed in key tumor suppressor genes, including *TP53* and *NOTCH*, in both actinic keratoses and invasive cSCC.[14]

Early loss-of-function mutations in *TP53* impair DNA damage–induced cell-cycle arrest and apoptosis, permitting clonal expansion of genomically unstable keratinocytes. Concurrently, inactivating mutations in *NOTCH1* and *NOTCH2*, now recognized as central epidermal tumor suppressors, further enhance proliferation and impair terminal differentiation.[14,15]

Additional recurrent driver events involve signaling pathways such as RAS/MAPK and PI3K/AKT, telomere maintenance mechanisms (including *TERT* promoter mutations), and cell-cycle regulators (e.g., *CDKN2A* and *RB*), as well as alterations in chromatin-remodeling complexes (e.g., SWI/SNF, *ARID2*). Together, these changes promote progression from actinic keratosis to in situ carcinoma and ultimately to invasive and metastatic disease.[16,17]

Recent genomic studies further highlight the exceptionally high mutational burden of cSCC and a branched evolutionary architecture, whereby UV-imprinted field cancerization of chronically sun-exposed skin provides a reservoir of mutated keratinocyte clones.[18] From this background, aggressive cSCC subclones may emerge under additional selective pressures, including immunosuppression, chronic inflammation, and microenvironmental factors.

1.4 Histologic subtypes of cutaneous squamous cell carcinoma

Cutaneous squamous cell carcinoma encompasses a heterogeneous group of tumors characterized by varying degrees of keratinocyte differentiation and architectural growth patterns. While most cSCCs share common histopathologic features, several distinct

histologic subtypes have been described, reflecting divergent morphologic differentiation pathways and, in some cases, differing clinical behavior.[1,4,7]

The conventional (keratinizing) subtype represents the most common form of cSCC. It is composed of atypical squamous cells arranged in nests, cords, or sheets extending from the epidermis into the dermis, with variable degrees of keratinization and formation of keratin pearls. Tumors may range from well-differentiated lesions, closely resembling normal squamous epithelium, to poorly differentiated carcinomas with marked cytologic atypia and minimal keratin production.

The acantholytic (adenoid) subtype is characterized by prominent acantholysis within tumor nests, resulting in pseudoglandular spaces. Although historically considered a distinct variant, this pattern is now regarded as a morphologic phenotype rather than a separate biological entity.

The spindle cell (sarcomatoid) cSCC is composed predominantly of elongated, spindle-shaped tumor cells with minimal squamous differentiation. These tumors often exhibit a fascicular growth pattern and may lack overt keratinization, frequently requiring immunohistochemical staining for cytokeratins to establish the diagnosis. Spindle cell cSCC commonly arises in chronically sun-damaged skin or in previously irradiated areas and represents a diagnostically challenging variant due to its resemblance to true sarcomas or other spindle cell neoplasms.

The desmoplastic cSCC is characterized by infiltrative strands and cords of atypical squamous cells embedded within a dense desmoplastic stroma. This growth pattern often results in ill-defined tumor margins and deep tissue infiltration. The desmoplastic subtype may coexist with perineural involvement and frequently demonstrates subtle cytologic atypia, potentially leading to under-recognition on superficial biopsies.

The verrucous carcinoma represents a well-differentiated, low-grade variant characterized by exophytic, papillomatous architecture, pushing rather than infiltrative margins, and minimal cytologic atypia. Despite its locally aggressive growth, verrucous carcinoma rarely metastasizes, and its distinction from conventional cSCC has important diagnostic and therapeutic implications.[1]

The adenosquamous carcinoma is a rare variant defined by the presence of both squamous and glandular differentiation within the same tumor. This subtype is uncommon in the skin and must be distinguished from metastatic adenocarcinoma or primary adnexal neoplasms.

Finally, keratoacanthoma-type cSCC is characterized by a rapidly growing, dome-shaped lesion with a central keratin-filled crater and well-differentiated squamous epithelium. Histologically, these lesions display well-differentiated squamous proliferation with glassy eosinophilic cytoplasm, pushing rather than infiltrative margins, and a symmetrical architecture. Although keratoacanthoma has historically been regarded as a distinct benign entity with potential for spontaneous regression, current NCCN guidelines consider it within the spectrum of cutaneous squamous cell carcinoma.

1.5 Histopathologic prognostic features and risk stratification

Although histologic subtypes provide valuable morphologic classification, increasing evidence suggests that specific histopathologic features, rather than histologic subtype alone, play a more decisive role in determining tumor aggressiveness and clinical outcome.

Among tumor-related factors, tumor thickness and depth of invasion represent the most powerful and consistently validated prognostic indicators. Increasing thickness, particularly beyond 2 mm, and invasion extending beyond the dermis into subcutaneous tissue or deeper structures are strongly associated with higher risks of local recurrence, regional nodal metastasis, and disease-specific mortality. Thompson et al. demonstrated that invasion

beyond subcutaneous fat confers the highest relative risk for both metastatic spread and disease-specific death, underscoring the central prognostic relevance of depth of invasion.[19]

The degree of histologic differentiation is another well-established prognostic factor, with poorly differentiated tumors (G3) showing significantly higher rates of recurrence and metastasis compared with well (G1) or moderately (G2) differentiated lesions.[7,19] The loss of squamous differentiation likely reflects underlying genomic instability and aggressive tumor biology.

Perineural invasion (PNI) is a critical adverse feature associated with increased risks of local recurrence, nodal metastasis, and disease-specific mortality. The prognostic impact of PNI seems to be influenced by nerve caliber and extent of involvement, with invasion of larger or named nerves conferring particularly high risk.

Lymphovascular invasion, although less frequent, similarly reflects early dissemination potential and is associated with poorer outcomes, supporting its inclusion among high-risk pathologic features.

Tumor size, particularly lesions exceeding 20 mm in greatest dimension, independently correlates with increased risks of recurrence, metastasis, and disease-specific death. In addition, tumors arising in specific anatomic locations – including the ear, lip, temple, and other regions of the head and neck – are consistently associated with worse outcomes, likely reflecting complex anatomic and lymphatic factors that facilitate tumor spread.

