THE EVOLVING ROLE OF IMMUNOTHERAPY IN NON-MELANOMA SKIN CANCERS

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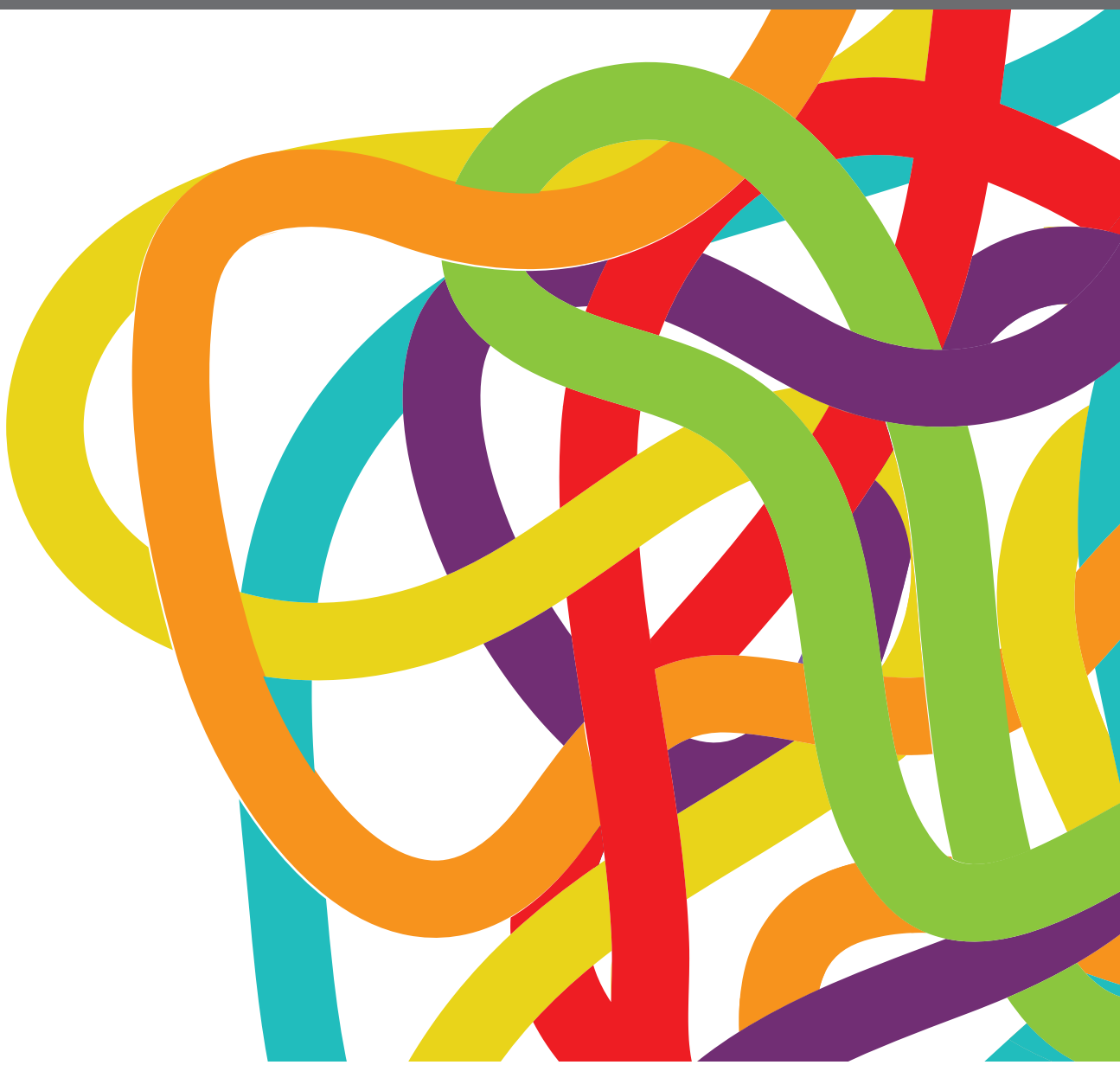

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THE EVOLVING ROLE OF IMMUNOTHERAPY IN NON-MELANOMA SKIN CANCERS

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EDITORIAL
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Editorial: The Evolving Role of Immunotherapy in Non-Melanoma Skin Cancers

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Keywords: non melanoma skin cancer, immunotherapy, PD-1, immune suppression, prognosis

Editorial on the Research Topic

The Evolving Role of Immunotherapy in Non-Melanoma Skin Cancers

Non Melanoma Skin Cancers (NMSCs) represent the most common form of cancer in Caucasians, whose incidence continues to increase all over the world (1, 2). Traditionally, NMSC referred mainly to skin tumours deriving from keratinocytes, thus including basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC), that represent the most frequent subtypes. In addition to BCC and cSCC, this group also includes Merkel cell carcinoma (MCC), dermatofibrosarcoma protuberans, adnexal carcinoma, atypical fibroxanthoma, soft tissue sarcomas including angiosarcoma and, in particular, Kaposi sarcoma, and primary cutaneous lymphoma.

From an epidemiological point of view, BCC represents the most frequent malignant tumour type in humans, followed by cSCC. On the other hand, other NMSCs such as Merkel cell carcinoma or primary cutaneous lymphoma are very rare although their incidence is rapidly increasing. NMSCs mainly affects elderly people, and the most frequent cutaneous sites of development are the head and neck area (BCC, cSCC and MCC). Immunosuppression is an important risk factor for developing these tumours.

Significant differences can be found between these tumour types in terms of disease course and survival. More than 90% of patients with cSCC are disease-free after surgery at a 5-year follow-up, however, a percentage of patients ranging from 1.9% to 4.6% develop disease recurrence or progression (3–5). BCC is more frequent overall than cSCC, and it is characterised by a very indolent disease course, with only 1% of cases progress to advanced disease (6). MCC is characterised by a highly aggressive disease course, as more than half of patients show metastatic disease at the initial diagnosis (7, 8). Survival of metastatic patients in the era pre-check point inhibitors was poor, with 18% at 5 years for distant metastatic disease (9, 10).

Surgery represents the treatment of choice, often associated with radiotherapy. However, in case of locally advanced or metastatic forms (1% of BCCs, in 5% of cSCCs and up to 50% of MCCs) these traditional therapeutic approaches are not sufficient for complete disease control.

In case of advanced disease, systemic therapy is a possible choice. With the advent of immune-checkpoint inhibitors (ICIs), previously unexplored horizons have opened up for these pathologies.

Although the majority of NMSC are treated with conventional surgery and/or radiotherapy, a small percentage of patients progress to locally advanced or metastatic disease, mainly due to patient

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negligence, comorbidities, or immunosuppression. In these circumstances, systemic treatment may be indicated. Until recently, effective therapy remained an area of significant unmet clinical need. Improved understanding of molecular and immune pathogenesis has been critical to driving and developing new therapeutic advances, particularly towards immunotherapy.

The rationale for the application of immunotherapy in NMSC is based on three group of factors: molecular, pathologic and clinical (11–13).

Tumour mutational burden (TMB) measures the quantity of somatic mutations found in a tumour and has been attributed to both endogenous factors and environmental damage. A number of clinical trials have revealed that TMB is correlated with the rate of response to anti-PD-1/PD-L1 blockade (11). Both BCC and cSCC show a marked UV-signature, thus it is conceivable that these cancers exhibit the highest TMB among all other cancer types. The increased expression of neo-antigens which is associated with a high TMB, which likely results in higher levels of tumour neo-antigens that may be targets for the immune system.

As a second point, from a clinical perspective, the mentioned high incidence of NMSC, in particular cSCC and MCC, with conditions of immune-suppression as well as the poor disease course of these cases highlights the relevance of the host immune response in the development and evolution of these diseases. Even if immune environment plays a major role in both BCC and cSCC, probably cSCC presents a higher immunogenicity than BCC in spite of its higher TMB. This theory could also explain the higher incidence of cSCC in immune-suppressed and transplant patients. As a third point, from a pathologic point of view, these tumours are characterised by a significant expression of the PD-1/PDL-1 axis both in tumour cells and microenvironment in the immune infiltrate (12). Moreover, PD-L1 levels had prognostic clinical relevance in as much as patients with a tumour microenvironment type characterised by high expression of both PD-L1 and TILs had the longest survival. Also concerning cutaneous T-cell lymphoma, it has been shown that specific subtypes of these diseases express PD-1 at high levels (14).

All NMSCs are theoretically suitable for immunotherapy; albeit the robustness of their immunological response is quite different. Moreover, in the face of a powerful immune reaction, many patients progress or do not respond to modern immunotherapy due to resistance or immunoescape mechanisms. To date, Cemiplimab for cSCCs (3) and Avelumab (15) for MCCs have been approved by European Medicines Agency (EMA); recently Food and Drug Administration (FDA) approved Cemiplimab for locally advanced Basal cell carcinoma (LaBCC) (16) and other drugs are studied through several clinical trials.

The aim of this Research Topic is to provide clinicians an overview of innovative systemic treatments for NMSC, mainly oriented towards immunotherapy. Adjuvant and neoadjuvant settings, as well as future therapeutic directions, will also be highlighted.

AUTHOR CONTRIBUTIONS

PiQ wrote the text, MG and PaQ contributed to drafting and revising the article. All the authors gave final approval of the version to be published, agreed to the submitted journal, and agree to be accountable for all aspects of the work.

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CASE REPORT
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Case Report: Clinical Experience With Avelumab in Patients With Metastatic Merkel Cell Carcinoma and Brain Metastases Treated in Europe

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Merkel cell carcinoma (MCC) is a rare and aggressive skin cancer that can metastasize rapidly. In patients with metastatic MCC (mMCC), brain metastases are uncommon but are associated with poor prognosis; furthermore, there is limited published literature regarding treatment of these patients, and no specific regimens are currently recommended by guidelines. Avelumab, an anti–programmed death ligand 1 monoclonal antibody, was the first approved treatment for patients with mMCC. Here, we present 4 cases of patients with mMCC and brain metastases treated with avelumab. Patient age ranged from 48 to 70 years, and all patients received avelumab as second-line therapy following disease progression with platinum-based chemotherapy. Patient cases 1 and 2 received avelumab alone and experienced rapid disease progression according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1). In patient case 3, avelumab alone resulted in a prolonged complete response by RECIST 1.1 of 1 brain metastasis and partial response by RECIST 1.1 of a second brain metastasis. After 11 months of avelumab treatment, the patient received concurrent stereotactic radiosurgery that resulted in complete response of the second metastasis. Patient case 4 achieved a partial response by RECIST 1.1 with avelumab plus stereotactic radiosurgery. These results suggest that avelumab followed by radiotherapy or with concurrent radiotherapy may be an effective treatment option for patients with mMCC and brain metastases.

Keywords: Merkel cell carcinoma, brain metastases, avelumab, immunotherapy, stereotactic radiosurgery

Abbreviations: CK, cytokeratin; CT, computed tomography; ECOG PS, Eastern Cooperative Oncology Group performance status; MCC, Merkel cell carcinoma; mMCC, metastatic Merkel cell carcinoma; MRI, magnetic resonance imaging; OS, overall survival; PD, progressive disease; PD1, programmed death 1; PD-L1, programmed death ligand 1; PET, positron emission tomography; Q2W, every 2 weeks; RECIST 1.1, Response Evaluation Criteria in Solid Tumors version 1.1; SRS, stereotactic radiosurgery.

INTRODUCTION

Merkel cell carcinoma (MCC) is a rare neuroendocrine tumor associated with UV radiation exposure, clonal integration of the Merkel cell polyomavirus, and immunosuppression (1). MCC commonly occurs in sun-exposed areas of the body such as the head and neck region (1). However, in approximately 4-5% of all patients and 28-40% of those with clinically detectable nodal disease, the primary lesion cannot be identified; these cases are associated with a more favorable prognosis (1–3).

MCC is an aggressive disease that can metastasize early (4); at diagnosis, approximately 26% and 8% of patients have nodal and distant metastatic MCC (mMCC), respectively (2). Metastases usually arise in the lymph nodes, skin, bone, lung, or liver (4). Brain metastases are less common and occur in approximately 7-13% of patients with distant mMCC (4–6). In patients with MCC, the occurrence of brain metastases is associated with a poor prognosis, with a median overall survival (OS) of approximately 2 years without neurosurgery (6). There are limited published data on patients with mMCC and brain metastases, and many trials in MCC exclude this subset of patients (6). Furthermore, there are no treatment options recommended by guidelines specifically for patients with mMCC and brain metastases (4, 7); however, a possible survival benefit has been suggested for patients who receive surgery or radiotherapy (6).

Avelumab, an anti–programmed death ligand 1 (PD-L1) monoclonal antibody, became the first approved treatment for mMCC based on promising results in the phase 2 JAVELIN Merkel 200 trial (8, 9). Patients with brain metastasis were excluded from this trial. Prior to approval, avelumab showed clinical benefit in a real-world setting in patients with mMCC and limited treatment options (including immunocompromised patients and those with treated brain metastases) in the global expanded access program (10).

Here, we report the clinical experiences of 4 patients with mMCC and brain metastases treated with avelumab in Europe.

PATIENT CASES

The patient cases are summarized in **Table 1**.

Patient Case 1

A 70-year-old woman from Italy with hyperuricemia, arterial hypertension, and hypothyroidism presented with pain and a growing thigh mass and was diagnosed with mMCC of the left thigh and multiple nodal metastases (inguinal and paraaortic/iliac lymph nodes) in October 2016. The patient had a family history of cancer (gastric cancer and leukemia) and had previously received a bilateral total knee prosthesis and undergone a right saphenectomy. Between November 2016 and March 2017, the patient received 6 cycles of chemotherapy (cisplatin 25 mg/m^2 plus etoposide 100 mg/m^2 on days 1-3 every 21 days), with no relevant acute toxicity.

On April 5, 2017, the patient presented with worsened left leg edema, and a subsequent whole-body positron emission tomography (PET) scan showed an increase in the number of metastases of fluorodeoxyglucose uptake compared with the previous PET scan in November 2016, indicating disease progression. On April 12, 2017, a whole-body computed tomography (CT) scan, including the brain, showed progressive disease (PD) by Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) with increased size of the left leg edema and left inguinal lymph node metastases. The patient subsequently received 3 cycles of chemotherapy (VAC regimen: vincristine 2 mg plus doxorubicin 50 mg/m^2 plus cyclophosphamide 1000 mg/m^2 every 21 days); however, the patient experienced toxicity and further PD with an increase in the dimensions and number of abdominal lymph node and pelvic metastases, and cutaneous and subcutaneous nodules. At this time, the patient also developed 1 asymptomatic brain metastasis (largest diameter, 18 mm on October 2, 2017; **Figure 1**). Due to the asymptomatic nature of the brain metastasis, neurosurgery was not considered.

The patient began treatment with avelumab (10 mg/kg intravenously every 2 weeks [Q2W]) on October 4, 2017; at this time, the patient had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 1. Avelumab was well tolerated, with no adverse events reported, and the patient had reduced pain during the first 2-3 infusions; however, after a total of 4 infusions, the treatment was stopped on November 14, 2017, because of further local (left thigh cutaneous lesion) and systemic (brain metastasis) clinical and radiological progression. The patient then received palliative care and died approximately 4 weeks later.

Patient Case 2

A 48-year-old woman from Italy with a family history of cancer (biliary tract) was diagnosed with mMCC of the right inguinal region with nodal (right lumboaortic, retrocrural, inguinal, and common external iliac) and mammary gland metastases in February 2017. The patient had moderate pain which was controlled with paracetamol. No primary cutaneous lesion was identified. Immunohistochemical analysis showed expression of synaptophysin, cytokeratin (CK) 20, and high levels of Ki67 (70%). Between March and July 2017, the patient underwent 6 cycles of chemotherapy (cisplatin 30 mg/m^2 plus etoposide 100 mg/m^2 on days 1-3 every 21 days), with no relevant toxicity.

On August 18, 2017, a CT scan showed PD by RECIST 1.1 of the nodal, lung, and bone regions and the appearance of an asymptomatic meningeal metastasis close to the ethmoid region. Due to the unusual location of the brain metastasis, the patient was considered too high risk for neurosurgery. The patient was enrolled in the avelumab global expanded access program on August 30, 2017 and began treatment with avelumab (10 mg/kg intravenously Q2W); at this time, the patient had an ECOG PS of 2. Avelumab was well tolerated, with a reduction in pain and no adverse events reported; however, despite initial stable disease, the patient experienced rapid PD (including the meningeal metastasis) by RECIST 1.1 after 5 infusions. Avelumab treatment was

TABLE 1 | Summary of patient cases.

	Patient case 1	Patient case 2	Patient case 3	Patient case 4
Sex	Female	Female	Female	Male
Age, years	70	48	67	66
Comorbidities	Hyperuricemia, arterial hypertension, hypothyroidism (treated with hormone replacement therapy)	None	None	High blood pressure, depression, dyslipidemia
Date of diagnosis of mMCC	October 2016	February 2017	January 2017	March 2016 (confirmed in June 2017)
Site of primary lesion	Left thigh	Unknown	Unknown	Unknown
Site of metastases at baseline	Inguinal and paraaortic/iliac nodes	Right lumboaortic, retrocrural, inguinal, and common external iliac nodes	Left axilla, neck, and supraclavicular nodes	Retroclavicular, retropectoral, and right axillary nodes
Treatment before avelumab	Cisplatin + etoposide	Cisplatin + etoposide	Carboplatin + etoposide, surgery	Surgery, radiotherapy, carboplatin + etoposide
Date brain metastases identified	October 2, 2017	August 18, 2017	May 9, 2018	December 31, 2017
No. of brain metastases	1	1	2	1
Symptoms associated with brain metastases	None	None	None	None
ECOG PS at start of avelumab treatment	1	2	0	1
Date of first avelumab dose	October 4, 2017	August 30, 2017	June 26, 2018	December 26, 2017
Avelumab treatment	2L monotherapy	2L monotherapy	2L + SRS	2L with concurrent SRS
Approximate duration of avelumab treatment at last follow-up, months	1	2	17	15
Best response to avelumab per RECIST 1.1	Progressive disease	Stable disease	Complete response in 1 metastasis; partial response in 1 metastasis*	Partial response†
Toxicity associated with avelumab	None	None	Sinus node disease probably related to avelumab treatment	None
Subsequent treatment	Palliative care	Radiotherapy + chemotherapy, palliative care	Radiotherapy	Nivolumab with concurrent SRS
Avelumab treatment ongoing at last follow-up	No	No	No	No
Vital status at last follow-up (date)	Died	Died	Alive (November 2020)	Alive (March 2021)

2L, second line; mMCC, metastatic Merkel cell carcinoma; RECIST 1.1, Response Evaluation Criteria in Solid Tumors version 1.1; SRS, stereotactic radiosurgery.