Host-related factors further modify prognosis. Immunosuppression, especially in solid organ transplant recipients, is associated with higher rates of recurrence, metastatic progression, and cSCC-related mortality, even in tumors lacking classic high-risk histopathologic features. For this reason, NCCN considers immunosuppression an independent high-risk factor warranting intensified management and surveillance.

Current guidelines emphasize an integrated assessment of tumor depth, differentiation, perineural and lymphovascular invasion, size, anatomic location, and immune status to identify patients at increased risk for adverse outcomes and to inform staging, nodal evaluation, adjuvant therapy, and follow-up strategies.[1]

1.6 Staging systems for cutaneous squamous cell carcinoma

Accurate staging of cutaneous squamous cell carcinoma is central to prognostic assessment, risk stratification, and treatment planning. The formal anatomic staging of cSCC is defined by the American Joint Committee on Cancer (AJCC) TNM system, which is designed to classify tumors into categories that are prognostically distinct, homogeneous, and monotonic with respect to clinical outcomes. According to AJCC principles, tumor (T) classification is primarily based on anatomic extent, including tumor size, depth of invasion and bone invasion, while nodal (N) and metastatic (M) staging follow standard oncologic criteria.

However, multiple outcome-based analyses demonstrated important limitations of AJCC tumor staging when applied to cSCC. Validation studies of the AJCC 7th edition revealed that advanced T categories (T3–T4), characterized by bone involvement, were exceedingly rare in clinical practice, whereas most adverse outcomes, including local recurrence, nodal metastasis, and disease-specific death, clustered within AJCC T2 tumors. This resulted in substantial heterogeneity within intermediate stages and limited prognostic discrimination, as tumors with markedly different biological behaviors were assigned the same AJCC T category.

To address these shortcomings, investigators at Brigham and Women's Hospital developed an alternative staging system specifically designed for cutaneous squamous cell carcinoma. Through retrospective cohort analyses, four histopathologic features were identified as independent predictors of poor outcome: tumor diameter ≥2 cm, poor differentiation, perineural invasion, and invasion beyond subcutaneous fat.[20] Based on the cumulative

number of these adverse features, tumors were stratified into low-risk (T1, T2a) and high-risk (T2b, T3) categories, thereby subdividing the heterogeneous AJCC T2 group.

This approach was subsequently validated in a larger cohort study directly comparing AJCC, Union for International Cancer Control (UICC), and Brigham and Women's Hospital (BWH) staging systems.[21] In that analysis, only 5% of tumors were classified as high stage (T2b/T3) according to the BWH system, yet these accounted for approximately 60% of all poor outcomes, including 70% of nodal metastases and more than 80% of disease-specific deaths. In contrast, AJCC staging concentrated most adverse events within lower T categories, confirming limited homogeneity and monotonicity. Collectively, these studies demonstrated that the BWH system provided superior prognostic discrimination for aggressive cSCC compared with AJCC staging alone.

To address some of the limitations of earlier staging systems, the 8th edition of AJCC Cancer Staging Manual later introduced a dedicated classification for cutaneous squamous cell carcinoma of the head and neck and incorporated several biologically meaningful parameters into tumor staging.[22] These included formal integration of depth of invasion, refined definitions of clinically significant perineural invasion (defined by involvement of nerves measuring ≥ 0.1 mm in caliber or by invasion of deep or named nerves), and a more detailed characterization of bone involvement. Collectively, these changes improved the biologic relevance and prognostic performance of AJCC staging compared with earlier editions, although residual heterogeneity within intermediate-stage tumors persisted.

Recognizing the complementary strengths of these frameworks, the National Comprehensive Cancer Network (NCCN) adopted AJCC staging as the formal anatomic classification while emphasizing a feature-based risk stratification model for clinical decision-making. NCCN guidelines categorized tumors as low-risk, high-risk, or very high-risk based on tumor size, depth of invasion, differentiation, perineural involvement,

anatomic location, recurrence status, and host-related factors such as immunosuppression.[1] Importantly, NCCN acknowledged that tumors with early AJCC stage could nonetheless behave aggressively and therefore required intensified evaluation, including imaging, nodal assessment, and adjuvant radiotherapy.

Contemporary assessment of cutaneous squamous cell carcinoma should therefore rely on an integrated interpretation of these systems to accurately identify patients at increased risk for recurrence, metastasis, and disease-specific mortality.

1.7 Surgical management of the primary cutaneous squamous cell carcinoma

Surgical excision represents the cornerstone of treatment for primary cSCC and remains the modality with the highest likelihood of achieving durable local control. The primary goal of surgery is complete tumor removal with histologically negative margins while preserving function and minimizing morbidity, particularly in anatomically sensitive areas.

The choice of surgical technique and the extent of resection are guided by tumor-related risk factors, anatomic location, and patient characteristics. Wide local excision is the most commonly employed approach for primary cSCC and is appropriate for the majority of low-risk tumors. In contrast, tumors with high-risk features, lesions located in cosmetically or functionally critical areas (such as the eyelids, nose, lips, ears and genital region), or tumors with ill-defined clinical borders require more meticulous margin control.

Current clinical guidelines recommend risk-adapted surgical margins. For low-risk cSCC, excision with clinical margins of 4–6 mm is generally sufficient to achieve histologic clearance in most cases. In contrast, high-risk tumors require wider margins, typically at least 6–10 mm, reflecting the increased likelihood of subclinical extension.

Mohs micrographic surgery (MMS) plays a central role in the management of selected cSCCs, particularly those arising in the head and neck region, recurrent tumors, and lesions exhibiting high-risk histopathologic features. MMS offers the advantage of complete

circumferential and deep margin assessment, enabling maximal tissue preservation while ensuring oncologic clearance. This approach is especially valuable for tumors with aggressive growth patterns, perineural involvement, or poorly defined clinical margins, where conventional excision carries a higher risk of residual disease.