**Patient achieved a partial response in 1 brain metastasis with avelumab, this metastasis then progressed (not confirmed by RECIST 1.1) and the patient received SRS with avelumab and later achieved a complete response by RECIST 1.1 in that metastasis.*

†Patient achieved a complete response following subsequent nivolumab treatment.

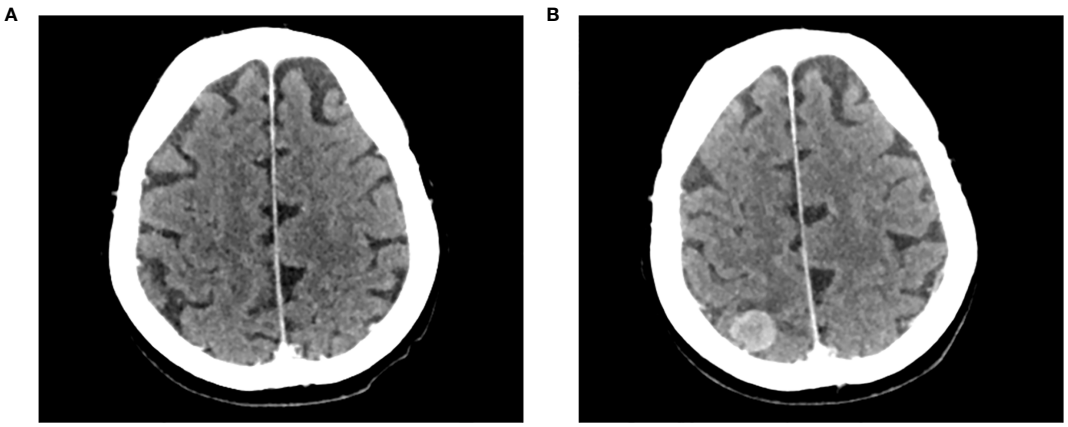

FIGURE 1 | Development of an asymptomatic brain metastasis in patient case 1. Computed tomography brain scans of patient case 1 prior to initiating treatment with avelumab **(A)** on August 16, 2017, and **(B)** on October 2, 2017.

subsequently stopped on October 25, 2017. The patient then underwent radiotherapy (39 Gy in 13 fractions) for the inguinal metastasis and received subsequent chemotherapy (oral etoposide 50 mg on days 1-14 every 28 days); however, by February 2018, the patient had experienced further PD. The patient was then referred to palliative care and died approximately 5 months later.

Patient Case 3

A 67-year-old British woman with a history of congenital jejunal diverticular bleeding (treated with resection) was diagnosed with mMCC in January 2017, after presenting with a large left axillary mass and lymphoedema; no primary site was identified. The patient did not have a family history of cancer. Immunohistochemical analysis showed expression of synaptophysin, CD56, CK7, and CK20 and no expression of thyroid transcription factor 1, calcitonin, or CDX-2. A PET-CT scan revealed high fluorodeoxyglucose uptake in the left axilla (largest node, 39 mm), neck, and supraclavicular nodes. No primary skin lesion was identified. The patient received 4 cycles of neoadjuvant chemotherapy (carboplatin AUC 5 plus etoposide 120 mg/m^2 on days 1-3 every 28 days) and experienced a partial response by RECIST 1.1 after 3 months (July 2017); however, the patient subsequently experienced PD. In September 2017, the patient underwent surgical dissections of the level I-V nodal regions in the left side of the neck and a level I-III axillary node. Following surgery, a PET-CT scan showed no fluorodeoxyglucose-avid disease. The patient declined adjuvant radiotherapy due to the risk of the left arm lymphoedema worsening.

In April 2018, a PET scan showed PD by RECIST 1.1 in the left supraclavicular (30-mm node) and axilla regions, and a brain magnetic resonance imaging (MRI) scan on May 9, 2018, showed the presence of 2 asymptomatic brain metastases in the right medial temporal lobe adjacent to the optic chiasm (29 mm×25 mm) and left occipital lobe (33 mm×31 mm; **Figure 2A**). Because of the location of the temporal lobe metastasis, it was not possible to safely undertake neurosurgery or stereotactic radiosurgery (SRS).

On June 26, 2018, the patient began avelumab treatment (10 mg/kg intravenously Q2W); at this time, the patient had an ECOG PS of 0. After 4 months of avelumab treatment, an MRI scan on October 17, 2018, showed a complete response by RECIST 1.1 of the occipital metastasis and a partial response by RECIST 1.1 of the temporal lobe metastasis (reduced to 21 mm). On January 21, 2019, an MRI scan showed a substantial decrease in the size of the temporal lobe metastasis to 5 mm×3 mm; the left occipital lobe metastasis was cystic with no enhancement (**Table 2**; **Figure 2B**).

On April 29, 2019, a brain MRI scan showed that the anterior medial part of the right temporal lobe metastasis was larger (maximum transverse dimension of 21 mm) and surrounded by increased white matter edema (**Figure 2C**). There was no change in the area of cystic encephalomalacia in the left occipital lobe compared with January 2019. Given this observed progression in the right temporal metastasis, avelumab treatment was paused (from May 14 to June 23, 2019), and the patient began treatment with fractionated SRS for this metastasis from May 31 to June 5, 2019 (25.5 Gy in 3 fractions).

On September 4, 2019, an MRI scan showed the right temporal metastasis had reduced in size (maximum transverse dimension of 8 mm; **Figure 2D**). In October 2019, clinical evaluation showed enlargement of the left-sided neck mass, and a PET scan showed a left-sided lower neck mass of 30 mm, fluorodeoxyglucose uptake in the small left upper cervical node, and a new right upper cervical node. The patient stopped avelumab treatment on November 25, 2019 and received external beam radiotherapy to the bilateral neck region on December 2, 2019, to improve localized control of the disease progression (40 Gy in 15 fractions in 3 weeks [1 fraction per day]). The patient then resumed avelumab treatment on January 13, 2020, for 2 cycles (last dose February 24, 2020). On January 25, 2020, an MRI scan showed a complete response by RECIST 1.1 of the brain metastases with no residual enhancement of the right temporal lobe. On February 24, 2020, a CT scan showed a

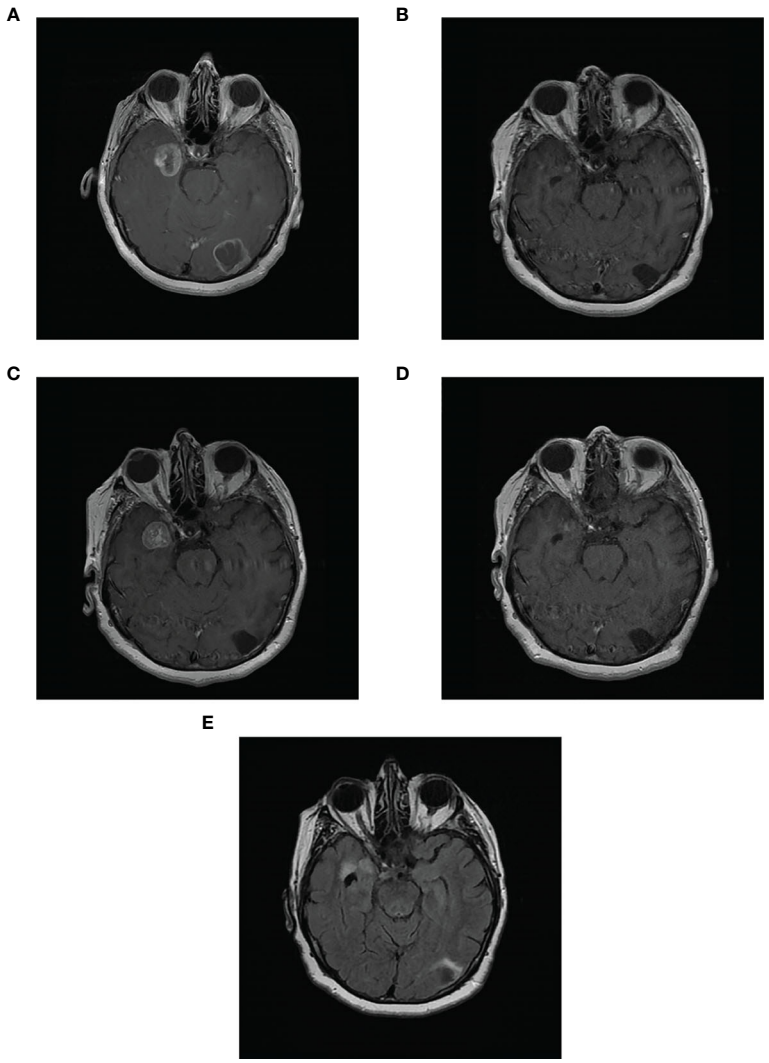

FIGURE 2 | Complete response of 2 asymptomatic brain metastases in patient case 3. Magnetic resonance imaging scans of patient case 3: **(A)** with 2 brain metastases in the left occipital lobe and right temporal lobe prior to starting avelumab (May 9, 2018); **(B)** Complete response in occipital lobe and partial response in temporal lobe metastases after 8 months of avelumab (January 21, 2019); **(C)** PD in temporal lobe metastasis after 10 months of avelumab (April 29, 2019; patient began concurrent SRS on May 31, 2019); **(D)** substantial decrease in size of temporal lobe metastasis after SRS and 15 months of avelumab (September 4, 2019); **(E)** Complete response in both metastases (June 16, 2020; avelumab treatment stopped on February 24, 2020).

complete response by RECIST 1.1 in all metastases including in the extracranial regions (i.e., the neck).

In March 2020, the patient had experienced 3 recent drop attacks; cardiac investigations, including an implantable heart monitor, detected sinus pauses of ≤28 seconds due to sinus node disease. Cardiac MRI and echocardiogram were normal, as were troponin, B-type natriuretic peptide, and creatine kinase concentrations; the patient had no risk factors for cardiac disease. Therefore, it was likely that this adverse event was related to avelumab treatment. The patient received a dual chamber pacemaker on April 7, 2020. MRI scans on June 16 (**Figure 2E**) and October 28, 2020 showed volume loss and no enhancement of the brain metastases. At last follow-up, on November 28, 2020, a body CT scan showed no recurrence of metastases. The patient is delighted with the outcome of her treatment and, having been off treatment for over a year, continues to be able to maintain an active lifestyle.

Patient Case 4

A 66-year-old man from Algeria but treated in France with a history of high blood pressure, depression, and dyslipidemia presented with an asymptomatic, left parotid tumefaction in July 2015. He had no family history of cancer and no relevant prior interventions. An exofacial left parotidectomy, including a biopsy of areas II and IV, was performed in March 2016. This biopsy showed a high-grade neuroendocrine carcinoma that was considered to be a potential metastasis. A whole-body PET scan and a cervical, thoracic, and abdominal CT scan had negative findings at the

TABLE 2 | Timeline of treatments received and brain metastases size in patient case 3.

Date of MRI scan; treatment received	Dimensions of brain metastases, mm	
	Right temporal lobe	Left occipital lobe
May 9, 2018; prior to starting avelumab	29×25	33×31
June 26, 2018; started avelumab (10 mg/kg every 2 weeks)		
July 23, 2018; 1 month of avelumab	27×19	32×35
October 17, 2018; 4 months of avelumab	21×9	NA (focal volume loss and no residual enhancement)
January 21, 2019; 8 months of avelumab	5×3	NA
April 29, 2019; 10 months of avelumab	21 (maximum transverse dimension)	NA
May 14, 2019 to June 23, 2019; avelumab paused		
May 31, 2019 to June 5, 2019; received SRS (25.5 Gy in 3 fractions over a week)		
September 4, 2019; 14 months of avelumab*	8 (maximum transverse dimension)	NA
January 25, 2020; 16 months of avelumab†	NA (no residual enhancement)	NA
June 16, 2020	NA (no intracranial mass or abnormal contrast enhancement)	NA
October 28, 2020	NA (focal volume loss; no mass or enhancement)	NA (focal volume loss; no mass or enhancement)

MRI, magnetic resonance imaging; NA, not applicable; SRS, stereotactic radiosurgery.

**The patient stopped avelumab treatment on November 25, 2019, and received external beam radiotherapy to the bilateral neck region on December 2, 2019, to improve localized control of the disease progression [40 Gy in 15 fractions in 3 weeks (1 fraction per day)].*

†The patient resumed avelumab treatment on January 13, 2020, for 2 months (last avelumab dose February 24, 2020).

time, and no primary tumor site was found. Adjuvant radiotherapy (60 Gy in 30 fractions) was performed on the surgical area from May to July 2016. In June 2017, the patient presented with right axillary adenopathy, and a whole-body PET scan and a cervical, thoracic, and abdominal CT scan found substantial retroclavicular, retropectoral, and right axillary node metastases. A biopsy of the right axillary node was used to diagnose MCC.

From August 7 to October 18, 2017, the patient received 4 cycles of chemotherapy (carboplatin AUC 5 plus etoposide 100 mg/m^2 on days 1-3 every 28 days). In November 2017, a CT scan found node, muscle, and pararectal disease progression. In December 2017, the patient presented with pain and dysesthesia of the right arm due to right axillary adenopathy. Second-line avelumab treatment (10 mg/kg Q2W) was started on December 26, 2017, and was well tolerated (no adverse events were reported and the pain and dysesthesia of the right arm rapidly improved); at this time, the patient had an ECOG PS of 1. On December 31, 2017, a brain MRI scan showed an asymptomatic left cerebellar metastasis measuring <1 cm; neurosurgery was not considered to be necessary, and the patient received concurrent SRS (20 Gy in 1 fraction) of the cerebellar metastasis and began palliative radiotherapy (30 Gy in 10 fractions) of the right axillary mass.

In March 2018, a CT scan showed an extracranial partial response according to RECIST 1.1 (68% decrease), and an MRI scan showed regression of the cerebellar metastasis. Avelumab was discontinued in January 2019 after 25 cycles (13 months) of treatment, due to the persistent partial response and the difficulties for the patient to travel to receive avelumab.

Six months after avelumab treatment was stopped (July 2019), a brain MRI scan showed recurrence of the cerebral metastasis, justifying resumption of avelumab and 1 dose of SRS (16 Gy in 1 fraction). At this time, the patient was still experiencing an extracranial partial response. After 2 months, the patient switched from avelumab to nivolumab (anti–programmed death 1 [PD-1]; 480 mg IV every month) to reduce the patient's travel burden. In July 2020, an MRI scan showed that the patient remained in extracranial partial response and had also achieved partial response of the brain metastases; however, radionecrosis of the previously irradiated cerebral metastasis was found, and the patient was subsequently treated with corticosteroids 0.5 mg/kg with good resolution. At last follow-up (March 2021), an MRI scan showed both intracranial and extracranial complete response by RECIST 1.1.

DISCUSSION

MCC is a rare tumor, but incidences have increased in recent years with approximately 5000 new cases of MCC annually in the US and Europe (11, 12). Patients with mMCC have a poor prognosis, with historical 5-year OS rates of 35% and 14% for nodal and distant disease, respectively (2). Current guidelines for the treatment of mMCC recommend enrollment in a clinical trial or systemic therapy with an anti–PD-1/PD-L1 antibody (4).

In 2017, avelumab became the first approved treatment for mMCC based on the results of the JAVELIN Merkel 200 trial (8, 9). Initially, this approval was based on primary analysis results from a cohort of patients with mMCC who received avelumab as second-line or later treatment after disease progression with chemotherapy (part A) (8) and preliminary data from a subset of patients who received avelumab as first-line treatment, which was initiated subsequently (part B) (9). After 3 years of follow-up from part A of the trial (N=88), the objective response rate was 33.0% (95% CI, 23.3-43.8) and median duration of response was 40.5 months (95% CI, 18.0-not estimable). Median progression-free survival and OS was 2.7 months (95% CI, 1.4-6.9) and 12.6 months (95% CI, 7.5-17.1), respectively (8, 13). After ≥15 months of follow-up in part B (N=116), the objective response rate was 39.7% (95% CI, 30.7-49.2), and median duration of response was 18.2 months (95% CI, 11.3-not estimable). Median progression-free survival and OS was 4.1 months (95% CI, 1.4-6.1) and 20.3 months (95% CI, 12.4-not estimable), respectively (14). The JAVELIN Merkel 200 trial excluded patients with

active central nervous system metastases (8), and limited data are available for the treatment of these patients.

In the cases reported here, patient age ranged from 48 to 70 years, 3 of the 4 patients were female, and 2 had comorbidities. All patients had asymptomatic brain metastases and received avelumab as second-line therapy following disease progression with platinum-based chemotherapy. Avelumab treatment was well tolerated in 3 patients; however, 1 patient (case 3) developed sinus node disease that was likely related to avelumab treatment.

MCC is an aggressive cancer, and disease progression and development of metastases can occur early (4). In the cases reported here, slower tumor growth and the use of SRS appeared to correlate with better response to subsequent avelumab treatment. Two of the 4 patients experienced rapid progression, with brain metastases identified approximately 6 months (patient 2) and 12 months (patient 1) after initial mMCC diagnosis, and further disease progression with avelumab. In the remaining 2 patients, progression appeared more gradual, with brain metastases diagnosed approximately 16 months (patient 3) and 22 months (patient 4) after initial diagnosis. With avelumab treatment alone, patient 3 experienced a complete response in 1 brain metastasis and partial response in a second brain metastasis; the second metastasis then progressed, but further treatment with avelumab and SRS led to a complete response. Patient 4 experienced a partial response with avelumab plus concurrent SRS, and subsequently achieved a complete response after switching to nivolumab treatment. Patient ECOG PS at the time of starting avelumab treatment did not appear to be associated with a better response.

Although no treatment options are recommended by guidelines for patients with mMCC and brain metastases (4, 7), radiotherapy is commonly used and has been associated with a survival benefit (6). Furthermore, SRS with concurrent immunotherapy is recommended by the European Society for Medical Oncology for patients with melanoma and brain metastases (15). In a recent study of 262 patients with melanoma and brain metastases, radiotherapy combined with either immunotherapy or targeted therapy was associated with a significantly reduced risk of death *vs* systemic therapy (median OS 16.8 *vs* 6.9 months, respectively) (16). In the cases reported here, SRS administered 11 months after starting avelumab treatment (patient 3) or at the same time as starting avelumab (patient 4) resulted in a complete response and a partial response, respectively, and both patients were alive at last follow-up. Neurosurgery has also been associated with prolonged survival in patients with mMCC and brain metastases (6); however, in the cases reported here, patients did not undergo neurosurgery due to the asymptomatic nature of the metastases and their locations being considered too high risk for resection. Additionally, combining immunotherapy and SRS does not appear to increase toxicity compared with SRS alone (17).

Furthermore, patients with nodal mMCC and no known primary tumor location have been shown to have longer OS compared with those with a known primary tumor site (2, 18). This may be due to a more active immune response in some patients that is able to eliminate the primary tumor (18). For the 2 patients with the longest OS in this series, cases 3 and 4, no primary tumor was identified. However, the dataset is small, and immune markers were not analyzed; therefore, further research is needed to investigate the potential mechanisms involved.

In summary, published data for patients with mMCC and brain metastases are limited. We report the clinical experiences of 4 patients with mMCC and brain metastases treated in Europe with avelumab after prior disease progression with chemotherapy. In this small series, we show that avelumab can have intracerebral and systemic activity and, when combined with radiotherapy (both brain SRS and radiotherapy to other sites), can produce lasting disease control. Although the optimal timing of SRS administration warrants further investigation, the combination of SRS and avelumab, along with more gradual disease progression, appeared to be associated with improved survival. Further research into potential predictors of response and prolonged survival in this subset of patients is needed. Although clinical trials in this small subset of patients are unlikely to be feasible, prospective data on the use of SRS plus immunotherapy in patients with mMCC and brain metastases along with data from trials investigating this combination in patients with other more common tumor types will provide further insight into this treatment strategy. Overall, our findings indicate that the use of SRS with immunotherapy may be an effective treatment option for patients with mMCC and brain metastases.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors contributed to data collection and interpretation and writing of the manuscript. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Case Report: Exceptional Response to Avelumab After Failure of Electrochemotherapy in a Patient With Rapidly Progressive, PD-L1-Negative Merkel Cell Carcinoma

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This case report shows, for the first time, a patient experiencing a complete response after one dose of avelumab following extensive disease progression with prior electrochemotherapy (ECT) treatment. We suggest that ECT may help to establish a tumor microenvironment favorable to immunotherapy. Merkel cell carcinoma (MCC) is a highly aggressive skin cancer with seldom durable chemotherapy responses. ECT has recently emerged as a potential treatment option for several malignancies, including MCC. Avelumab, an anti-programmed cell death-ligand 1 (PD-L1) monoclonal antibody, became the first approved treatment for patients with metastatic MCC. ECT has been shown to activate the immune response, but it is still unknown how ECT may affect patient's response to subsequent immunotherapy. We report a case of a patient with MCC who presented with a rapidly growing skin nodule of the right cheek and experienced extensive disease progression following surgical debulking and ECT treatment. The patient received a flat dose of 800 mg avelumab intravenously every 2 weeks showing complete tumor regression after only one dose. Immunohistochemical analysis of surgical and post-ECT biopsies collected from the primary lesion revealed tumor expression of programmed cell death protein-1 (PD-1), but not PD-L1. Analysis of the tumor samples also revealed no expression of Merkel cell polyomavirus (MCPyV). Comparison of the biopsies showed a decrease in myeloid and T-cell markers after ECT but an increase in major histocompatibility complex (MHC) class I expression on tumor

cells. Additionally, the patient experienced an increase in neutrophil-to-lymphocyte ratio and lactate dehydrogenase values post-ECT, which subsequently decreased with avelumab treatment. As of 30 October 2019, the patient was still receiving avelumab treatment and had an ongoing complete response. In this case report, a patient with PD-L1-negative and MCPyV-negative MCC who had disease progression following ECT experienced complete tumor regression with avelumab treatment, suggesting, for the first time to our knowledge, that ECT may help to establish a tumor microenvironment favorable to immunotherapy *via* a potential abscopal effect. Tumor-intrinsic PD-1 expression and modulation of MHC class I antigens after ECT may contribute to the clinical efficacy of avelumab in this context.

Keywords: skin neoplasms, immunotherapy, electrochemotherapy, MCC, avelumab, Merkel cell carcinoma, ECT, case report

INTRODUCTION

Merkel cell carcinoma (MCC) is a rare and highly aggressive neuroendocrine tumor associated with clonal integration of the Merkel cell polyomavirus (MCPyV), ultraviolet (UV) radiation exposure, advanced age, and immunosuppression (1, 2). Approximately 65% of patients with MCC present with local disease, and approximately 26 and 8% of patients present with nodal and distant metastatic disease, respectively (3).

MCC can grow rapidly, and treatment options are limited; the current standard of care for patients with localized MCC is surgery with or without adjuvant radiation therapy (1). Although MCC is considered chemo-sensitive, responses to chemotherapy are rarely durable; median overall survival with chemotherapy is approximately 10 months (1, 2). Recently, emerging evidence suggests that electrochemotherapy (ECT; electrical pulses administered alongside chemotherapy) may be an effective treatment option for patients with MCC, although published literature is limited to case reports (4–6). Furthermore, immune checkpoint inhibitors (ICIs), including the anti-programmed cell death-ligand 1 (PD-L1) monoclonal antibody avelumab, are currently recommended for the treatment of patients with metastatic MCC (mMCC) based on promising results including durable responses observed in clinical trials (1, 7–9).

Here, we report the case of an 80-year-old man who presented with rapidly growing skin nodule of the right cheek. A surgical debulking was performed and the microscopical histopathological examination showed small cells with a round-oval nucleus and scarce cytoplasm. Immunohistochemical analysis results were consistent with MCC. The postsurgical physical examination revealed an irregular purplish lesion near the right preauricular region, close to the surgical scar. Computed tomography (CT) of the face, neck, chest, and abdomen revealed malignant disease in the preauricular region but showed no distant metastases.

After surgical debulking in January 2019, the patient experienced relapse in April 2019. He then initiated ECT without results since the lesion increased in size. When the lesion reached dimensions of 8.5 × 10 cm, the patient started avelumab therapy.

After receiving one dose of avelumab, the patient experienced a complete response following extensive disease progression with prior ECT treatment. To our knowledge, this has never been documented in literature before.

MATERIALS AND METHOD

Ki-67, synaptophisin, chromogranin-A, TTF1, CK7, CK AE1-AE3, PD-1, PD-L1, MHC class I, HLA-DR, CD14, CD68, CD163, CD3, CD4, CD8, Granzyme B, and CD20, were investigated by immunohistochemistry (IHC). Briefly, sections 2.5/3-μm thick were cut from paraffin blocks, dried, de-waxed, rehydrated, and unmasked (with Dako PT-link, EnVision™ FLEX Target Retrieval Solution, High/Low pH). Antibodies were incubated with a commercially available detection kit (EnVision™ FLEX+, Dako, Denmark) in an automated Immunostainer (Dako Immunostainer Link 48). IHC for PD-L1 were made using Ventana Benchmark Ultra IHC/ISH System immunostainer (Ventana Medical Systems, Tucson, AZ, USA) following manufacturer instructions. Antibody dilutions, clones, and specifics are reported in detail in **Table 1**.

The expression of inflammatory markers (PD-1, PD-L1, MHC class I, HLA-DR, CD14, CD68, CD163, CD3, CD4, CD8, Granzyme B, and CD20) on tumor cells and within the tumor microenvironment was evaluated using a semiquantitative scoring system according to a previous analysis by Milione and colleagues (10). Scoring considered the staining intensity (I) in a three-tiered scale (1, less intense than the control; 2, intensity superimposable to the control according to the manufacturer

Abbreviations: CD, cluster of differentiation; CK, cytokeratin; CT, computed tomography; ECOG, Eastern Cooperative Oncology Group; ECT, electrochemotherapy; FDG-PET, 18-fluorodesossyglucose-positron emission tomography; HLA, human lymphocyte antigen; ICI, immune checkpoint inhibitor; IHC, immunohistochemistry; iRECIST, Response Evaluation Criteria in Solid Tumors guideline for immunotherapy; IV, intravenous; LDH, lactate dehydrogenase; MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus; MHC, major histocompatibility complex; mMCC, metastatic Merkel cell carcinoma; mTOR, mechanistic target of rapamycin kinase; NLR, peripheral blood neutrophil-to-lymphocyte ratio; ORR, objective response rate; PD-1, programmed cell death protein-1; PD-L1, programmed cell death-ligand 1; TTF1, thyroid transcription factor 1; UV, ultraviolet.

TABLE 1 | Antibody sources and dilutions.

Antigens	Dilution	Code Number	Clone	Source
KI-67 (M)	1/400	M7240	Mib-1	Dako, Agilent, Denmark
Synaptophisin (M)	1/200	M7315	Dak-Synap	Dako, Agilent, Denmark
Chromogranin-A (M)	1/100	M0869	Dak-A3	Dako, Agilent, Denmark
TTF1(M)	1/2000	M3575	8G7G3	Dako, Agilent, Denmark
Cytokeratin 7 (M)	1/200	M7018	OV-TL	Dako, Agilent, Denmark
Cytokeratin (M)	1/100	M3515	AE1/AE3	Dako, Agilent, Denmark
PD-1 (M)	1/50	ab52587	NAT105	Abcam
PD-L1 (M)	Prediluted	740-4859	SP142	Ventana Medical System-Roche
MHC class I (M)	1/4000	ab6405	OX18	Abcam
HLA-DR(M)	1/500	MS-133-P0	LN3	Thermo Fisher Scientific
CD14 (M)	1/500	ab133335	EPR 3653	Abcam
CD68 (M)	1/3000	M0814	KP1	Dako, Agilent, Denmark
CD163 (M)	1/200	NCL-L-CD163	10D6	Leica Biosystems
CD3 (P)	1/400	A0452	Polyclonal	Dako, Agilent, Denmark
CD4 (M)	1/300	M7310	4B12	Dako, Agilent, Denmark
CD8 (M)	1/20	M7103	C8/144B	Dako, Agilent, Denmark
Granzyme B (M)	1/50	M7235	GrB-7	Dako,Agilent, Denmark
CD20	1/400	M0755	L26	Dako,Agilent, Denmark

M, Monoclonal; P, Polyclonal; TTF-1, Thyroid transcription factor-1.

indications; 3, more intense than the control). Extension (E) was defined as the percentage of positive cells for each marker (0, 0%; 1, <25%; 2, 25–50%; 3, 51–74%; 4, 75–100%). A final score was determined from the product of I E.

CASE DESCRIPTION

In January 2019, an 80-year-old man, came to the physician's attention due to the appearance of a nodular, fixed lesion of 1.5 × 1.5 cm in dimension, localized in the right cheek. The lesion appeared around 3 months before (October 2018) he came to the hospital and was initially interpreted as a cystic lesion. The patient reported preauricular pruritus, sense of tension, and sporadic pain during chewing. In January 2019, due to the preauricular localization of the lesion and its infiltrative features with suspected highly aggressive cutaneous characteristics, the clinicians proposed a surgical debulking with a radical intention. At the time of the surgical proposal, clinical conditions were suitable with chronological age, with an Eastern Cooperative Oncology Group (ECOG) performance status score of 1. In anamnesis, the patient presented grade 1 arterial hypertension (controlled with medical chronic therapy), mild grade 1 hypercholesterolemia, mild cognitive impairment, and non-clinically significant mitral valve insufficiency (patient performed annual cardiologic follow-up).

Familial anamnesis was negative for neoplastic disorders. The patient's history excluded both professional exposure to neoplastic risk factors or any other patient-dependent risk factors, such as smoking habits, or ethnicity. The patient's history did not present features suspicious for hereditary/syndromic familial presentation of the neoplastic event.

After surgical debulking of the lesion (in January 2019), the patient presented localized pain associated with persistence of homolateral hearing loss and homolateral (right) preauricular sensitivity deficit. Microscopical histopathological examination showed small cells with a round–oval nucleus and scarce cytoplasm. IHC staining confirmed the expression of cytokeratin (CK)20, CK7, chromogranin A, synaptophysin, and high levels of Ki67 (80%), whereas thyroid transcription factor 1 (TTF1) was not expressed; these IHC results are consistent with MCC (11). A diagnosis of MCC extended at the surgical resection margin was confirmed (**Figure 1A**). Additionally, MCPyV was not present. After 2 months, the patient returned for postsurgical restaging and a physical examination, which revealed an irregular purplish lesion of approximately 1.5 × 1.5 cm situated near the right preauricular region, close to the surgical scar (**Figure 1B**). CT of the face, neck, chest, and abdomen revealed malignant disease in the preauricular region (**Figure 1C**), including two pseudonodular areas, but showed no distant metastases.

At disease relapse, in April 2019, the patient presented progressive increment of pain and loss of appetite due to chew-related pain. Together with dimensional increment of the lesion, discomfort worsened in terms of sense of tension in preauricular and later cervical areas, and loss of appetite. The patient began ECT, consisting of an intravenous (IV) bolus infusion of bleomycin 15,000 IU/m^2 administered 8 min before delivery of electroporation by means of hexagonal array electrodes (5,000 Hz) connected to an electric pulse generator (Cliniporator; IGEA Clinical Biophysics; Carpi, Italy). In June 2019 (at the first post-ECT assessment), the treated lesion had increased in size (3.5 × 4.5 cm). Subsequently, the patient received regular follow-ups in order to distinguish whether the increase in lesion size was due to postprocedural inflammation or to disease progression.

At the end of June 2019, the lesion measured 5.0 × 7.0 cm and was erythematous. After 1 week (at the beginning of July 2019), the erythematous lesion had increased substantially in size (8.5 × 10 cm) in the preauricular region and extended to the lateral cervical region (**Figure 2A**). In July 2019, together with the symptoms experienced in April 2019, the patient reported pain

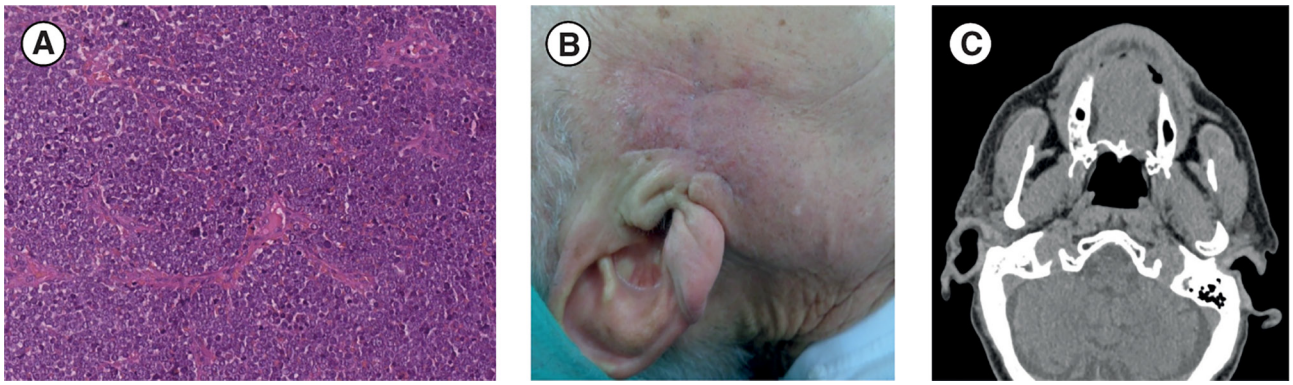

FIGURE 1 | **(A)** Diagnosis: histological image of hematoxylin and eosin. section (scale bar: 50 μm) shows small tumor cells with a round-oval nucleus and poor cytoplasm that are very densely arranged in a diffuse pattern of growth. **(B, C)** Post-debulking surgery restaging: **(B)** Post-debulking clinical presentation with a purplish lesion (approximately 1.5 × 1.5 cm) situated near the right preauricular region close to the surgical scar. **(C)** Face and neck CT scan (axial projection) showing residual disease in the right preauricular region. CT, computed tomography.

and pruritus in lateral cervical area due to tumor cutaneous infiltration. CT scans confirmed disease progression and revealed extensive infiltrates, including in the pseudonodular areas, in the subcutaneous tissues of the bilateral lateral cervical region, and in the right preauricular area (**Figure 2B**); 18-fluorodesossyglucose-positron emission tomography (FDG-PET) confirmed pathological accumulation in the preauricular area extending to the bilateral lateral cervical and sternal level (**Figure 2C**), as confirmed by the whole body PET-FDG (**Figure 2D**). Additionally, biopsy and IHC staining of the preauricular cutaneous area showed a high-grade neuroendocrine tumor, confirming progression (**Figure 2E**).

Given the extensive progression, a decision was made to start a regimen of avelumab flat dose 800 mg IV every 2 weeks in August 2019; at this time, the lesion was 13.0 × 15.0 cm. After the first administration of avelumab, a substantial reduction in lesion size was observed with no measurable lesion remaining (**Figure 3A**). After three administrations of avelumab, a complete response was confirmed according to iRECIST (modified Response Evaluation Criteria in Solid Tumors guideline for immunotherapy) (12) (**Figures 3B, C**). As of 30 October 2019, the patient was still continuing with avelumab flat dose 800 mg IV every 2 weeks, with CT scans every 3 months; he had an ongoing complete response, with no evidence of new lesions or progressive disease (**Figure 3D**).