Histopathologic evaluation of the surgical specimen is critical not only for confirmation of complete excision but also for definitive risk stratification. Assessment of margin status, depth of invasion, degree of differentiation, and the presence of perineural or lymphovascular invasion directly influences subsequent management decisions. Positive or close surgical margins are associated with an increased risk of local recurrence and warrant further intervention. When feasible, re-excision is preferred to achieve clear margins. In cases where re-excision is not possible due to anatomic constraints or patient-related factors, adjuvant radiotherapy represents an alternative strategy for local control. Thus, definitive surgical excision serves both therapeutic and diagnostic purposes in the management of cSCC.

1.8 Nodal assessment in cutaneous squamous cell carcinoma

Assessment of regional lymph nodes represents a critical component of staging and risk stratification in cutaneous squamous cell carcinoma, as the presence of nodal metastasis is one of the strongest predictors of disease-specific mortality. The risk of lymph node involvement varies widely according to tumor-related and host-related factors and is generally low in patients with low-risk primary tumors but increases substantially in the presence of high-risk features.

Initial nodal evaluation relies on careful clinical examination of the regional lymphatic basins, with particular attention to size, consistency, fixation, and tenderness of lymph nodes. In patients with low-risk cSCC and no clinical suspicion of nodal disease, routine imaging or invasive nodal assessment is not recommended. In contrast, patients with high-

risk or very high-risk tumors require a more thorough evaluation, as occult nodal metastases may be present despite a clinically negative examination.

Ultrasound (US) examination of regional lymph nodes is widely used as a first-line imaging modality in high-risk cSCC. Ultrasound allows detection of morphologic features suspicious for metastasis, including cortical thickening, loss of the fatty hilum, round shape, and abnormal vascular patterns, and facilitates image-guided fine-needle aspiration (FNA) or core biopsy when indicated. Compared with clinical examination alone, ultrasound improves sensitivity for early nodal involvement, particularly in the head and neck region.

Cross-sectional imaging with computed tomography (CT) or magnetic resonance imaging (MRI) is indicated in selected patients, especially those with clinically suspicious lymph nodes, bulky primary tumors, deep invasion, or extensive perineural involvement. Positron emission tomography combined with CT (PET-CT) may be considered in patients with very high-risk tumors or when advanced locoregional or distant disease is suspected, although its routine use in clinically node-negative patients remains controversial.

In patients with clinically and radiologically negative lymph nodes but a substantial estimated risk of occult metastasis, sentinel lymph node biopsy (SLNB) may be considered. SLNB aims to detect subclinical nodal disease at an early stage and is generally reserved for selected high-risk tumors, such as those with multiple adverse histopathologic features or advanced risk-based staging categories. The role of SLNB in cSCC remains an area of active investigation, and its use is typically individualized within a multidisciplinary setting.

1.9 Treatment of locoregional disease

Management of locoregional cutaneous squamous cell carcinoma is guided by the extent of nodal involvement, resectability of disease, and the presence of adverse pathologic features. Locoregional disease includes clinically or radiologically involved regional lymph nodes,

with or without residual or recurrent primary tumor, and represents a distinct clinical entity associated with a significantly worse prognosis compared with localized cSCC.

When regional nodal metastases are identified and disease is considered surgically resectable, surgical management of the nodal basin represents the preferred initial approach. Therapeutic lymph node dissection is performed according to the anatomic drainage of the primary tumor and aims to achieve complete removal of involved nodal disease. Pathologic evaluation of the nodal specimen provides essential prognostic information, including the number of positive nodes, size of metastatic deposits, and the presence of extranodal extension (ENE). In cutaneous squamous cell carcinoma of the head and neck with locoregional progression, treatment typically consists of excision of the primary tumor combined with ipsilateral or bilateral neck dissection, depending on the laterality of nodal involvement. In cases of intraparotid lymph node metastases, parotidectomy (typically superficial parotidectomy) is performed, together with ipsilateral neck dissection when clinically indicated.

Adjuvant radiotherapy is commonly indicated following nodal dissection in patients with high-risk pathologic features, such as multiple involved lymph nodes (≥2), large nodal metastases (>3 cm), extranodal extension or incomplete nodal disease resection with positive surgical margins. In this setting, radiotherapy improves locoregional control and reduces the risk of nodal recurrence. The addition of concurrent systemic therapy is considered in cases of extranodal extension or incompletely excised nodal disease.

Radiotherapy may also be considered for patients with unresectable nodal disease or for those who are not surgical candidates due to medical comorbidities, with either definitive or palliative intent depending on disease extent.

In cases of recurrence following prior surgery and radiotherapy, systemic therapy may be considered.

Overall, treatment decisions are determined within a multidisciplinary setting and take into account disease burden, prior treatments, and patient-related factors.

1.10 Radiotherapy

Radiotherapy plays an important role in the management of cutaneous squamous cell carcinoma and may be delivered with adjuvant, definitive, or palliative intent, depending on disease extent, resectability, and pathologic risk factors.[23]

In the adjuvant setting, radiotherapy is indicated following surgical excision of the primary tumor in the presence of high-risk features, including close or positive margins when re-excision is not feasible, extensive perineural invasion, or deep invasion. Conventional fractionation using 1.8–2 Gy per fraction is most commonly employed, with total doses corresponding to a BED_{10} of approximately 60–79 Gy. In clinical practice, this typically translates into total doses of 60–66 Gy.[24] Hypofractionated regimens may be considered in selected patients, particularly older individuals or those unable to undergo a protracted treatment course, and generally deliver BED_{10} values ranging from approximately 48 to 70 Gy.[25]

Definitive external beam radiotherapy (EBRT) represents an alternative to surgery in patients with unresectable disease, those who are not surgical candidates, or when surgery would result in unacceptable functional or cosmetic morbidity. With conventional fractionation, definitive treatment of the primary tumor typically delivers BED_{10} values of approximately 70–84 Gy, corresponding to total doses of 60–70 Gy, depending on tumor size and extent. Hypofractionated definitive schedules may be used in selected cases, particularly for small tumors or in frail patients, provided adequate biologic dose is achieved.[26]

For regional nodal disease, postoperative radiotherapy is indicated after lymph node dissection in the presence of adverse nodal features. Gross residual nodal disease is typically

treated to doses of 66–70 Gy, whereas dissected nodal basins are commonly treated to approximately 60 Gy using conventional fractionation. Elective irradiation of at-risk nodal basins may also be considered using standard fractionation schedules.[1]

Radiotherapy may also be employed in the management of satellitosis and in-transit metastases, either as adjuvant treatment following resection or as definitive therapy in unresectable disease. In these settings, total doses generally range from 50–60 Gy after resection to 60–70 Gy for unresected disease.

1.11 Systemic therapy

Systemic therapy is primarily indicated in cutaneous squamous cell carcinoma in the setting of unresectable or recurrent locally advanced disease, regional progression not amenable to curative local treatment, and distant metastatic disease. In this context, immunotherapy has largely supplanted traditional cytotoxic chemotherapy as the preferred systemic approach, owing to its more favorable safety profile, lower risk of treatment-related toxicity, and improved tolerability, particularly in the predominantly elderly and comorbid patient population.

Immune checkpoint inhibitors (ICIs), such as cemiplimab and pembrolizumab, represent the preferred first-line agents, supported by prospective clinical trial data demonstrating meaningful response rates and durable disease control. In selected circumstances, cemiplimab may also be administered in the neoadjuvant setting.

Nivolumab, as well as cosibelimab-ipdl, may be considered as secondary options.

For patients who are ineligible for immune checkpoint inhibitors, who experience disease progression during or after immunotherapy, or who have specific contraindications, alternative systemic treatment options may be considered.

Traditional chemotherapy, either as single-agent cisplatin or in combination regimens (e.g., carboplatin and paclitaxel), represents a potential option in selected patients, particularly

when rapid disease control is required. However, responses are generally transient, and treatment-related toxicity limits its use, especially in elderly or frail patients.

Epidermal growth factor receptor (EGFR) inhibitors, such as cetuximab, have demonstrated activity in cSCC and may be considered in selected circumstances, either alone or in combination with chemotherapy.

Participation in clinical trials is strongly encouraged whenever available, particularly for patients with refractory disease or uncommon clinical scenarios.

In selected patients with locoregional disease, systemic therapy may be integrated with surgery or radiotherapy, either in the neoadjuvant, concurrent, or salvage setting. The optimal sequencing and combination of systemic agents with local treatments continues to be investigated and should be individualized based on tumor characteristics and patient factors.

1.12 Rationale, role and molecular mechanisms of immunotherapy

The immune system plays a central role in tumor immune surveillance, a process by which emerging malignant cells are recognized and eliminated through coordinated innate and adaptive immune responses. Effective antitumor immunity relies on the activation of cytotoxic T lymphocytes capable of identifying tumor-associated antigens presented on major histocompatibility complex (MHC) molecules.

To prevent uncontrolled immune activation and autoimmunity, peripheral immune tolerance mechanisms tightly regulate T-cell activity. Among these mechanisms, immune checkpoint pathways function as inhibitory signals that attenuate T-cell responses once antigen clearance has been achieved. The programmed cell death-1 (PD-1) receptor and its ligand PD-L1 represent a key immune checkpoint axis involved in the maintenance of peripheral tolerance. PD-1 is expressed on activated T cells and, upon engagement with PD-L1

expressed on antigen-presenting cells, delivers inhibitory signals that reduce T-cell proliferation, cytokine production, and cytotoxic function.

However, tumor cells expressing PD-L1 may take advantage of this checkpoint-mediated negative regulation to suppress T-cell activation and evade immune surveillance. Agents targeting the PD-1 pathway, acting as immune checkpoint inhibitors, block the interaction between PD-1 and PD-L1, and restore antitumor surveillance, allowing cytotoxic T cells to eliminate tumor cells.[27]

These agents have demonstrated durable clinical responses with a favorable toxicity profile compared with cytotoxic chemotherapy in the treatment of several cancers, including, but not limited to, melanoma, colorectal cancer, and non-small cell lung carcinoma (NSCLC).

Cutaneous squamous cell carcinoma is a tumor characterized by a high tumor mutational burden, largely driven by ultraviolet-induced DNA damage leading to the generation of multiple neoantigens. Moreover, SCC development is associated with the expansion of regulatory T cells (Tregs; CD4+, CD25+, FOXP3+) that suppress exacerbated immune responses and maintain self-tolerance.[28,29] Experimental depletion of CD25+ Tregs significantly reduced SCC development in murine models, while their accumulation within the tumor microenvironment may contribute to increased aggressiveness of cSCC.[30,31] Together, these biological features provide a strong rationale for the use of immune checkpoint inhibitors, and immunotherapy has emerged as the cornerstone of systemic treatment for advanced cSCC.

Cemiplimab (Libtayo®) is a high-affinity, highly potent human monoclonal antibody directed against PD-1. It was approved in 2018 by the U.S. Food and Drug Administration (FDA) and in 2019 by the European Medicines Agency (EMA) for the treatment of locally advanced and metastatic cSCC, not amenable to surgery or radiotherapy. Its approved indications currently also include locally advanced or metastatic basal cell carcinoma

progressing on or intolerant to Hedgehog pathway inhibitors, as well as other solid tumors in specific regulatory settings, namely NSCLC and cervical cancer.

Among the other ICIs available in the United States for cSCC treatment, pembrolizumab and nivolumab are also authorized in Europe for head and neck squamous cell carcinoma, while cosibelimab-ipdl has not been authorized by the EMA.

Cemiplimab demonstrated promising clinical outcomes, with early response and a favorable safety profile. However, most studies reported an objective response rate between 40 and 60%, and immune-related adverse events were still observed, suggesting that a better understanding of PD-1-mediated immunosuppressive mechanisms is needed to predict patients' response and improve treatment efficacy.[32–34]

1.13 Purpose of the study

The purpose of the study was to evaluate whether there were any differences between patients affected by advanced cSCC responding to cemiplimab and non-responders in terms of patient- and tumor-related clinical and histopathological features. The objective was to define whether some of these features could be considered as predictors of response to immunotherapy.

2. PATIENTS AND METHODS

2.1 Study design and patient selection

This was a prospective observational study, enrolling patients with advanced cSCC, referred to the Institutional Tumor Board for Skin Cancer of Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Italy and treated with cemiplimab (Libtayo®) at the Department of Medical Oncology from November 2022 to December 2025.