In order to better understand the immune profile of the tumor microenvironment and its relationship with tumor cell features, a comparative IHC analysis was performed in two tumor samples, respectively obtained during debulking surgery and after ECT. The final scores obtained from the product of I × E, as described in the *Material and Methods* section, are summarized in **Table 2**. **Figure 4** shows the images related to the most important data resulting from immunohistochemical analysis performed on bioptic samples at diagnosis **(Column A)** and after ECT **(Column B).**

Interestingly, in both the surgical and post-ECT samples, programmed cell death protein-1 (PD-1) was found to be expressed at a low level on tumor cells, and PD-L1 was not expressed either on tumor cells or within the tumor microenvironment. Additionally, fewer myeloid cells were present in the post-ECT sample compared with the surgical sample, as shown by the decrease in CD68 and CD163 expression in the tumor microenvironment. Major histocompatibility complex (MHC) class I antigen expression on tumor cells increased in the post-ECT sample compared with the surgical sample, while CD3 and CD4 markers in the tumor microenvironment substantially decreased.

The absolute count of neutrophils and lymphocytes and lactate dehydrogenase (LDH) serum values were collected at each visit and after each ECT or avelumab treatment. The derived peripheral blood neutrophil-to-lymphocyte ratio (NLR) was also evaluated. With these data, we aimed to correlate blood-toxicities or complications and to investigate the potential prognostic significance and/or predictive value of NLR or LDH markers on treatment response.

At first diagnosis (January 2019), the basal NLR value was 2.45, and LDH was 256 U/L. These values increased after ECT in parallel with disease progression and increased tumor size until August 2019. The patient then showed a reduction in NLR and LDH values concurrent with achieving a complete response after the first dose of avelumab (September 2019); these values continued to decrease after three cycles of avelumab, when complete response was radiologically confirmed (**Supplementary Table 1** and **Supplementary Figure 1**). As of October 2020, NLR and LDH values were continuing to decrease.

TIMELINE

See **Supplementary Table 2** for the timeline.

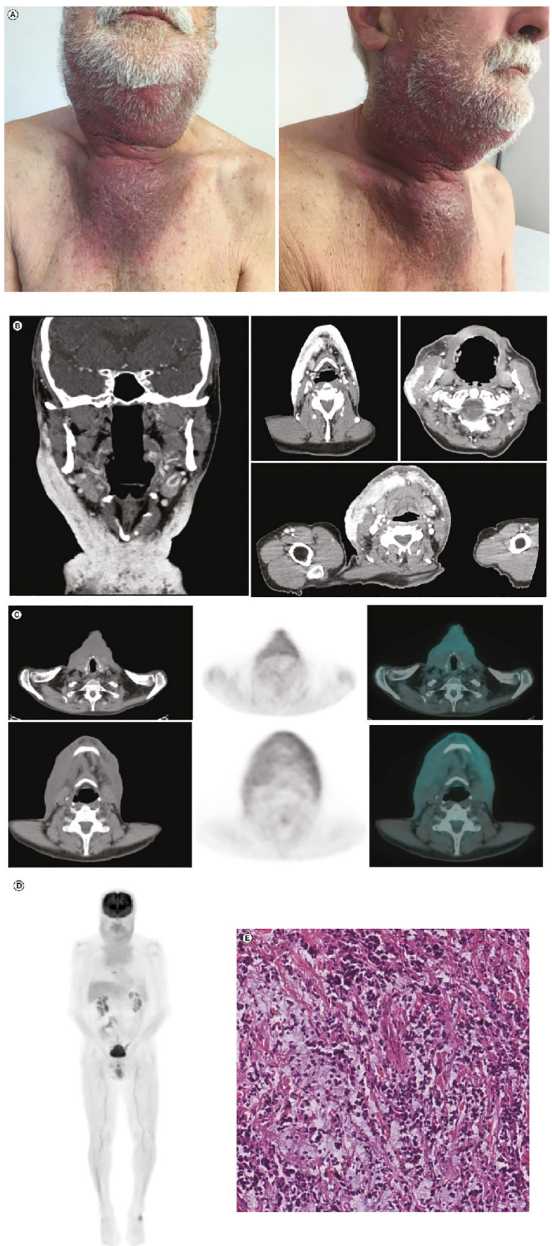

FIGURE 2 | Post-ECT restaging: **(A)** Post-ECT clinical presentation with an extensive, dark lesion (approximately 8.5 × 10.0 cm) in the preauricular and laterocervical regions. **(B)** Coronal (leftmost panel) and axial (right panels) CT scans showing extensive infiltrates in the subcutaneous tissues of the bilateral laterocervical region and right preauricular area. **(C)** Left to right: CT scan, FDG-PET scan, and CT and FDG-PET fusion images showing pathological accumulation in the preauricular area extending until the bilateral laterocervical and sternal level. **(D)** Total-body FDG-PET image confirming significant FDG uptake in mandibular, laterocervical, and sternal regions. **(E)** Hematoxylin and eosin stain (scale bar: 50 μm) of the tumor sample taken after ECT. Compared with the tumor sample collected before ECT, there was a decrease in neoplastic cellularity and edematous stroma. CT, computed tomography; ECT, electrochemotherapy; FDG-PET, 18-fluorodesossyglucose-positron emission tomography; MCC, Merkel cell carcinoma.

DIAGNOSTIC ASSESSMENT

In this study, the following diagnostic tests were performed: physical examination, IHC, neutrophil count, CT of the head, neck, thorax, and abdomen, and FDG-PET. The primary diagnostic challenge was to evaluate the immune-context of the patient before treatment with avelumab. The diagnosis in October 2018 was of MCC extended at the surgical resection margin.

DISCUSSION

Incidences of MCC have increased in recent years; approximately 5,000 new cases of MCC occur each year in the USA and Europe (13, 14). MCC is associated with clonal integration of the MCPyV (approximately 80% of cases) or UV radiation exposure and commonly occurs in patients who are elderly and fair skinned (11). MCC usually presents as a rapidly growing purple nodule situated in the upper region of the body, including the shoulders, head, and neck. Diagnosis of MCC is dependent on IHC staining patterns, including the expression of CK20 and a lack of TTF1 (11). Most patients with MCC present with local disease, for which surgery with or without concomitant radiotherapy is the current recommended treatment (1). Unfortunately, disease progression can occur quickly, and recurrence is common; approximately one-third of patients with MCC develop distant metastases (1).

For patients with mMCC, current guidelines recommend enrollment in a clinical trial or systemic therapy with an ICI (1). In 2017, avelumab became the first approved treatment for mMCC based on the results of the JAVELIN Merkel 200 trial (7, 9). Avelumab binds to PD-L1, preventing its interaction with PD-1 and subsequent T-cell exhaustion (15). Durable responses have been observed with first- and second-line or later avelumab in patients with mMCC. In part A of JAVELIN Merkel 200, 88 patients with mMCC and progressive disease after chemotherapy received avelumab. After ≥36 months of follow-up, the objective response rate (ORR) was 33.3%, including complete responses in 11.4%; median duration of response was 40.5 months (16). In part B, 116 patients with mMCC who were naive to systemic therapy received avelumab; after a median follow-up of 21.2 months, the ORR was 39.7%, and 30.2% of patients had a response that lasted ≥6 months (17). Another ICI, pembrolizumab (anti-PD-1 antibody), has shown encouraging results in patients with advanced MCC. In the KEYNOTE-017 trial, 50 patients with stage IIIB or IV MCC who were naive to systemic therapy received pembrolizumab, and after a median follow-up of 14.9 months, the ORR was 56.0%, including complete responses in 24.0% (8). In this case report, treatment with avelumab led to a complete response that was ongoing as of 10 April 2020.

Recently, ECT has emerged as a potential treatment option for MCC. ECT allows the delivery of non-permeant chemotherapy, such as bleomycin, into cells through

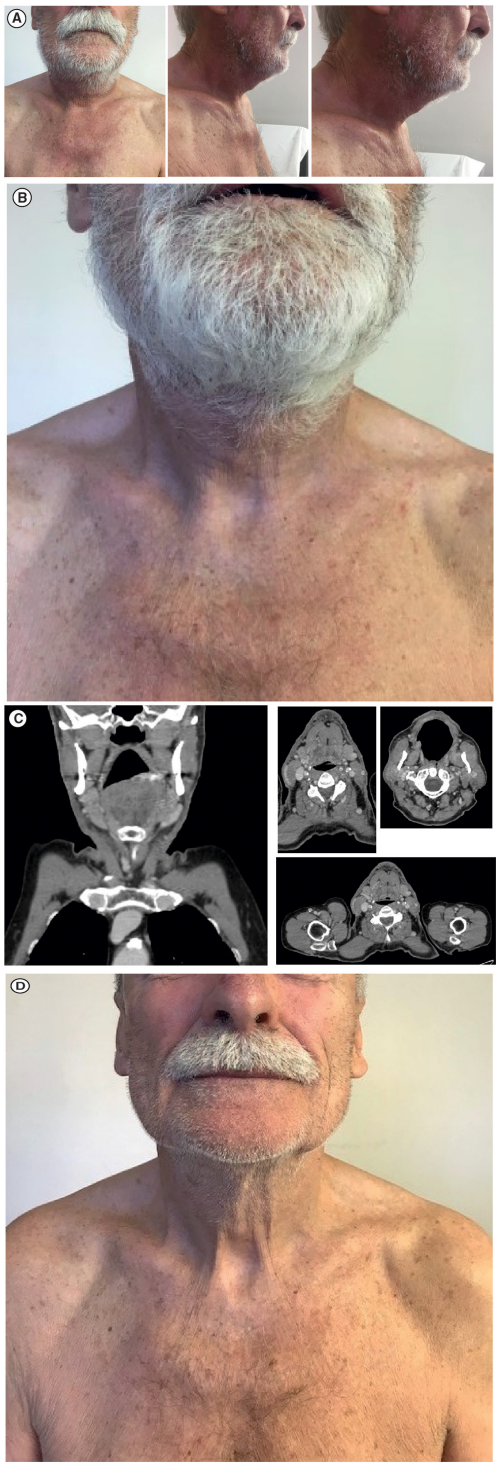

FIGURE 3 | Response to avelumab treatment: **(A)** Clinical presentation of substantial measurable reduction in lesion size after one dose of avelumab. **(B)** Clinical presentation of confirmed complete regression of the tumor mass after three doses of avelumab. **(C)** Face and neck coronal (leftmost panel) and axial (right panels) CT scans showing complete radiological response. **(D)** Clinical presentation of ongoing complete response on 30 October 2019. CT, computed tomography.

TABLE 2 | Expression of inflammatory markers using a semiquantitative scoring system that considers staining intensity (I) on a three-tiered scale (1, less intense than control; 2, intensity superimposable to control according to manufacturer indications; 3, more intense than control), as well as extension (E), defined as the percentage of positive cells for each marker (0, 0%; 1, <25%; 2, 25–50%; 3, 51–74%; 4, 75–100%) (10). Total score = I E.

Marker (score)	Surgical biopsy		Post-ECT biopsy	
	Expressed on tumor cells	*Expressed in tumor microenvironment*	*Expressed on tumor cells*	*Expressed in tumor microenvironment*
PD-1	9	0	6	0
PD-L1	0	0	0	0
MHC class I	2	4	4	0
HLA-DR	2	4	2	0
CD14	0	2	0	2
CD68	0	4	0	2
CD163	0	4	0	2
CD3	0	6	0	1
CD4	0	6	0	2
CD8	0	2	0	2
Granzyme B	0	2	0	0
CD20	0	2	0	2

ECT, electrochemotherapy; HLA, human lymphocyte antigen; MHC, major histocompatibility complex; PD-1, programmed cell death protein-1; PD-L1, programmed cell death-ligand 1.

administration of short, intense electric pulses that increase cell membrane permeability, thereby enhancing the cytotoxic activity of chemotherapy (4). ECT, most commonly used with bleomycin, is well tolerated and has been shown to be an effective treatment strategy in several tumor types, including non-melanoma skin cancers (4). Complete responses have been reported with ECT in some patients with MCC; however, published literature remains limited (4–6). In the case presented here, ECT was chosen due to the small area of local relapse and absence of distant lesions; previous clinical data described the efficacy of ECT in small and locally relapsed MCC lesions of head and neck origin (5, 6). Additionally, the location of the lesion (preauricular region) and its proximity to the surgical scar meant that treatment with radiotherapy had an increased risk of radiotherapy-related toxicities and the potential to be less effective due to altered vascularization in the area. However, in this patient, treatment with ECT was followed by extensive disease progression, highlighting the need for further research into the use of ECT in patients with MCC.

Preclinical data has shown that ECT activates the immune system, can induce immunogenic cell death, and may lead to an abscopal effect, wherein an antitumor response is elicited outside the primary target of treatment (18). However, little is known about how the immunogenic mechanisms elicited by ECT may affect patient response to subsequent immunotherapy.

In the present case report, IHC analysis of surgical and post-ECT biopsies indicated a substantial remodeling of the immune contexture after ECT. In particular, we observed decreased expression of T-cell and myeloid-cell markers, but an increase in tumor cell expression of MHC class I antigens. The latter suggests that antigen presentation by tumor cells increased after ECT, which may have improved tumor recognition by the

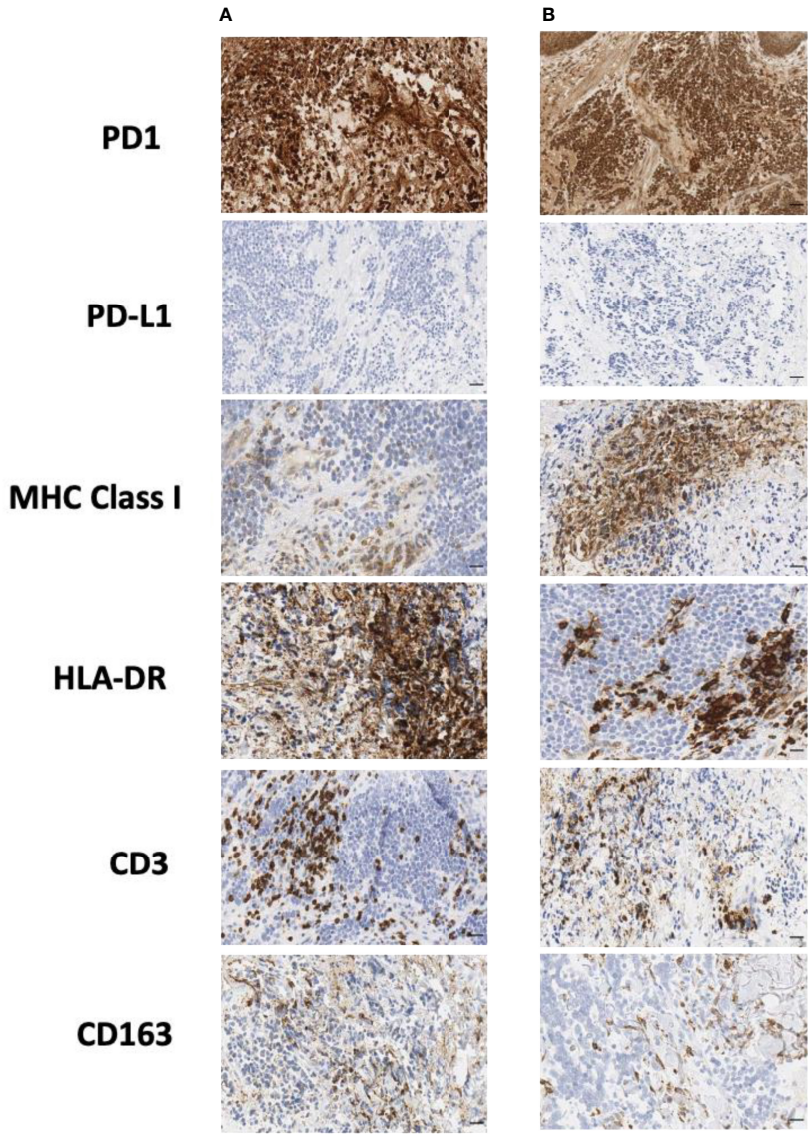

FIGURE 4 | Immunohistochemical staining of biopsy samples taken **(A)** during surgery and **(B)** after ECT. CD, cluster of differentiation; ECT, electrochemotherapy; HLA, human lymphocyte antigen; MHC, major histocompatibility complex; PD-1, programmed cell death protein-1; PD-L1, programmed cell death-ligand 1. Scale bar: 50 μm.

reactivated adaptive immune system following subsequent avelumab treatment. Furthermore, a post-ECT reduction in infiltrating T cells may not have compromised the efficacy of anti-PD-L1 treatment, consistent with recent analyses of T-cell clonotype differences before and after anti-PD-1 therapy in basal cell carcinoma or squamous cell carcinoma (19) and in the context of neoadjuvant PD-1 blockade in melanoma (20). Results of these studies suggest that the antitumor T cells reactivated by ICI treatment are not the exhausted T cells already present in pretherapy lesions but are instead a newly recruited population of T cells. Additionally, the reduction in T-cell markers observed in the post-ECT sample contrasted with previous evidence indicating that ECT promotes CD3+ and CD8+ T-cell infiltration of treated lesions (21). A potential explanation for our findings may be the extensive disease progression observed after ECT; the rapidly growing tumor may have outpaced the ability of the immune system to maintain infiltrating T cells at the level observed at surgical excision of the primary lesion.

Of note, in this patient, PD-1 was expressed on a subset of tumor cells. As shown initially in melanoma, tumor cell-intrinsic PD-1 expression may foster tumor growth by activation of the mechanistic target of rapamycin kinase (mTOR) signaling pathway upon binding to PD-L1 (22). Therefore, in this case, interruption of the PD-1/PD-L1 axis by avelumab may have

counteracted potential protumoral signaling by PD-1. However, a recent study has shown that, in immunodeficient murine models, engagement of the PD-1/PD-L1 axis due to coexpression of both PD-1 and PD-L1 on tumor cells can suppress tumor growth due to inhibition of the protein kinase B (AKT) and extracellular signal-regulated kinase (ERK) pathways (23). Blockade of the PD-1/PD-L1 interaction in this model promotes tumor growth in the absence of adaptive immunity. These contrasting findings suggest that the biological functions of the PD-1/PD-L1 axis, when receptor and ligand are both expressed on tumor cells, may be dependent on tumor context, and that engagement or interruption of this interaction may have different effects depending on the specific tumor setting where immunotherapy targeting PD-1 or PD-L1 is employed.

The use of PD-L1 and MCPyV as predictive biomarkers in MCC has not yet been established. In the case reported here, the patient's tumor was both PD-L1-negative and MCPyV-negative, and an exceptional complete response was achieved with avelumab after ECT. However, this PD-L1-negative result may be due to heterogeneous expression of PD-L1 within the tumor. Similarly, responses to avelumab have also been observed, irrespective of PD-L1 or MCPyV status, in patients with mMCC enrolled in the phase II JAVELIN Merkel 200 trial (16), suggesting that these biomarkers may have limited utility to predict a response to ICI treatment.

In recent years, the potential prognostic significance of NLR and LDH levels in patients with advanced tumors has been investigated (24, 25); however, the role of NLR and LDH levels remains unknown for patients with MCC treated with immunotherapy. Neutrophilia indicates a systemic inflammatory response, whereas lymphopenia has been associated with impaired cell-mediated immunity; elevated pretreatment NLR is associated with poorer prognosis in advanced tumors (24). In the case reported here, although the patient had elevated NLR and LDH values prior to avelumab treatment, a rapid decrease was observed, concurrent with complete tumor response. These findings suggest that the treatment sequence of ECT followed by immunotherapy alters the inflammatory markers present in tumor cells, as well as in peripheral blood. Further studies are needed to determine the roles of NLR and LDH as potential biomarkers to consider when selecting patients for immunotherapy treatment (26).

PATIENT PERSPECTIVE

Already after the first administration of avelumab, the patient reported sudden regression of sense of tension, pain, and also conditioning loss of appetite. After the third administration of avelumab, the patient reported complete symptom regression. Since the start of avelumab, the patient reported optimal tolerance without significative toxicities and presented objective features of amelioration: cutaneous and subcutaneous grade 2 later cervical erythema, which was documented before starting the systemic treatment, macroscopically reduced after the first administration, and completely disappeared after three cycles.

The patient provided consent to the use of his data for the present case report.

CONCLUSION

In conclusion, we report an exceptional complete response in a patient with PD-L1–negative and MCPyV-negative MCC after relatively few administrations of avelumab and following extensive disease progression with ECT treatment. This case report, although inherently anecdotal in its nature, suggests that ICIs are a potential therapeutic option for patients with MCC who are non-responsive to ECT and that the treatment sequence of ECT followed by immunotherapy may improve clinical outcome. More data are needed to identify how immunogenic mechanisms after ECT may affect response to subsequent ICI treatment. However, the findings reported here suggest that promotion of antigen presentation, changes in inflammatory markers, and perhaps interruption of tumor-intrinsic PD-1/PD-L1 signaling may contribute to the efficacy of avelumab treatment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval were not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

MT participated in the clinical management of the patient and contributed to data analysis and interpretation, paper writing, manuscript editing, and final approval. LC, MM, MU, and AA contributed to the histological analysis, data analysis and interpretation, manuscript writing, editing, and approval. NP and FC participated in the clinical management of the patient, data analysis and interpretation, and manuscript editing and approval. RM contributed to the histological analysis, data analysis and interpretation, paper writing, editing, and approval. AO, GB, AM, JC, MB, FB, and SP participated in the clinical

management of the patient, data analysis and interpretation, and manuscript editing and approval. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.628324/full#supplementary-material

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REVIEW
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Immune Check Point Inhibitors in Primary Cutaneous T-Cell Lymphomas: Biologic Rationale, Clinical Results and Future Perspectives

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Primary cutaneous T-cell lymphomas (PCTCL) are the most common types of cutaneous lymphomas, with Mycosis fungoides as the most frequent subtype. Besides early stages which usually have a good prognosis, advanced stages remain a great therapeutic challenge with low survival rates. To date, none of the currently available therapeutic options have significantly improved the outcomes of advanced cutaneous lymphomas. Recent studies have demonstrated that immune-checkpoint molecules, such as PD-1 and CTLA-4, play part in the proliferation pathways of neoplastic T-cells, as well as in other tumors. Hence, the potential role of immune-checkpoint-inhibitors in treating cutaneous lymphomas has been investigated in the last years. Herein, we outline the current knowledge regarding the role of immune-checkpoint molecules in PCTCL, their signaling pathways, microenvironment and therapeutic inhibition rationale. Moreover, we review the published data on immunotherapies in PCTCL and summarize the currently ongoing clinical trials in this field.

Keywords: Cutaneous T-cell lymphomas, immunotherapy, Mycosis fungoides, immune-checkpoint-inhibitors, Sézary syndrome, nivolumab, pembrolizumab

INTRODUCTION

Primary cutaneous lymphomas (PCL) are a family of rare non-Hodgkin's lymphomas (NHL) characterized by monoclonal proliferation of malignant lymphocytes in the skin. Among them, 75% are represented by Cutaneous T-cell lymphoma (CTCL), with Mycosis Fungoides (MF) as the most common subtype, while 25% are Cutaneous B-Cell lymphoma (CBCL) (1). Rarer yet more aggressive, defined by the triad of T-cell leukemic evolution, lymphadenopathy and erythroderma, is the Sézary syndrome (SS) form, which can develop as a final manifestation of MF or appear *de novo* (2). Recently it has been suggested that due to their different T-cell subsets

origin SS and MF may represent two distinct pathological entities (3). In the recent years, there has been a growing interest in the understanding of molecular and immunological mechanisms that play a role in tumor development and progression: for instance, it has been well documented that the host's immune system acts as an active player in modulating the defense response against tumor progression (4). These findings have led to an unprecedent development of new immune-based therapies in the oncology field (5). To date, the world of immuno-oncology, which comprehends all those treatments aimed at manipulating the host's immune system in order to stop tumor proliferation, has achieved remarkable results in several tumors, such as melanoma, lung, kidney, and bladder cancer (6–9). Interestingly, recent studies on the pathogenesis of CTCL have also identified potential immunological targets for therapeutic approaches aimed at enhancing cell-mediated immunity (10). In particular, the role of immune checkpoint antibodies against PD1 (Programmed cell death protein 1) has been subject of inquiry in the last few years, as it has been proved that by targeting inhibitory PD-1 molecules expressed by exhausted T cells, these drugs can revitalize antitumor T cells and lead to impressive clinical responses (11, 12). In this review of the literature, we outline the current evidence on the interactions between CTCL and the immune system, review the published data on immunotherapies for CTCL and summarize the noteworthy ongoing clinical trials in this field.

PRIMARY CUTANEOUS T-CELL LYMPHOMAS: AN OVERVIEW

The cluster of primary cutaneous T-cell lymphomas (PCTCL) encompasses several lymphomatous entities with common defining underlying features (13). Among them, MF is the most common subtype, representing around 55% of the cases, with an incidence rate of about 5.6 per million people and a stable trend in the last two decades (14). Regardless of the traditionally described histopathological variants (i.e., folliculotropic, pagetoid reticulosis, granulomatous slack skin), the current 2007 staging system is based on a tumor-node-metastasis-blood involvement (TNMB) classification and correlates the clinical features with the prognosis (15). Early stages (IA, IB, IIA), characterized by long-standing erythematous scaly patches/plaques, typically located in the bathing trunk areas, usually show an indolent course: even though the 5-year disease free survival is high (i.e., varying from 98% to 89%), there is still considerable morbidity from pain, itching, discomfort, and disfigurement (16–20). Moreover, according to an Italian retrospective study on 1,422 MF patients, 29.7% of early-stage disease develops a disease progression (18). Advanced stages are conversely identified by skin tumors (stage IIB) or erythroderma (stage III), while blood (stage IVA1), nodal (stage IVA2) and visceral involvement (stage IVB) define the most severe extracutaneous forms (16, 19, 21). Regardless of the clinical onset, MF patients can later develop systemic manifestations of SS (22). The survival rates dramatically drop in the most advanced stages, with 5-year OS rates falling from 56% in IIB to 18% in IVB stages (16). Along with the clinical features, other factors contribute to the biological evolution of the disease: for example, age over 60, large-cell transformation and increased LDH values have been described as independent unfavorable variables (23). Still today prompt diagnosis remains a great challenge for clinicians, as almost 9 out of 10 cases show a significant time delay between symptoms onset and confirmed diagnosis (19).

CURRENT THERAPIES FOR CTCL

All the most recent published treatment guidelines agree on a stage-driven strategy, in consideration of clinical presentation, symptom burden and patient's comorbidities. However, due to the lack of strong evidence from clinical trials, there is currently no unanimous agreement on the sequence by which treatments should be administered: in fact, the choice of any specific therapy should primarily take into account several factors such as disease subtype, patient age/comorbidities, disease extension and treatment availabilities (24). The main goal of therapy is to improve the quality of life, by reducing symptoms, as durable complete remission is rarely achieved (24). With the exception of few selected stage IA patients, in which expectant policy and watchful waiting may be considered, treatment is recommended in all other cases. In early stages (IA, IB, IIA) first line options include skin direct therapies (SDT) such as topical corticosteroids, topical bexarotene, ultraviolet phototherapy, radiation therapy and the recently EMA-approved topical chlormethine (25). Those refractory to the first line may be considered for systemic therapies, such as retinoids, Interferon alpha, Total Skin Electron Beam therapy (TSEB) or low-dose methotrexate, which conversely represent first-line treatments for stage IIB MF (25, 26). Stage III patients may benefit from extracorporeal phototherapy (ECP), whilst refractory and stage IV patients have been traditionally treated with chemotherapy regimens (gemcitabine, pegylated liposomal doxorubicine, CHOP and CHOP-like polychemotherapy) (26). Patients with advanced MF or SS still have an unmet clinical need of effective treatments, due to low response rates, short-lived improvements, concomitant immunosuppression, and often severe drug-related side effects. Overall survival rates in SS are still low, varying from 7.5 to 22.4 months (27). Allogeneic stem cell transplantation (alloSCT), particularly using reduced-intensity conditioning, remains the only treatment option with curative intention for few selected patients (28). Notably, new options have become available in the last years. The anti-CD52 monoclonal antibody Alemtuzumab has shown significant clinical activity in patients with previously treated advanced MF/SS and constitutes a second-line option for patients with advanced disease, although with less efficacy in tumor-stage MF and large cell transformation types (29, 30). The ALCANZA trial led to the approval of the anti-CD30 monoclonal antibody Brentuximab Vedotin in patients with CD30+ MF, showing an ORR lasting at least 4 months of 56% compared to 13% in the control arm in which MTX or bexarotene were administered (31). Moreover, the MAVORIC trial compared the anti-CCR4 antibody

Mogamulizumab with Vorinostat, showing a significantly higher ORR in the former arm (28% *vs* 5%) and resulting in mogamulizumab approval for patients with high Sezary cell burden (32). Among histone deacetylase inhibitors, Vorinostat and Romidopsin have been approved by FDA as second line therapies for CTCL patients, whilst they are currently not available in Europe (33, 34). To date, regardless of the encouraging results of some trials on the aforementioned drugs, there is still no curative therapy that has represented a major breakthrough in the outcomes of CTCL. Ultimately, the latest therapeutic frontier has been set in motion by new studies regarding the potential role of immune-checkpoint-inhibitors in CTCL. Fully understanding the tumor microenvironment and its relationship with the host's immune system is crucial to develop new effective and highly specific immunotherapies.

THE ROLE OF TUMOR MICROENVIRONMENT IN PRIMARY CUTANEOUS LYMPHOMA

Since the introduction of so-called immunoediting theory an increasing number of studies have focused on the interaction between the malignancy and the microenvironment: non-immune cells (such as antigen-presenting cells), cells exerting immunosuppressive action or activated T-lymphocytes against neoplastic cells (35). All the studies have highlighted such interactions between the tumour and its microenvironment that are fundamental for MF/SS progression. Globally, in the advanced stages there is a switch from an anti-tumour (Th1) phenotype to a tumorigenic (Th2) one. In the early 2000s it was hypothesised that dendritic cells (DCs) may play an important role in CTCL progression (36–42). Indeed, an accumulation of immature DCs has been thought to be related to MF progression, owing to the immune-suppressive actions that immature DCs may play on activated T-cells, leading to anergy. Another debated category of cells are immune-suppressive cells such as T-reg cells or myeloid derived suppressor cells (MDSCs). In pioneering studies the former has been proposed as the normal counterpart of MF cells, evidence confuted later by the introduction of more specific immunohistochemistry antibody (43–50). Today, the role of Tregs seems to be related to a therapeutic response but it is still far to be fully understood (51–53). Studies on MDSCs are few and hypothesise a role in MF progression as well as the fact that MDSCs can be a marker of treatment response (42, 54, 55). Another intriguing category of cells are tumour-infiltrating lymphocytes (TILs). TILs try to control malignant T-lymphocytes and the main problem is that currently no specific markers can be used to distinguish benign from malignant T-cells. In advanced stages it has been proposed that an accumulation of exhausted anti-tumour cells may be one of the events leading to immune-suppression in MF/SS (56). Furthermore, in contrast to the plasticity of malignant T-cells that can express different phenotypes, TILs may have a constrained one (57). Consequently, malignant T-cells may have the ability to elude the control of the immune system. Eosinophils, macrophages, and endothelial cells may play a role in MF/SS progression. In hematologic malignancies it has been proposed that macrophages may recruit eosinophils *via* the production of vascular endothelial growth factors (VEGFs) (58, 59). However, it's still unclear whether eosinophils may exert an anti-tumour or tumorigenic role (60). Eosinophils within MF/SS infiltrate are rare. Most of the studies on the role of eosinophils provide contrasting results. Indeed, some groups have observed a significantly higher number of eosinophils in the advanced stages, while other studies have not found correlations between the eosinophil level and the disease stage (61–65). Currently, most studies suggest that eosinophils may play a tumorigenic role in MF/SS or may not exert an anti-tumour action at all. The role of macrophages in CTCLs is clearly tumorigenic and mounting evidence has proven that a polarisation to M2 (CD163+) macrophages is related to disease progression. M2 macrophages have an immune suppressive role leading to MF/SS progression. M2 macrophage accumulation starts in early MF phases and increases in the plaque and tumour stages (66–69). Some Authors have observed an accumulation of periostin-stimulated macrophages in plaque-stage MF that may lead to formation of the tumour lesions, while M2 macrophages may play an important role in maintaining an immunosuppressive tumour microenvironment later. Moreover, the interaction between neoplastic T-cells and the microenvironment also involves keratinocytes, fibroblasts and endothelial cells. A loop has been hypothesised between neoplastic elements and keratinocytes as well as fibroblasts that may lead to a permanent activation of STAT proteins with the production of tumorigenic (Th2) molecules (70). STAT overexpression determines a feedback loop between keratinocytes, stromal, and malignant T-cells leading as a consequence to a Th2 polarization of the inflammatory milieu and an empowerment of STAT overexpression (71). Evidence that endothelial cells can play an important role in MF progression has been clearly observed. By comparing MF infiltrate with healthy donor skin different groups have been proven to have an increase in microvascular density in MF (42, 72–75). Moreover, markers of both neo-angiogenesis such as VEGFA or lymph-angiogenesis (VEGF-C) have been observed as overexpressed in MF/SS highlighting the concept that during MF progression both an increase in blood and in lymphatic vessels can be advantageous to tumour survival and spread (42, 73). In conclusion, in CTCL a switch from an anti-tumour to a tumorigenic phenotype can help the disease to survive and spread to the lymph-nodes and the internal organs. Indeed, the accumulation of exhausted anti-tumour T cells and the increase in immuno-suppressive cells may lead to a cascade of events including an empowerment of immune-suppressive cytokine release as well as an increase in neo-angiogenesis which has the consequence of providing an advantage to the disease.

THE PD-1/PDL-1 AXIS IN PRIMARY CUTANEOUS LYMPHOMA

The immunological interactions between Programmed cell death receptor 1 (PD-1) and its ligands (PD-L1 and PD-L2) expressed

on cell membranes have been well documented in the scientific literature, as engagement of PD-1 with PD-L1/PD-L2 has shown to prevent T-cell activation and proliferation, weakening immune response (76). These findings have represented a breakthrough in the immuno-oncology field, leading to the understanding that tumor infiltrating T-cells are often functionally impaired due to high expression of PD-1 levels, while malignant cells can escape immune surveillance by expressing PD-L1 (77). Similarly, overexpression of other inhibitory checkpoint receptors, such as the B7-ligand known as CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4), has been proved to lessen immune surveillance in tumors (78). Hence, throughout the years, antibodies targeting PD-1 and CTLA-4 have been developed for treating several tumors, with the aim of restoring PD-1+ T-cell function and eventually halting tumor proliferation (79). In the growing field of immuno-oncology, studies have been carried out in order to achieve a thorough understanding of PD-1 and CTLA-4 expression in cutaneous lymphomas as well (80). The results have been diverse and noteworthy. Firstly, it has been shown that PD-1 and CTLA-4 are expressed by malignant cutaneous T-cells in MF and SS, while PD-L1 levels are high in dendritic cells *émigrés* from the skin but low in T-cells themselves (56, 81, 82). The different proportion of PD-1 expressing T-cells in MF and SS groups, reported as 13% in the former and 89% in the latter, has provided further evidence for considering them as two distinct entities (83). Secondly, PD-1 expression can help differentiate SS patients, in which PD-1 is highly expressed on neoplastic CD4 + cells, from patients affected by other inflammatory dermatoses, in which PD-1 is more often expressed by CD8+ cells (84). Klemke et al. proved that loss of CD7 and increased PD-1 expression in > 50% of the lymphocytic infiltrates discriminates SS from other erythrodermic inflammatory dermatoses (85). Kantekure et al. have also suggested that PD-1 expression seems to increase with lymphoma progression, correlating with an enhanced immunosuppressive microenvironment (10). However, while the progressive nature of immunosuppression in CTCL is well recognized, the mechanisms that underlie the immune impairment remain essentially unknown (10). The major part seems to be played by the interaction between PD-1 and its ligands PD-L1/PD-L2, as it leads to the transduction of a signal which inhibits the T-cell function, attenuating the immune response and the antitumor activity (86). Besides CTCL, this increased PD-1 expression has been also reported in several other models of defective immune function, including chronic viral infections (87–90). Conversely, high number of tumor-infiltrating CD8+ T cells in MF lesions correlates with a more favorable outcome (91). Moreover, the understanding that CTCL cells, as well as other cancer cells, are capable of evading immune surveillance has been documented by detecting a reduced TH1-response and an enhanced TH2-switch in MF lesions (92–94). All these aspects, along with the immunosuppression observed during disease progression and the evidence of common alterations in immune checkpoint related genes, have brought clinicians to theorize a therapeutic role of immune check point inhibitors in treating CTCL (95, 96). Herein we summarize the current available results, as far as anti-PD1 and anti-CTLA4 therapies for CTCL are concerned.