Inclusion criteria:

- Histopathological diagnosis of cutaneous squamous cell carcinoma
- Advanced tumor, not amenable to surgery or radiation therapy, or metastatic disease
- Age ≥ 18 years
- Signed informed consent

Exclusion criteria:

- Contraindications to receive cemiplimab
- Previous treatment with anti-PD-1 or anti-PD-L1 therapy
- Concurrent malignancy, unless indolent or non-life threatening (e.g., basal cell carcinoma)
- History of solid organ transplantation
- Pregnancy

Objective response rate (ORR), defined as the proportion of patients achieving either a partial or complete response, was selected as the primary clinical endpoint. Responders were defined as those patients achieving at least partial response as best response to treatment according to both RECIST v1.1 criteria for radiological measurable disease and/or WHO criteria for externally visible tumors.[35]

Patients were assessed for response to therapy every 3 weeks in the case of externally visible tumors. Radiological evaluation was performed every 12 weeks or as clinically indicated.

The following patient-related clinical features were recorded and analyzed: age; sex; Eastern Cooperative Oncology Group Performance Status (ECOG PS); comorbidities: hypertension, coronary artery disease, atrial fibrillation, chronic obstructive pulmonary disease, cerebrovascular disease, diabetes mellitus, dyslipidemia and statin use, chronic kidney disease, hematopoietic disorders, autoimmune diseases, and neuropsychiatric disorders.

Tumor-related features of interest were also analyzed: site of the primary cSCC lesion; histopathological characteristics of the tumor (size, grade, thickness and perineural invasion); TNM staging according to AJCC 8th edition.

Collected data regarding the clinical history included previous treatments (surgery, radiotherapy); relapse; administration of antibiotics within the 3 months preceding the start of immunotherapy; date of cemiplimab initiation; date and reason for discontinuation; response to the treatment; treatment-related adverse events and toxicity grade according to the Common Terminology Criteria for Adverse Events (CTCAE).

Cemiplimab was administered intravenously at the dose of 350 mg every 3 weeks. The treatment was discontinued in case of disease progression or unacceptable toxicity or after 2 years of treatment.

2.2 Statistical analysis

Descriptive statistics were used to summarize baseline variables. Continuous variables were expressed as median and interquartile range (IQR), whereas categorical variables were reported as counts and percentages.

Statistical analyses were performed using the Mann-Whitney U test for ordinal variables, the χ^2 (chi-square) test for association between categorical variables, and Fisher's exact test when expected frequencies were fewer than five.

A univariate logistic regression analysis was performed to evaluate whether patient-related or treatment-related variables were associated with response to immunotherapy. Odds ratios

(ORs) with 95% confidence intervals (CIs) were calculated. Variables of clinical relevance and those showing potential association in the univariate analysis ($p < 0.20$) were included in a multivariate logistic regression model to identify independent predictors of response. Due to the limited sample size, separate multivariate models were constructed to avoid overfitting. A p value < 0.05 was considered statistically significant. Statistical analyses were performed using Jamovi software (version 2.7.23).

2.3 Ethical considerations

The study was conducted in accordance with the ethical standards of the international rules of Good Clinical Practice and the 1964 Declaration of Helsinki and its later amendments. The research protocol received approval by the institutional Ethics Committee.

Every patient, before being enrolled in the study, was asked to sign informed consent. The participants were informed about the purpose of the research, risks and anticipated benefits, and any available alternative procedures. The participants were also informed of the right to abstain from the study or to withdraw consent to participate at any time without reprisal.

The data collected were kept by the investigators, under the protection and responsibility of the coordinator, and will be kept for a maximum of 5 years. The medical information obtained for each patient was considered confidential. Only the investigators had access to the data and results prior to their publication.

3. RESULTS

3.1 Patient population

Between November 2022 and December 2025, a total of 35 patients with advanced cSCC were treated with cemiplimab and enrolled in the study. The median age at treatment initiation was 81 years (IQR 71.5 – 87 years) and 26 patients (74.3%) were male. Several patients presented with relevant comorbidities. These included hypertension (*n* = 22; 62.9%), coronary artery disease (*n* = 4; 11.4%), atrial fibrillation (*n* = 4; 11.4%), chronic obstructive pulmonary disease (*n* = 3; 8.6%), cerebrovascular disease (*n* = 6; 17.1%), diabetes mellitus (*n* = 8; 22.9%), dyslipidemia (*n* = 10; 28.6%), chronic kidney disease (*n* = 5; 14.3%), hematopoietic disorders (*n* = 5; 14.3%), autoimmune diseases (*n* = 2; 5.7%; rheumatoid arthritis and ulcerative colitis), and neuropsychiatric disorders (*n* = 9; 25.7%), including depression, schizophrenia, dementia, Parkinson's disease, and Alzheimer's disease. At treatment initiation, most patients had a good performance status, with 23 patients (65.7%) presenting an ECOG performance status of 0–1, while 12 (34.3%) had an ECOG ≥2. A detailed breakdown is shown in Table 1.

Table 1. Baseline demographic and clinical characteristics of the study population.

	Overall cohort, *n* (%) or median (IQR)
Study population	35
Age, years	81 (71.5-87)
Male sex	26 (74.3%)
ECOG PS 0-1	23 (65.7%)
ECOG PS ≥2	12 (34.3%)
Hypertension	22 (62.9%)
Coronary artery disease	4 (11.4%)
Atrial fibrillation	4 (11.4%)
Chronic obstructive pulmonary disease	3 (8.6%)
Cerebrovascular disease	6 (17.1%)
Diabetes mellitus	8 (22.9%)
Dyslipidemia (statin use)	10 (28.6%)
Chronic kidney disease	5 (14.3%)
Hematopoietic disorders	5 (14.3%)
Autoimmune diseases	2 (5.7%)
Neuropsychiatric disorders	9 (25.7%)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; IQR, interquartile range.

3.2 Tumor characteristics and clinical history

The head and neck region was the most common primary tumor site, accounting for 25 cases (71.4%), followed by the trunk ($n = 4$; 11.4%), the genital and perineal region ($n = 4$; 11.4%), and the lower extremity ($n = 2$; 5.7%).

Tumor differentiation grade reported in pathology reports was G1 in 7 patients (20%), G2 in 14 (40%), G3 in 11 (31.4%). In six patients with recurrent disease, a progressive tumor dedifferentiation was observed across successive resections, with tumors initially reported as G1 and G2 later presenting as G3 in subsequent pathological reports. Perineural invasion was documented in 17 cases (48.6%) and excluded in 5 cases (14.3%). For some patients whose prior resections had been performed at other institutions, information on tumor

grading (*n* = 3; 8.6%) and perineural invasion (*n* = 13; 37.1%) was unavailable, as these prognostic factors had not been documented in the corresponding histopathological reports. According to the AJCC TNM staging system, at the time of treatment initiation, two patients (5.7%) had stage II disease, both presenting with multiple locally recurrent lesions and therefore considered unsuitable for further curative surgery or radiotherapy; thirteen patients (37.1%) had stage III disease; and twenty (57.1%) had stage IV disease. Nineteen patients (54.3%) presented nodal involvement (N+), while only one (2.9%) had radiologically detected distant metastases.

Regarding previous surgical management, 28 patients (80%) had undergone surgical resection of the primary tumor before starting immunotherapy. The median number of surgical procedures prior to immunotherapy was 2 (IQR 1–3). A history of tumor recurrence of the primary cancer was documented in 18 patients (51.4%). Among surgically treated patients, 18 out of 28 (64.3%) had a history of microscopically positive margins (R1) or macroscopically incomplete resections (R2). Furthermore, regional lymph node dissection had been performed in 7 patients (20%). In addition to surgery or as exclusive treatment, radiotherapy had been delivered in 10 patients (28.6%) prior to immunotherapy. Chemotherapy had been administered in 3 patients (8.6%). Full details are summarized in Table 2.

Table 2. Tumor characteristics, staging, and previous oncologic treatments at cemiplimab initiation.

	Overall cohort, *n* (%) or median (IQR)
Primary tumor site:	
• Head and neck	25 (71.4%)
• Trunk	4 (11.4%)
• Genital and perineal region	4 (11.4%)
• Lower extremity	2 (5.7%)
Tumor differentiation grade:	
• G1	7 (20.0%)
• G2	14 (40.0%)
• G3	11 (31.4%)
• Unknown	3 (8.6%)
Perineural invasion:	
• Yes	17 (48.6%)
• No	5 (14.3%)
• Unknown	13 (37.1%)
AJCC stage II	2 (5.7%)
AJCC stage III	13 (37.1%)
AJCC stage IV	20 (57.1%)
Nodal involvement (N+)	19 (54.3%)
Distant metastasis	1 (2.9%)
Recurrent primary tumor	18 (51.4%)
Previous primary-tumor surgery	28 (80.0%)
Number of previous surgical procedures	2 (1-3)
R1/R2 resection among surgically treated patients	18/28 (64.3%)
Regional lymph node dissection	7 (20.0%)
Previous radiotherapy	10 (28.6%)
Previous chemotherapy	3 (8.6%)

Abbreviations: AJCC, American Joint Committee on Cancer; IQR, interquartile range; R1, microscopically positive margin; R2, macroscopically incomplete resection.

3.3 Treatment exposure and response

Cemiplimab was administered intravenously at a dose of 350 mg every 3 weeks.

The median duration of treatment was 5 months (IQR 3–11.5), with a median of 5 cycles administered (IQR 3.5–18.5).

A complete response (CR) was observed in 7 patients (20%), while 8 patients (22.9%) achieved a partial response (PR) as best response. Stable disease (SD) was not reported in any patient (0), whereas 20 patients (57.1%) experienced progressive disease (PD). The ORR and the disease control rate (DCR) were both 42.9%.

At data cutoff, 2 patients (5.7%) were still receiving treatment. Among the remaining 33 patients, treatment discontinuation occurred due to disease progression (*n* = 18; 51.4%), worsening of general condition (*n* = 2; 5.7%), death (*n* = 4; 11.4%), and achievement of sustained clinical response (*n* = 9; 25.7%). Only one death was related to cancer progression, while none were attributable to treatment.

Three patients, having experienced disease progression during cemiplimab treatment, were subsequently re-evaluated for surgical management, despite having been previously considered unsuitable for surgery. Following surgical resection, disease control was achieved; however, these responses were attributed to surgery rather than immunotherapy, and these patients were therefore classified as non-responders for response analysis. Two of them resumed immunotherapy after surgery.

3.4 Safety and adverse events

Nine patients (25.7%) experienced at least one treatment-related adverse event.

All adverse events were mild to moderate (grade 1–2) according to the CTCAE. The most frequent toxicity was skin toxicity (17.1%), presenting as grade 1 pruritus or grade 1–2 rash. Diarrhea (grade 1–2) and fatigue (grade 1–2) were each reported in two patients (5.7%). One patient experienced grade 1 mucositis and one developed grade 2 adrenal insufficiency. Two

patients developed pneumonitis, one of whom required hospitalization; however, given the presumed bacterial etiology and response to antibiotics, these events were not considered immune-related. No patients discontinued treatment due to toxicity, and no treatment-related deaths (grade 5 adverse events) were observed. Further details are provided in Table 3.

Table 3. Cemiplimab treatment exposure, response, discontinuation, and safety.

	Value, n (%) or median (IQR)
Cemiplimab dose and schedule	350 mg intravenously every 3 weeks
Treatment duration, months	5 (3-11.5)
Number of cycles	5 (3.5-18.5)
Best response:	
• Complete response (CR)	7 (20.0%)
• Partial response (PR)	8 (22.9%)
• Stable disease (SD)	0 (0.0)
• Progressive disease (PD)	20 (57.1%)
Objective response rate	42.9%
Disease control rate	42.9%
Treatment status and discontinuation reason:	
• Ongoing treatment at data cutoff	2 (5.7%)
• Disease progression	18 (51.4%)
• Worsening general condition	2 (5.7%)
• Death	4 (11.4%)
• Sustained clinical response	9 (25.7%)
Safety:	
• At least one treatment-related adverse event	9 (25.7%)
• Skin toxicity	6 (17.1%)
• Diarrhea	2 (5.7%)
• Fatigue	2 (5.7%)
• Mucositis	1 (2.9%)
• Adrenal insufficiency	1 (2.9%)
• Discontinuation due to toxicity	0 (0.0)
• Treatment-related deaths	0 (0.0)

3.5 Comparison between responders and non-responders

Baseline characteristics, as well as tumor- and treatment-related variables were compared between responders (CR + PR) and non-responders (SD + PD).