RESULTS OF CLINICAL TRIALS

To date only few studies related to safety and efficacy of ICI use in treating CTCL have been published. Two open-label trials have shown some significant results. The former is a phase I study conducted by Lesokhin et al. in which nivolumab, administered at dosage of 1 or 3 mg/kg every 3 weeks, showed a good tolerability profile in 81 patients with hematologic malignancies (97). Specifically, in the T-cell lymphoma subset, thirteen patients were affected by MF, five by PTCL (Peripheral T-cell lymphoma) and five by other T-cell lymphomas. The ORR was 15% in patients with MF and 40% in those with PTCLs. 73% (i.e., 17/23) of these patients experienced some kind of adverse events (AEs), most commonly mild fatigue, rash, and pruritus, while 5 patients experienced ≥ grade 3 reactions. The latter is a phase II study in which 24 patients with pre-treated MF (n=9) and SS (n=15) received Pembrolizumab 2 mg/kg every 3 weeks for up to 2 years (12). In this case, the ORR was 38%, with two CRs (complete responses) and seven PRs (partial responses). The median response follow-up time was 58 weeks. Four patients discontinued treatment due to immune-related side effects, while 53% of the patients with SS experienced cutaneous flare reactions. This occurrence was found to be associated with high PD-1 expression on Sézary cells. Furthermore, interesting preliminary clinical data were obtained in a phase 1b study in which 12 patients with relapsed/refractory PTCL and CTCL received pembrolizumab in combination with pralatrexate, a dihydrofolate reductase inhibitor, or decitabine, a cytidine analog, or both pralatrexate and decitabine (98). One patient achieved CR, two had PR, one stayed in SD (stable disease) and two experienced PD (progression disease). All responses were seen in the triple combination arm of pembrolizumab, pralatrexate and decitabine. This result suggests that the integration of pembrolizumab on an epigenetic backbone is safe and may improve the outlook in patients with PTCL and CTCL. Attention has also been focused on personalized treatments based on genomic features. A recent study by Beygi et al. hypothesized that genomic alterations of PD-L1, detected through Next Generation Sequencing techniques, may help predict response to PD-1 targeting therapy in CTCLs: in fact, the identification of PD-L1 structural variants (SVs) as potential genomic biomarkers of response to PD-1 axis inhibition proved to be helpful in assessing the response to Pembrolizumab in 3 patients with CTCL (99). However, the authors acknowledged the need of further larger studies in order to fully explore the predictive value of PD-L1 alterations in CTLCs. As for CTLA-4 inhibiting antibodies, current data are even more limited, as its efficacy in CTCL has yet to be determined. To date, only two case reports have showed positive results. In a case report by Bar-Sela, a 44-year-old male with MF and melanoma, exhibited a complete resolution of MF cutaneous lesions after treatment with ipilimumab for advanced melanoma (100). In another case

report, Sekulic et al. described the rapid response of a SS patient with a rare gene fusion between the extracellular/transmembrane domain of CTLA-4 (which has a high affinity for binding ligands) and the intracytoplasmic domain of PD-1 (101). Ultimately, combination of ipilimumab with nivolumab has been experienced in T-cell lymphomas. In a phase I study of eleven patients, the efficacy of the combination was not superior to nivolumab monotherapy with an ORR of 9% and only 1 PR observed (102). Altogether, these findings regarding the role of PD-1 axis in CTCLs confirm the great need for further investigations in this field (103). Here we outline a synopsis of the currently published studies and the ongoing clinical trials (103, 104) (**Tables 1**, **2**).

DISCUSSION AND FUTURE PERSPECTIVES

The role of immune checkpoint inhibitors in the treatment of CTCL still represents a unique challenge in immuno-oncology, as the exact role of PD-1 and its ligands in tumor microenvironment of patients with CTCL is not fully understood and may differ from other tumors (105–107). This peculiarity is related to the fact that the tumor itself arises from CD4+ T-cells, a population responsible for priming of the cytotoxic response; therefore, it has been speculated that targeting immune checkpoints would have implications on the functionality of both helper and cytotoxic T cells (108). Hence, as for the PD-1 axis, a substantial difference can be noted between solid tumors and CTCL: in the former group, neoplastic cells express PD-L1 which binds to PD-1 on T-cells, inhibiting their activity. Therefore, targeting PD-1 with anti-PD-1 antibodies can prevent this inhibitory interaction, restoring T-cell function. Conversely, the peculiarity of PD-1 expression in CTCL resides in the fact that the proliferating neoplastic itself is a CD4+ T-cell. In this specific case, targeting PD-1 with anti-PD-1 antibodies could have a double effect: on the one hand, this could restore the antineoplastic function of TILs as in solid tumors, while on the other, this could promote the proliferation of the neoplastic T-cell population (109, 110). Several questions have been raised and still need to be answered. O'Malley et al. showed that over 86% of malignant T-cells in patients with CTCL express PD-1, compared with 16% of benign T cells, suggesting that preferential expression of PD-1 by malignant T cells may underlie worsening of clinical disease in a subset of patients treated with PD-1 blockade (111). Saulite et al. also emphasized that blocking PD-1 in SS reduces Th2 phenotype of non-neoplastic T-cell and may paradoxically enhance tumor proliferation (105). Similarly, Sivanand et al. brought attention to the controversy that, if expression of PD-1 on malignant T-cell has an inhibitory function, PD-1 blockade can potentially

TABLE 1 | Summary of the published results from the main studies on immunotherapy in CTCL.

Target	Drug	Study Type	N° of pts	Inclusion	ORR	Disease outcome
PD-1 (Lesokhin)	Nivolumab	Phase I open-label dose-escalation, cohort-expansion basket	13	Heavily pretreated MF	15%	Duration of response up to 81 weeks
PD-1 (Khodadoust)	Pembrolizumab	Phase II	24	MF/SS patients (23 of 24 with stage IIB to IV) and heavily pretreated	38%	8 durable responses (median DOR not reached > 58 weeks)
PD-1 (Marchi)	Pembrolizumab in combination with epigenetic drugs	Phase 1b Three arms (4 patients per arm): **A**: pembrolizumab + pralatrexate **B:** pembrolizumab + pralatrexate + decitabine **C:** pembrolizumab + decitabine	12	Relapsed/refractory TCL (5 PCTL, 3 AITL*, 1 ATLL°, 2 MF and 1 SS). **Angioimmunoblastic T-cell lymphoma* *°Adult-T-cell lymphoma/ leukemia*	6 out of 12 patients evaluable for response at the time of analysis	*Arm B*: 2/4 (CR, PR)
PD-1 (Beygi)	Pembrolizumab	Case report on 3 patients **Pt.1** Pembrolizumab + IFNg 6 cycles, Pembrolizumab alone 36 cycles; **Pt.2** Pembrolizumab 2 cycles **Pt.3** Pembrolizumab 6 cycles	3	Pt.1 Stage IIB MF Pt.2 Stage IVB MF Pt.3 Stage IIB MF	***Duration of response:*** Pt.1 12 weeks (first round), 110 weeks (second round in combination with RT) Pt.2 12 weeks Pt.3 9 weeks	Pt.1 SD Pt.2 Discontinuation due to immune-related pneumonitis Pt.3 PD
CTLA-4 (Bar-Sela)	Ipilimumab	Case report	1	Stage IA MF	CR	–
CTLA-4 (Sekulic)	Ipilimumab	Case report	1	Stage IVA SS	PR 6 weeks	Death 3 months after last dose

ORR, Overall response rate; Pt, patient; Arm A, Arm B, Arm C.

TABLE 2 | Summary of the currently ongoing trials on immunotherapy in CTCL.

Study	Type of study	Drug	Inclusion criteria	Start date	Primary completion	Study completion
NCT03063632	Phase II	Pembrolizumab + Interferon-gamma	Relapsed-Non respondent (stage IB-IVB) MF, SS and Advanced Synovial Sarcoma	Oct 13, 2017	Apr 8, 2021	Apr 8, 2022
NCT03278782	Phase I/II	Pembrolizumab + Romidepsin	Relapsed-refractory- non respondent peripheral T-cell Lymphoma	Nov 14, 2017	Nov 30, 2021	Nov 30, 2021
NCT02581631	Phase I/II	Nivolumab + Brentuximab Vedotin	Relapsed-refractory- non respondent NHL CD30+	Dec 18, 2015	Jan 16, 2020	Aug 30, 2021
NCT02978625	Phase II	Talimogene Laherparepvec followed by Talimogene Laherparepvec + Nivolumab	Refractory T-cell and NK Cell Lymphomas, Cutaneous SCC, Merkel Cell Carcinoma, and Other Rare Skin Tumors.	Sept 18, 2017	June 1, 2022	June 1, 2022
NCT03011814	Phase I/II	Durvalumab as a single agent or with Lenalidomide	Relapsed/refractory PTCL including CTCL	March 8, 2017	June 8, 2022	June 8, 2022
NCT03357224	Phase II	Atezolizumab	Relapsed or refractory stage IIb-IV MF-SS	Sept 24, 2018	Sept, 2021	June, 2025

promote tumor growth (110). Another topic of discussion is the heterogeneity of results as far as PD-1 expression on T-cell in MF and SS is concerned: in fact, while some authors have reported an augmented expression in a substantial proportion of both MF and SS patients, others have described it as more relevant in SS only (111, 112). Moreover, recent studies about neoantigen heterogeneity have emphasized the role of mutational load in CTCL: Iyer et al. have proved that as MF progresses, the tumor accumulates somatic mutations and evolves to produce multiple genetic subclones (113). Sivanand et al. suggested that this process has a double effect, as on one hand it leads to higher neoantigen expression and increased opportunities for the neoplasm to be recognized by the immune system, while on the other the increasing subclonal distribution of neoantigens can direct the immune system to discrete subpopulations of the most immunogenic tumor cells (114). This in turn may shield the less immunogenic subclones from the antitumor attack and limit efficacy of immunotherapy in MF (114, 115). As for the other main actor in the ICI class (i.e., CTLA-4), even less evidence has been found so far: in fact, while Querfeld et al. observed a promising higher expression of CTLA-4 in CTCL, Anzengruber et al. reported no significant differences with healthy controls (116, 117). To date, as the combination of anti-PD-1+anti-CTLA-4 showed no benefit over anti-PD1 alone, there are no current active trials on the efficacy of CTLA-4 in CTCL (118). All evidence considered, it remains challenging to come to a univocal conclusion on the efficacy of ICI in CTCL. New interesting data may come from other less characterized, yet worthy of mention, ICI molecules: for example, FRCL3 (Fc receptor-like 3), TIGIT (T-cell immunoreceptor with Ig and ITIM domains), BTLA (B and T Lymphocyte Associated), ICOS (Inducible T-cell costimulator) and LAG-3 (Lymphocyte-activation gene 3) have been found to be significantly upregulated in CTCL and this finding could represent a new frontier in the research of new target therapies (108, 117, 119, 120). Recently, new findings regarding ICOS expression in CTCL cells seem to provide the preliminary basis for therapeutic trials, as anti-ICOS antibody–drug conjugates proved antitumor potential against CTCL cell lines and patient-derived xenografts (121). Nevertheless, no *in vivo* studies testing the blockage of these molecules have been started so far. Finally, it is worth mentioning that few authors have listed T-cell lymphomas among the potential, yet very rare, immune-related adverse events following ICIs use: for instance, in the 2012-2018 FAERS (Food and Drug Administration Adverse Events Reporting System) pharmacovigilance database a 0.02% incidence of T-cell lymphoma post-ICIs use, with a 17% mortality, was registered (122). It has been speculated that this phenomenon might be associated with rebound overexpression of PD-1 after the treatment, however actual mechanisms remain still unknown and further studies are needed to better characterize this paradoxical occurrence (123, 124). In conclusion, this literature review highlights the potential role of immune-check point inhibitors in CTCL, according to the current available data. Altogether, the outlook of using ICI in this field seems to be less favorable compared to the one observed in other tumors, such as melanoma (6). Carrying out new research, aimed at disentangling the complex relationship between CTCL and the host's immune system, may hopefully lead to a more detailed understanding of immunological targetable molecules, in order to provide patients with innovative therapeutic chances.

AUTHOR CONTRIBUTIONS

PQ, MF, SR, and PF conceived and designed the presented review. GR, SG, AP, and AG wrote the manuscript with input from all authors. MS and LT analyzed the data. PQ, MF, SR, PF, GR, SG, AP, AG, MS, and LT contributed to the implementation of the research. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Case Report: Autoimmune Pemphigus Vulgaris in a Patient Treated With Cemiplimab for Multiple Locally Advanced Cutaneous Squamous Cell Carcinoma

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Background: Pemphigus vulgaris (PV) is a rare and severe autoimmune blistering disorder affecting the skin and mucous membranes, characterized by the production of autoantibodies against two desmosomal adhesion proteins, desmoglein 1 and 3. In patients with advanced squamous cell carcinoma of the skin unfit for surgery and radiotherapy, immune check-point inhibitors, including the anti-Programmed Death-1 (PD-1) agent cemiplimab have been successfully employed proving relevant clinical outcomes. Cemiplimab is a monoclonal antibody capable of inhibiting PD-1 signalling that has recently been approved for the treatment of patients with metastatic or locally advanced cutaneous squamous cell carcinoma. Although the peculiar setting of advanced CSCC involving elderly patients, rare and unusual skin immune-related adverse events such as PV could be observed in cemiplimab treated patients.

Case Report: A 95-year-old man without a history of autoimmune disease was treated with cemiplimab for multiple and advanced squamous cell carcinomas of the head obtaining a complete response to therapy. After seven cycles of cemiplimab administered every 21 days, the patient developed a mucocutaneous blistering eruption. Clinical diagnosis of PV was suspected on the basis of the diffuse involvement of trunk and extremities with large blisters and necrotic eschar. It was carried out an ELISA test, that showed high level of circulating antibodies against desmoglein 1, thus confirming the diagnosis of PV. For this reason, cemiplimab infusion was discontinued and complete resolution of skin lesions was obtained using oral prednisone 0,8 mg/kg/daily for four weeks. Once remission was achieved, a maintenance dose of 10 mg/day was administered, observing a good control of bullous disease and low value of desmoglein 1. Response to CSCC persisted also during cemiplimab discontinuation, until obtaining a complete remission still persisting at 9 months after the last cycle of therapy.

Conclusion: The case we observed is the first description of PV revealed from cemiplimab therapy, thus suggesting that cemiplimab could allow the arise of underlying autoimmune PV, through a mechanism both T and B-cell-mediated.

Keywords: Pemphigus vulgaris, desmoglein, immune check-point inhibitors, cemiplimab, anti-programmed-death-1, cutaneous squamous cell carcinoma

INTRODUCTION

Pemphigus vulgaris (PV) is a rare and severe autoimmune blistering disorder that affects the skin and mucous membranes (1). PV is characterised by the production of pathogenic autoantibodies directed against two desmosomal adhesion proteins, desmoglein Dsg1 and Dsg3 (also known as DG1 and DG3), which are present in the skin and mucosae (1). The binding of autoantibodies to Dsg proteins induces a separation of neighbouring keratinocytes *via* a process known as acantholysis, leading histologically to intraepidermal blisters, and clinically to blisters and erosions on the epithelium of the mucous membranes and/or the skin (1). Since the pathophysiology is driven by an autoimmune process, autoantibodies are the basis of diagnostic investigations and treatment strategies.

Cutaneous squamous cell carcinoma (CSCC) is a highly incident skin cancer that is often characterised by multifocal presentation and high rates of local recurrence after surgical excision. Advanced CSCCs include a small number of metastatic patients and more frequent cases of locally advanced disease unfit for both surgery and radiotherapy, which can only be treated by systemic therapy (2, 3). Immunotherapy with anti-programmed death ligand-1 (PD-1) agents is the gold standard in all current guidelines, and cemiplimab is the only recently approved anti-PD-1 antibody in Italy (4–6).

The increasing use of checkpoint inhibitors for the treatment of advanced skin cancers, with positive response to therapy, is related to an increase in adverse skin reactions. Among such side effects, diseases with autoimmune pathogenic mechanisms have also been described (7).

We report the first case of a patient who developed severe PV during cemiplimab therapy for locally advanced CSCC.

CASE DESCRIPTION

A 95-year-old man with no known history of autoimmune disease developed widespread mucocutaneous blistering during cemiplimab therapy, which was administered for multiple and advanced squamous cell carcinomas of the head.