No significant differences were observed between the two groups with regard to age, sex, ECOG performance status, or comorbidities. Statin use showed a trend toward association with response ($p = 0.062$), while tumor differentiation grade was significantly associated with treatment response, with a higher proportion of responses observed in G3 tumors ($p = 0.003$).

Table 4. Distribution of tumor differentiation grade according to response to cemiplimab.

G	Response to cemiplimab		Total
	Non-responders (SD + PD)	Responders (CR + PR)	
G1	6 (85.7%)	1 (14.3%)	7
G2	11 (78.6%)	3 (21.4%)	14
G3	2 (18.2%)	9 (81.8%)	11
Total	19	13	32
Fisher's exact test			***p* = 0.003**

No significant differences were observed between the two groups in terms of other histopathological features, including perineural invasion and tumor thickness. Previous surgical treatment ($p = 0.112$), number of previous surgical procedures ($p = 0.550$), history of positive margins (R1/R2) ($p = 0.703$), and recurrence status at baseline ($p = 0.315$), were not associated with response. Similarly, no significant associations were found with baseline tumor stage ($p = 0.645$) or nodal involvement ($p = 0.506$) prior to cemiplimab initiation. Prior oncologic treatments administered before the start of cemiplimab, including radiotherapy ($p = 0.712$) and chemotherapy ($p = 1$), were also not significantly associated with treatment response.

3.6 Logistic regression analysis

At univariate logistic regression analysis, tumor differentiation grade showed a significant association with response ($p = 0.006$).

Table 5. Univariate logistic regression analysis of the association between tumor differentiation grade and response to cemiplimab.

						95% Confidence Interval	
Predictor	Estimate	SE	Z	p	Odds ratio	Lower	Upper
Intercept	-4.84	1.747	-2.77	.006	0.00787	2.56e-4	0.242
G	2.00	0.734	2.73	**.006**	7.41447	1.76	31.224

Note. Estimates represent the log odds of "Response to cemiplimab" vs "No response to cemiplimab"

When dichotomized, G3 tumors demonstrated increased odds of response compared with G1-G2 tumors (OR 4.37, 95% CI 1.71–11.2, $p = 0.002$).

Table 6. Univariate logistic regression analysis of the association between dichotomized tumor differentiation grade and response to cemiplimab.

						95% Confidence Interval	
Predictor	Estimate	SE	Z	p	Odds ratio	Lower	Upper
Intercept	-2.92	0.921	-3.17	.002	0.0538	0.00885	0.327
G3 vs G1-G2	1.48	0.480	3.08	**.002**	4.3732	1.70841	11.195

Note. Estimates represent the log odds of "Response to cemiplimab" vs "No response to cemiplimab"

No other tumor-related variables were significantly associated with treatment response, although primary tumor site (head and neck versus others) showed a trend toward significance ($p = 0.098$).

Table 7. Univariate logistic regression analysis evaluating primary tumor site (head and neck vs other sites) as a predictor of response to cemiplimab.

						95% Confidence Interval	
Predictor	Estimate	SE	Z	p	Odds ratio	Lower	Upper
Intercept	-1.39	0.791	-1.75	.080	0.250	0.0531	1.18
H&N vs other sites	1.47	0.886	1.65	.098	4.333	0.7630	24.61

Note. Estimates represent the log odds of "Response to cemiplimab" vs "No response to cemiplimab"

However, statin use was associated with response ($p = 0.049$).

Table 8. Univariate logistic regression analysis evaluating statin use as a predictor of response to cemiplimab.

						95% Confidence Interval	
Predictor	Estimate	SE	Z	p	Odds ratio	Lower	Upper
Intercept	-0.754	0.429	-1.76	.079	0.471	0.203	1.09
Statin use	1.601	0.812	1.97	**.049**	4.958	1.009	24.37

Note. Estimates represent the log odds of "Response to cemiplimab" vs "No response to cemiplimab"

At multivariate logistic regression analysis, tumor differentiation grade remained an independent predictor of response (OR 4.04, 95% CI 1.54–10.6, $p = 0.005$), whereas primary tumor site was not significantly associated with response (OR 4.76, 95% CI 0.36–62.35, $p = 0.234$).

Table 9. Primary multivariate logistic regression model including tumor differentiation grade and primary tumor site as predictors of response to cemiplimab.

						95% Confidence Interval	
Predictor	Estimate	SE	Z	p	Odds ratio	Lower	Upper
Intercept	-4.04	1.481	-2.73	.006	0.0176	9.64e-4	0.320
G3 vs G1-G2	1.40	0.493	2.83	**.005**	4.0375	1.537	10.607
H&N vs other sites	1.56	1.312	1.19	.234	4.7637	0.364	62.352

Note. Estimates represent the log odds of "Response to cemiplimab" vs "No response to cemiplimab"

In a secondary multivariate model including tumor differentiation grade and statin use, tumor grade remained significantly associated with response (OR 4.21, 95% CI 1.26–14.06, $p = 0.019$), whereas statin use was not significantly associated (OR 1.13, 95% CI 0.10–13.29, $p = 0.925$).

Table 10. Secondary multivariate logistic regression model including tumor differentiation grade and statin use as predictors of response to cemiplimab.