He had undergone multiple resections of CSCCs of the head and neck region, three of which were performed during the preceding year. Over the last 6 months, he exhibited a local relapse in the right parotideal area, with a rapidly growing, ulcerated, and bleeding lesion extending to the zygomatic area close to the lower eyelid, the right cheek, and the mandibular area (**Figure 1**). Histological examination of the lesion showed infiltration of subcutaneous tissue, with a high proliferative rate (Ki-67, 90%) associated with wide necrotic and ulcerated areas, thus confirming the clinical impression of CSCC. Clinically, other smaller but similar lesions were localised in the patient's left zygomatic area, the left ear, and upper limbs. Computed tomography (CT) scan was negative for regional or distant metastases. The patient was initiated on immunotherapy with cemiplimab (350 mg flat dose every 3 weeks) in February 2020. Soon after the first cycle, the lesions became non-ulcerated and progressively thickened with crust evolution. The treatment

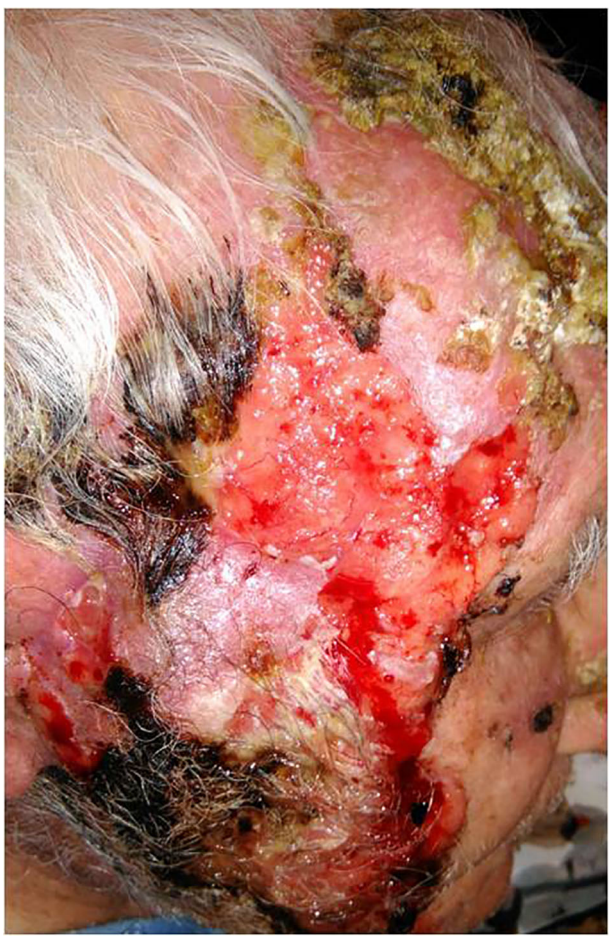

FIGURE 1 | Locally advanced locally cutaneous squamous cell carcinoma of the face extending to the zygomatic area close to the lower eyelid, the right cheek and the mandibular area, characterized by necrotic ulcerated areas and infiltration of subcutaneous tissue.

was well tolerated, except for occasional intermittent grade 1 pruritus after cycle 5, which was responsive to anti-histamines. After completion of seven cycles, ubiquitous splinter lesions appeared on the patient's body, first occurring in the lower limbs, then extending to the trunk and upper limbs, alongside similar lesions in the mucosa of the oral cavity.

Due to the onset of this severe skin reaction, classified as grade four (G4), cemiplimab therapy was permanently discontinued. Suspecting an adverse reaction to cemiplimab, the patient was administered systemic steroid therapies, with incomplete remission of the dermatosis. However, the patient relapsed shortly after dose reduction.

At our department, clinical examination of the patient revealed diffuse involvement of the trunk and extremities with large blisters over the skin, with serum content, excoriations, and large necrotic haemorrhagic eschar on the head (face and scalp) (**Figure 2**). The patient had multiple comorbidities, such as arterial hypertension, hypertensive cardiomyopathy, and dyslipidaemia. The dermatological history was negative for a previously arisen bullous eruption. The patient had no underlying skin or autoimmune disorders, no recent exposure to light or radiation, and was not under any new medications. The first clinical differential diagnosis included paraneoplastic pemphigus (PNP), PV, and bullous pemphigoid (PB).

Due to the possibility of an autoimmune bullous disease, we suggested testing for serum IgG autoantibodies against desmoglein 1, 3, BP180, and BP230. Enzyme-linked immunosorbent assays (ELISA) showed a positive reaction with desmoglein (Dsg) 1: index value 28 U/ml; (reference positive >20 U/ml), but not with Dsg 3, BP 180, or BP 230. Based on clinical features and laboratory investigations, a diagnosis of PV was made. Consequently, the patient received treatment with prednisone 0.8 mg/kg/daily for 4 weeks and topical steroids, with resolution of skin lesions. Oral steroid therapy dose was gradually narrowed down to 25 mg per day, resulting in complete remission of the dermatosis.

Approximately 5 months after the first observation, the patient showed significant recovery, and there was no recurrence of

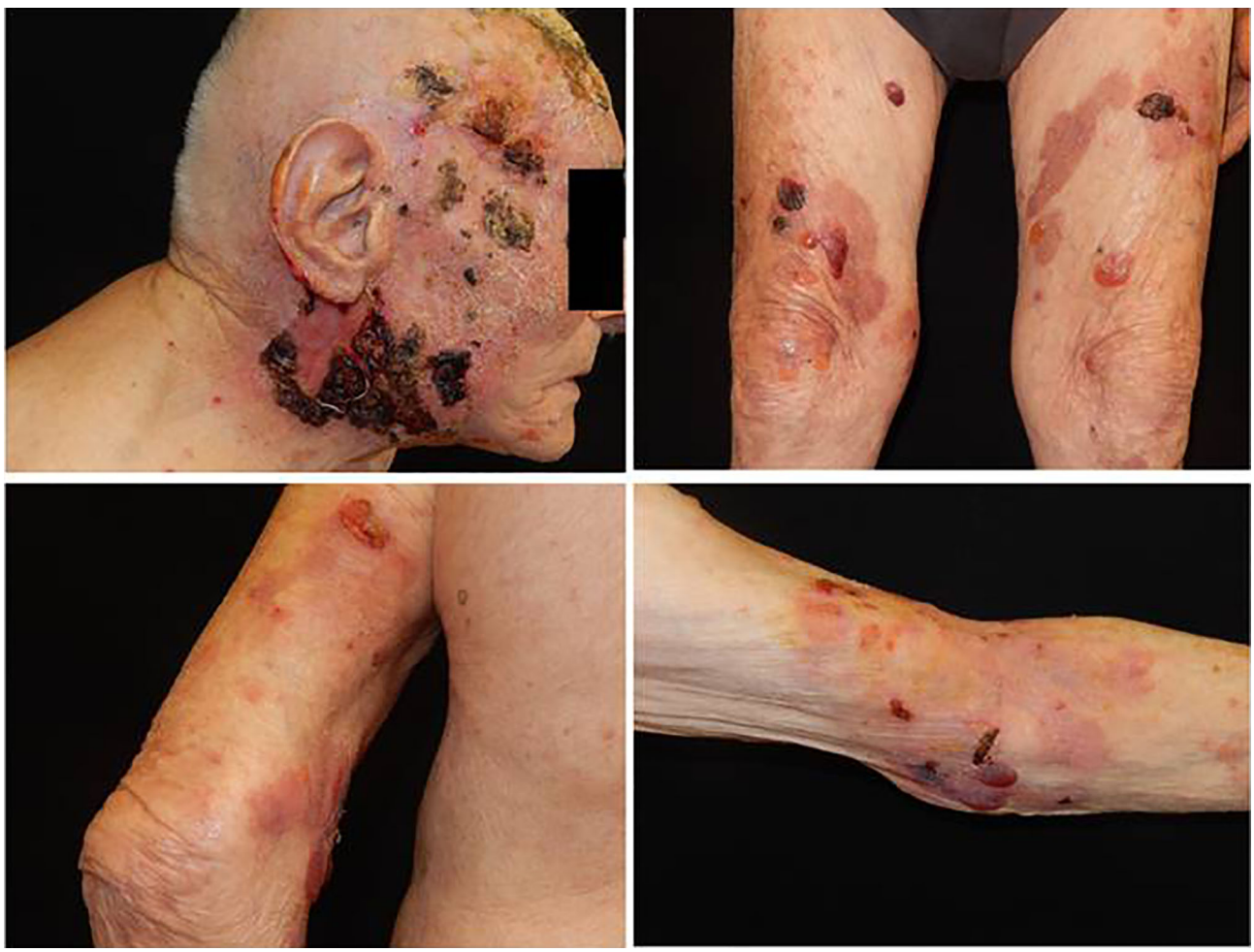

FIGURE 2 | A large necrotic haemorrhagic eschar on the head (face and scalp), with ulcers and serohematic scars associated with blisters over skin, with serum content, localized on the trunk and extremities.

dermatosis (**Figure 3**). Therefore, we reduced the dosage of prednisone to 12.5 mg per day.

DISCUSSION

A wide range of immune-related skin disorders have been observed in patients treated with immune checkpoint inhibitors (7). Immune checkpoint inhibitors (ICIs) represent a new class of anticancer agents. They belong to a class of drugs implicated in the inhibition of programmed cell death receptor 1 (nivolumab, pembrolizumab, and cemiplimab), PD-1 (atezolizumab, avelumab, and durvalumab), and cytotoxic T-lymphocyte-associated protein 4 (ipilimumab) (4). By blocking cytotoxic T-lymphocyte-antigen 4 (CTLA-4) and programmed cell death receptor 1 (PD-1) or its ligand; programmed death ligand 1 (PDL-1), they release negative inhibitory control of the immune system. This reverses T-cell suppression, thereby inducing an antitumor response (8).

Despite their efficacy against many malignancies, immuno modulatory antibodies non-specifically activate the immune system, which can lead to a new spectrum of immune-related adverse events (irAEs), like dermatologic toxicities (9). irAEs are described as a consequence of immune reactivation, with an unpredictable inflammatory response, loss of self-tolerance, and development of autoimmunity (10).

The precise mechanisms underlying the development of immune-related adverse events have not been fully elucidated but are postulated to be largely T cell-mediated. Monoclonal antibodies that target PD-1/PDL-1 pathways may induce immune-mediated adverse events possibly related to a reduction in regulatory T cells, leading to increased T-cell activation, B-cell proliferation, and synthesis of autoantibodies (7).

Cutaneous adverse events are reported in approximately 30%–40% of patients receiving immunotherapy. Their clinical expression can lead to pruritus, maculopapular rash, pigmentary changes, eczematous dermatitis, psoriasis, lichenoid dermatitis, vitiligo, and other inflammatory skin diseases (11). Autoimmune

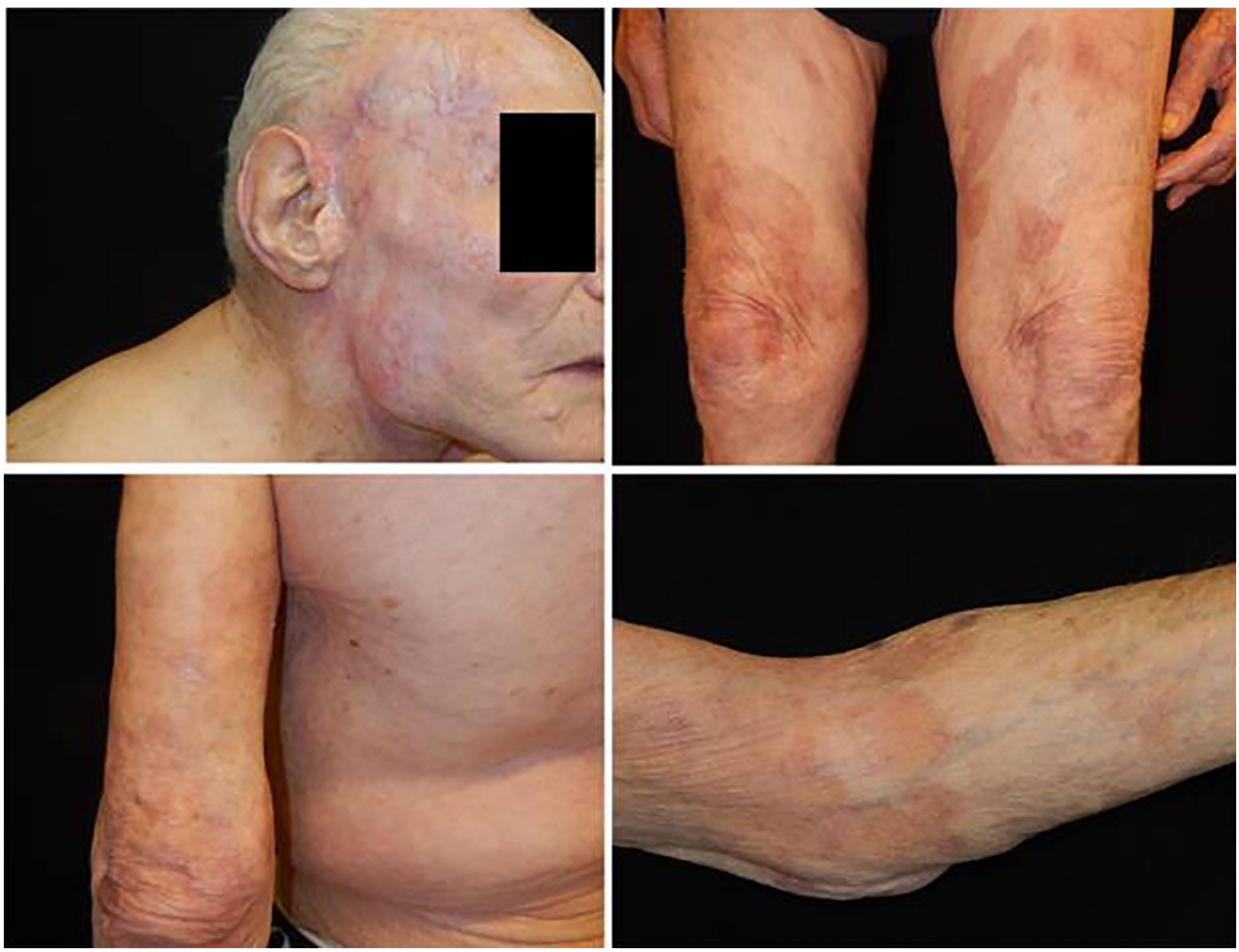

FIGURE 3 | Complete remission of cutaneous squamous cell carcinoma on the head, and absence of bullous lesions on the trunk and the extremities.

blistering disorders represent approximately 1% of cutaneous immune-related adverse events (12).

Previous literature has also reported occurrence of the present anti-PD-1/PD-L1 therapy-associated autoimmune blistering disease (13, 14). According to a recently published review, 21 cases of BP have been described in association with PD-1 inhibitors (10 cases were associated with pembrolizumab, 9 cases with nivolumab, one case with durvalumab, and one with atezolizumab) (14). A few other studies have described two cases of atypical PV in patients treated with anti-PD-1 (one related to nivolumab and one with pembrolizumab), one report of PNP with pembrolizumab, and two cases of mucous membrane pemphigoid (MMC) associated with pembrolizumab (15, 16). Among the data presented in our clinical experience, we observed two cases of PB related to PD-1 inhibitor therapy (one case with nivolumab and one with pembrolizumab) (data not published).

Cemiplimab, an immune checkpoint inhibitor (ICI), is a high-affinity potent human immunoglobulin G4 monoclonal antibody capable of inducing programmed cell death. It was approved by the Food and Drug Administration for the treatment of patients with metastatic CSCC or locally advanced CSCC that is not a candidate for surgery or radiation (2).

In this case study we observed that blistering lesions, indicative of bullous disease can occur during cemiplimab therapy for multiple and recurred CSCC. Based on the time of appearance of clinical lesions after initiation of immunotherapy with anti PD-1 and response to steroid therapy, we derived two hypotheses of diagnosis. The first hypothesis is concerned with a PNP revealed by use of cemiplimab therapy, as described in previous literature which states that PNP development has been related to the PD-1 pembrolizumab administration, used for a CSCC (15).

PNP is a severe autoimmune bullous disease, characterised by polymorphous skin lesions involving the mucosa and are associated with benign and malignant neoplasms, such as chronic lymphocytic leukaemia (30.2%), non-Hodgkin lymphoma (26.4%), carcinoma (18.9%), Castleman disease (9.4%), and thymoma (7.5%) (17). The criteria to make a diagnosis of PNP include, the presence of painful mucosal erosions associated with several polymorphous cutaneous eruptions and contemporary serum analysis for the presence of IgG autoantibodies against desmoglein 1 and desmoglein 3 (17). In our case, the absence of polymorphous lesions and mucous involvement, and the positive test for desmoglein 1 rather than desmoglein 3 allowed us to exclude the diagnosis of PNP.

According to the second hypothesis, it is conceivable that a diagnosis of PV without oral mucosal lesions can be induced by PD-1 therapy, whose possibility is confirmed on the basis of desmoglein 1 positivity. In particular, we noted that PV that appeared during cemiplimab administration showed typical manifestation with bullous eruptions, and can also occur at a later time point after the start of immunotherapy (5 months). In the literature, only one case of atypical pemphigus was reported in a patient during immunotherapy with nivolumab administration, for the treatment of urothelial carcinoma (16). Autoimmune skin diseases have been observed in many patients undergoing immunotherapy for cancer. The pathogenetic hypothesis lies in the mechanism of action of these drugs. The onset of autoimmune disease results from the inability to maintain immune self-tolerance. The immune mechanisms that lead to the breakdown of self-tolerance in PV during ICI therapy have not yet been fully elucidated. In order to explain the onset or recurrence of autoimmune diseases during therapy with ICI, it is hypothesised that there is close cooperation between innate immunity, adaptive immunity, T-regulatory, and T-memory cells (18). Furthermore, with particular reference to PV, for the production of anti-desmoglein antibodies, close communication between T and B lymphocytes is necessary. However, the immune mechanisms that lead to the breakdown of tolerance in PV during ICI therapy are not fully understood. From an immunological point of view, it is predicted that both the B and T lymphocyte compartments are involved in PV, or, alternatively, that the autoreactive B cells are widely expressed in patients with PV and ICI therapy can induce the block of tolerance, responsible for the autoimmunity trigger (19). It is also speculated that the antigen presentation of desmoglein by keratinocytes that have many mutations can potentially break tolerance to PV; it is even possible that there might be localised mutations in desmoglein that stimulate loss of tolerance.