						95% Confidence Interval	
Predictor	Estimate	SE	Z	p	Odds ratio	Lower	Upper
Intercept	-2.897	0.956	-3.0311	.002	0.0552	0.00848	0.359
G3 vs G1-G2	1.438	0.615	2.3403	**.019**	4.2142	1.26338	14.057
Statin use	0.119	1.259	0.0947	.925	1.1266	0.09552	13.288

Note. Estimates represent the log odds of "Response to cemiplimab" vs "No response to cemiplimab"

4. DISCUSSION

In this prospective observational real-world study, we evaluated clinical, pathological, and treatment-related factors associated with response to cemiplimab in patients with advanced cSCC. The main finding of our analysis is that tumor differentiation grade emerged as the only independent predictor of response. By contrast, no significant associations were observed for age, performance status, comorbidities, perineural invasion, nodal involvement, baseline stage, or previous local and systemic treatments.

Although histologic grade is traditionally regarded as a marker of aggressive tumor biology and adverse prognosis in cSCC, in our cohort poorly differentiated tumors were associated with a higher likelihood of response to PD-1 blockade. This finding is consistent with data reported in mucosal head and neck SCC treated with immune checkpoint inhibitors (pembrolizumab, nivolumab, ipilimumab and durvalumab), where high-grade tumors were associated with a significantly higher probability of achieving a clinically beneficial response compared with low-grade tumors.[36]

A possible explanation lies in the biological characteristics of more dedifferentiated tumors, which may be associated with greater genomic instability, higher tumor mutational burden, and enhanced neoantigen presentation, ultimately increasing immunogenicity and sensitivity to immune checkpoint inhibition. In this context, our results support the hypothesis that tumor biology may be more relevant than conventional clinicopathologic risk stratification in predicting response to immunotherapy.

Another relevant observation is that primary tumor site did not retain an independent association with response in multivariate analysis, although head and neck tumors showed a trend toward significance in univariate testing. This is noteworthy because head and neck cSCC is often considered, in clinical practice and in the literature, a subgroup with favorable responsiveness to immunotherapy, possibly due to the high UV-related mutational burden

and the predominance of chronically sun-damaged lesions in this region.[32] In our series, however, this effect did not remain significant after adjustment for tumor grade. This may suggest that the apparent predictive role of head and neck location is at least partially mediated by underlying biological variables rather than by anatomical site itself. At the same time, the wide confidence intervals observed in the multivariate model indicate that our study may have been underpowered to detect an independent effect of tumor location.

Statin use showed a trend toward a higher likelihood of response at univariate analysis. This finding is consistent with emerging evidence suggesting a potential beneficial effect of statins in patients receiving immune checkpoint inhibitors, possibly due to their immunomodulatory properties.[37] However, this association was not confirmed in multivariate analysis, suggesting that it may be influenced by confounding factors and limited sample size.

Our findings must also be interpreted in the context of the marked heterogeneity of the clinical trajectories observed in this population. Patients with advanced cSCC rarely follow a uniform oncologic pathway. Many had undergone multiple prior resections, recurrent disease, incomplete excisions, radiotherapy, nodal surgery, or systemic treatments before receiving cemiplimab. In some cases, immunotherapy was introduced after local treatment failure; in others, it was administered in a setting initially deemed not amenable to surgery, with subsequent re-evaluation of resectability during treatment. This dynamic and non-linear treatment sequence reflects real-world practice more accurately than highly selected trial populations, but it also complicates response assessment and outcome attribution. In particular, some patients who later achieved disease control after surgery had experienced progression during cemiplimab and were therefore appropriately classified as non-responders for the purpose of response analysis. This methodological choice was necessary

to preserve the internal consistency of the study and to avoid overestimating drug-related benefit.

A further relevant aspect of our cohort is the advanced age and frailty of the treated population. The median age at treatment initiation was 81 years, and a substantial proportion of patients presented with ECOG ≥2 and multiple comorbidities. This reflects the epidemiology of advanced cSCC in routine clinical practice, where patients are frequently elderly, medically complex, and sometimes only partially compliant with prolonged treatment and follow-up schedules. In this setting, the favorable toxicity profile observed in our study is clinically meaningful. No grade 3–4 treatment-related adverse events were recorded, and no patient discontinued therapy because of toxicity. Although the absolute number of adverse events was limited, these findings are consistent with the manageable safety profile reported for cemiplimab in pivotal studies and support its use even in fragile populations not typically represented in registration trials.

4.1 Limitations

The real-world nature of our cohort represents both a strength and a limitation. On the one hand, it increases the clinical relevance of the findings by reflecting daily oncologic practice, where treatment decisions are often influenced by comorbidities, functional decline, social context, and prior therapeutic history. On the other hand, the relatively small sample size, the heterogeneity of prior treatments, and the incompleteness of some histopathological data reduce the statistical power of the analysis and may limit the generalizability of the results. Information on tumor grade and perineural invasion was unavailable in a subset of patients previously treated at outside institutions, and the small number of cases may have prevented the detection of weaker but clinically meaningful associations.

4.2 Future Directions

Future studies should aim to validate these preliminary findings in larger, preferably multicenter cohorts. Increasing the sample size would provide greater statistical power, allow more robust multivariable analyses, and clarify the potential predictive role of tumor differentiation grade in response to cemiplimab. Larger studies should also investigate additional clinical and laboratory biomarkers, including lactate dehydrogenase (LDH) levels and the neutrophil-to-lymphocyte ratio (NLR), as potential predictors of treatment response and survival outcomes.[38] Furthermore, the analysis of the intratumoral and gut microbiomes may provide insight into their potential influence on the tumor immune microenvironment and the efficacy of PD-1 blockade. The integration of clinicopathological characteristics with inflammatory and biochemical markers may ultimately support the development of more accurate models for patient stratification and treatment selection.

5. CONCLUSION

Despite these limitations, our study provides a clinically relevant signal: poor differentiation may identify a subgroup of advanced cSCC more likely to respond to cemiplimab. If confirmed in larger cohorts, this finding may help refine treatment stratification and improve patient selection for immunotherapy. More broadly, our results suggest that histopathologic and biological features deserve greater attention as potential predictors of response, beyond conventional staging parameters alone.

Further studies with larger multicenter cohorts are needed to validate the predictive role of tumor grade and to clarify the interaction between anatomical site, histologic aggressiveness, host factors, and treatment outcomes. A better understanding of these relationships may ultimately support a more personalized use of immunotherapy in advanced cSCC.

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