The blocking of some pathways by these drugs results in reactivation of the immune system, thus leading to immune activation against skin target proteins by identifying them as antigens. As a confirmation for the above comments, cases of bullous disorders, such as PV and BP, have been reported in the literature after HAART therapy as a manifestation of IRIS, caused by a paradoxical production of auto antibodies against intercellular substances (desmoglein) and dermo-epidermal junction, respectively. The pathogenic mechanism may occur due to aberrant T−cell signalling to B cells, and secretion of immunoglobulin due to the sudden increase in CD4 T−cell count following initiation of HAART (20).

We assumed that cemiplimab immunotherapy could trigger a reactivation of T-cytotoxic immunity, similar to that observed in HIV-infected patients treated with antiretroviral therapy (HAART), in which a real immune reconstitution inflammatory syndrome (IRIS) is observed. We suggest that cemiplimab might have triggered the development of PV in our patient, and blockade of the PD-1/PD-L1 pathway may increase autoantibody production against the desmosomal protein Dsg1, through a process that is both T-cell-and B-cell-mediated.

To our knowledge, this is the first report on an association between PV and cemiplimab therapy. Its peculiarity also lies in the late occurrence of this immune-related adverse event in a very elderly patient. Due to the limited follow-up time and number of patients enrolled in cemiplimab clinical trials, real-world data on immune-related adverse events are becoming increasingly relevant. Of note, despite not being on active therapy, the tumour continued to decrease in size clinically and disappeared completely (**Figure 3**). Of note, for the entire observation period, the patient presented with an absence of bullous manifestation on the skin. In conclusion. During immunotherapy with checkpoint inhibitors, anti-PD-1/anti-PD-L1 autoimmune

disorders may occur; however, it is possible to have good control of dermatosis through steroid therapy without negative impact on outcomes.

This case highlights the importance of further studies to improve the understanding of the efficacy as well as skin adverse effect profile of PD-1 inhibitors in elderly patients with advanced CSCC.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the patient for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version. Conceptualization, RF, and RB. Data collection, RB, VM, RF, DQ, SS and MG. Methodology, RF and MG. Analysis, writing, and editing MG, RB, RF, VM, DQ, and SS. Supervision, RF.

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REVIEW
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Immunotherapy for the Treatment of Cutaneous Squamous Cell Carcinoma

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Cutaneous squamous cell carcinoma (CSCC) accounts for approximately 20% of all keratinocytic tumors. In most cases, the diagnosis and treatments are made on small, low-risk lesions. However, in about 5% of cases, CSCC may present as either locally advanced or metastatic (i.e. with locoregional lymph nodes metastases or distant localizations). Prior to the introduction of immunotherapy in clinical practice, the standard treatment of advanced CSCC was not clearly defined, and up to 60% of patients received no systemic therapy. Thanks to a strong pre-clinical rationale, clinical trials led to the FDA (Food and Drug Administration) and EMA (European Medicines Agency) registration of cemiplimab, a PD-1 inhibitor that achieved encouraging results in terms of objective response, overall survival, and quality of life. Subsequently, the anti-PD-1 pembrolizumab received the approval for the treatment of advanced CSCC by the FDA only. In this review, we will focus on the definition of advanced CSCC and on the current and future therapeutic options, with a particular regard for immunotherapy.

Keywords: immunotherapy, skin cancer, CSCC, cutaneous squamous cell carcinoma, cemiplimab, non-melanoma skin cancer, anti-PD-1 (programmed cell death-1 protein) monoclonal antibody, keratinocyte carcinomas

INTRODUCTION

Cutaneous squamous cell carcinoma (CSCC) is a non-melanoma skin cancer of keratinocytic origin, and accounts for approximately 20% of all keratinocytic cancers, standing as the second most common neoplasm after basal cell carcinoma (BCC) (1). The main risk factors are chronic exposure to ultraviolet (UV) radiation, followed by age, fair phototype and immunosuppression [specifically related to solid organ transplantation (2), chronic lymphocytic leukemia (3), and HIV infection (4)] (5). Other risk factors like the exposure to arsenic and polyaromatic hydrocarbons can be considered occupational (6).

CSCC is characterized by a high tumor mutational burden (TMB) (7) with a large amount of UV radiation-related mutations, most notably C>T and CC>TT dinucleotide mutations (8). However, genetic mutations that could lead to a targeted treatment are infrequent, and may include PIK3CA, fibroblast growth factor receptor 3 (FGFR3), BRAF and EGFR (9).

Some hereditary syndromes may increase the risk of developing CSCC such as xeroderma pigmentosum, epidermolysis bullosa, oculocutaneous albinism, Lynch syndrome, and Fanconi syndrome (1).

Due to the heterogeneity of clinical and histologic presentations, therapeutic options, and low mortality rates, accurate data on the incidence and prevalence of CSCC are not available to-date. In Australia, where the highest incidence of skin cancer is generally recorded, there are an estimated 387 cases per 100,000 (10). In the United States, more than 700.000 new cases of CSCC are diagnosed annually, and about 3900-8800 people die each year due to this disease (11). In Europe, the incidence of CSCC ranges across different latitudes from 9 to 96 per 100.000 for male individuals and 5 to 68 per 100.000 for females (12–15).

In more than 90% of cases, the prognosis is good and treatment consists of minimally invasive surgical procedures or, in selected cases, other local therapy modalities (16). In case of primary CSCC for which curative surgery is not indicated, definitive radiotherapy (RT) may be considered as a primary treatment. Despite the lack of randomized trials comparing the outcomes of RT *versus* surgery and other local therapy modalities, in a systematic review and pooled analysis of 7 observational studies for a total of 761 primary CSCCs, the local relapse with RT was as low as 6.4% (17). However, especially in the immunocompromised patient population, in case of social difficulties, lack of caregiver support, and/or in presence of multiple comorbidities, CSCC can manifest in locally advanced or metastatic forms representing an emerging clinical problem (5). In these cases, local treatments are no longer indicated to achieve an appropriate disease control. Until few years ago, the only available therapeutic options were chemotherapy and targeted therapy (i.e., EGFR inhibitors), with poor response rates and duration of response, and frequently at the cost of unacceptable toxicities for such a frail population. With the approval by the Food And Drug Administration (FDA) and European Medicines Agency (EMA) of the anti-PD-1 cemiplimab in 2018, and of the anti-PD-1 pembrolizumab by the FDA only in 2020, immunotherapy has become the standard of care for patients with CSCC who are not eligible for curative surgery or radiotherapy (18).

In this review, we will discuss the main criteria for the identification of CSCC patients who are at high risk of relapse, and the multidisciplinary definition of locally advanced CSCC, according to the most recent guidelines. In addition to that, the results of main systemic treatment regimens will be discussed, with a focus on immunotherapy, especially regarding the key findings on the new therapeutic options and future therapeutic landscapes.

IDENTIFICATION OF HIGH-RISK CSCC AND CLINICAL DEFINITION OF ADVANCED CSCC

In most cases, CSCCs are detected as small or early-stage lesions that have a low risk of recurrence after an appropriate surgical treatment (16). Specifically, the overall recurrence rate has been shown in several studies to be between 2.1% and 4.6% (19). Although only few CSCC have a high risk of local or distant recurrence, it is essential to identify the high-risk patient group for a proper diagnostic and therapeutic workup, and an individualized follow-up. Risk factors can be either tumor-related (clinical or pathological) or patient-related, as indicated by the European Dermatology Forum (EDF), European Association of Dermato-Oncology (EADO), and European Organization for Research and Treatment of Cancer (EORTC) guidelines (20, 21). However, the impact of each individual risk factor is not entirely clear. In a recent meta-analysis, published data on risk factors for recurrence, metastasis, and disease-specific death of CSCC were systematically analyzed. The main results of this work were summarized in **Table 1** (22). Briefly, tumor depth was associated with the highest risk ratio of local

TABLE 1 | Risk ratios for recurrence, metastasis, and disease-specific death for some of the most relevant high-risk factors (22).

High-risk factors	Risk Ratio for recurrence (95% CI)	Risk Ratio for metastasis (95% CI)	Risk Ratio for disease-specific-death (95% CI)
Tumor-related (clinical)			
Tumor diameter > 20 mm	3.22 (1.91-5.45)	6.15 (3.56-10.65)	19.10 (5.80-62.95)
Primary tumor site at:			
Temple	3.20 (1.12-9.15)	2.82 (1.72-4.63)	1.80 (0.22-14.79)
Ear	1.28 (0.56-2.90)	2.33 (1.67-3.23)	4.67 (1.28-17.12)
Lip	1.28 (0.41-3.97)	2.28 (1.54-3.37)	4.55 (1.41-14.69)
Tumor-related (pathological)			
Thickness > 6 mm	7.13 (3.04-16.72)	6.93 (4.02-11.94)	NR
Invasion beyond subcutaneous fat	7.61 (4.17-13.88)	11.21 (3.59-34.97)	4.49 (2.05-9.82)
Poor differentiation	2.66 (1.72-4.14)	4.98 (3.30-7.49)	5.65 (1.76-18.20)
Perineural invasion	4.30 (2.80-6.60)	2.95 (2.31-3.75)	4.06 (3.10-5.32)
Patient-related			
Immunosuppression	1.51 (0.81-2.81)	1.59 (1.07-2.37)	0.35 (0.05-2.58)

CI, confidence interval.

recurrence and metastasis, while a tumor diameter > 20 mm was associated with the highest risk ratio of disease-specific death (22).

There are several available staging systems for CSCC but each of them presents some important pitfalls and may not be able to fully provide an adequate risk stratification for all cases. The American Joint Committee on Cancer (AJCC) 8th edition classification does not perform well especially regarding T stage, as few tumors fit the criteria for T4, but most T2 tumors actually turn out to be associated with poor outcomes (23). Brigham and Women's Hospital (BWH) and the Breuninger systems are more accurate in stratifying the risk of T stage but are limited to the classification of primary tumors only (24). Finally, neither the AJCC nor the BWH staging systems consider immunosuppression, which is included as a major high-risk factor in the EADO and NCCN guidelines (20, 21, 25). Indeed, immunosuppression associated with conditions such as solid organ transplantation (26), HIV infection, and chronic lymphocytic leukemia (CLL), is not only a risk factor for increased incidence of CSCC, but also a risk factor for a more unfavorable outcome (20). Therefore, further efforts are needed to develop a dedicated classification for CSCC that could be more useful in daily clinical practice for risk stratification and early identification of high-risk CSCC (20).

Advanced CSCC is defined as a tumor for which neither surgery nor radiation therapy with curative intent is indicated (21). This broad definition is driven by the fact that there is no precise consensus on when CSCC can be considered advanced (27). In addition, contraindication to surgery or radiation therapy with curative intent may be due to several reasons which include not only the anatomic extent of the tumor, but also the patient's clinical condition, comorbidities, the risk of mutilation or severe functional loss due to the surgery, previous treatments performed, and patient preference (27).

The advanced form can be divided into locally advanced and metastatic (loco-regional and distant). Advanced forms are considered rare; it is estimated that only about 5% of total CSCC cases may become advanced, with the limitations of missing epidemiologic data (20). Unfortunately, while the definition of metastatic CSCC (mCSCC) implies the dissemination of tumor cells through locoregional lymph nodes or both distant lymph node and other visceral sites, there are no precise parameters for defining the locally advanced forms, and a multidisciplinary discussion is essential for defining the best diagnostic and therapeutic strategies. In general, a locally advanced CSCC (laCSCC) is a tumor which is no longer eligible for either surgery or curative radiation therapy due to multiple recurrences, large extension, bone erosion and/or deep infiltration beyond the subcutaneous tissue into muscles/nerve. Moreover, the definition of laCSCC could fit tumor masses where curative resection may lead to unacceptable complications, morbidity or deformity (27). Finally, multiple CSCCs related to genetic syndromes as xeroderma pigmentosum and those related to chronic conditions such as chronic lymphocytic leukemia (CLL) may be included in these criteria (27). Patient-related features, such as age, comorbidities and patient preferences, may also play a role in the choice of either surgery or immunotherapy.

The *Old* Therapeutic Options

Before immunotherapy, in addition to palliative radiotherapy, chemotherapy and targeted therapy with EGFR (Epidermal Growth Factor Receptor) inhibitors were the only available therapeutic options for advanced CSCC (21). In particular, chemotherapy can be considered in different treatment settings depending on the therapeutic purpose: (1) curative intent concurrent with radiation therapy, based on squamous cell carcinoma of the head and neck (HNSCC) clinical trials data. In fact, Pignon and colleagues published a meta-analysis conducted on 17,346 patients with HNSCC, demonstrating a survival benefit of concurrent chemoradiation (28). Notably, this benefit was not significant in the population over 70 years of age (28); (2) postoperative concomitant chemoradiation. A chemoradiation approach *versus* radiotherapy alone in a postoperative setting has been evaluated in a study including a population with at least one of the following high-risk features: intraparotid nodal disease, cervical nodal disease, primary tumor > 5 cm, primary tumor invading surrounding cartilage, skeletal muscle, or bone, and in-transit metastases. The study showed no differences between the two study arms in terms of either locoregional relapse or OS (29); (3) palliative intent, with questionable benefit in terms of quality of life (QoL) and overall survival (OS). Retrospective data showed that platinum derivatives appear to be the most active drugs in terms of progression-free survival (PFS) of 9.8 months and OS of 15.2 months (30). Other therapeutic options may be fluoropyrimidines (capecitabine), taxanes, bleomycin, adriamycin, and methotrexate, with PFS of approximately 5.5 months and OS of 10.9 months (30).

Regarding EGFR inhibitors, there are limited data in the curative and postoperative setting. Specifically, postoperative cetuximab concurrent with radiotherapy (n=29) *versus* radiation therapy alone (n=39) in patients with high-risk head and neck CSCC (high grade of differentiation, perineural or lymphovascular invasion, positive surgical margins, lymph node involvement, tumor recurrence, immunosuppression, localization to ear, cheek, lip), showed an advantage in terms of both freedom from local recurrence and freedom from distant recurrence (31). In the advanced setting, a phase II study including 36 patients with CSCC showed a response rate of 28% with a median duration of response of 6.8 months (32). Similar results were also observed with dacomitinib, with grade 3-4 adverse events being observed in 36% of patients, and 16% of patients discontinuing treatment because of drug-related toxicity (33). Finally, in a large retrospective case series, both chemotherapy and targeted therapy for the treatment of advanced CSCC showed response rates of less than 20%, with overall survivals of less than 20 months (34).

In summary, these treatment approaches were unsatisfactory, both in their impact on survival and quality of life (21), and a standard regimen for the treatment of advanced CSCC was not clearly defined, with up to 60% of patients with locally advanced CSCC not receiving any systemic therapy at all (35).

The *New* Therapeutic Options

The therapeutic paradigm of CSCC has been radically changed in recent years with the introduction of immunotherapy (21). For this reason, it is crucial to discuss each advanced case in a multidisciplinary setting to properly balance the risks and benefits of this treatment in a population commonly affected by severe comorbidities and to assess the most appropriate therapeutic strategy.

Immunotherapy is considered the breakthrough in the treatment of advanced CSCC. The available clinical evidence is supported by a strong preclinical rationale. UV radiation is the most relevant risk factor for CSCC, which in fact is among the tumors with the highest rate of somatic mutations (36). The high tumor mutational burden (TMB) sets the background for a large number of neoantigens that can be recognized by the immune system. The high number of somatic mutations found in CSCC provided the strong biological rationale for the development of immunological therapies. Indeed, several studies observed that CSCC is the tumor with the highest TMB (7), with a linear relationship between tumor mutational burden and immunotherapy efficacy (37). Moreover, CSCC is a typical tumor of the elderly, with a mean age of onset of 70 years, while it is extremely rare in subjects younger than 45 years of age. Some evidence suggests that the chance of obtaining benefit from immunotherapy may increase with age. In a study involving more than 500 melanoma patients treated with PD-1 inhibitors, the risk of disease progression decreased by 13% for each decade of age (38). Finally, CSCC is characterized by high expression of Programmed Death-Ligand 1 (PD-L1) (39). The interaction of this ligand with Programmed Death-1 (PD-1) results in the inhibition of the anti-tumor immune T cell response (40). This immune checkpoint is exploited by cancer cells to escape the immune response and is one of the mechanisms underlying the rationale for the use of PD-1 inhibitors in the treatment of CSCC.

One of the first clinical evidence supporting the use of the anti-PD-1 immunotherapy for the treatment of advanced CSCC was provided by the CARSKIN trial, where first-line therapy with pembrolizumab in patients with unresectable CSCC demonstrated an objective response rate at week 15 of treatment (ORR_{W15}) of 55% in PD-L1$^+$ patients *versus* 17% in PD-L1$^-$ patients (41). In the subsequent phase II KEYNOTE-629 trial, 105 patients with locally advanced, metastatic, or relapsed CSCC received pembrolizumab as a first-line treatment (13%) or subsequent to another systemic therapy (87%) achieving an ORR of 34% and disease control rate (DCR) of 52%. The safety profile was also acceptable and consistent with that observed in previous trials with pembrolizumab (42). As already mentioned, the results of this phase 2 trial led to the approval by the FDA of pembrolizumab for the treatment of advanced CSCC.

Before that, cemiplimab was approved by the FDA in 2018, and then by EMA, for the treatment of both mCSCC (nodal or distant metastases) and laCSCC (locally advanced) which are not eligible for curative surgery or radiation therapy, following the results of a phase 1 study that showed durable responses in 50% of 26 treated patients (18). These results were confirmed in the phase 2, open-label, non-randomized EMPOWER-CSCC 1 trial, where 193 patients with advanced, non-eligible for curative surgery or radiotherapy CSCC were enrolled. In the locally advanced CSCC group, patients were considered non-eligible for surgery if the anatomical location of the tumor would cause serious functional and aesthetic consequences (38% of cases). Other causes of inoperability were previous recurrences of the same lesion (32%) and the impossibility to obtain a complete surgical resection due to severe local invasiveness (26%). The most frequent cause of contraindication to radiotherapy was an unfavorable risk/benefit ratio (49% of cases) (18). At the last update presented at ASCO Annual Meeting 2020, the pooled ORR was 46.1% with a DCR of 72.5% (43). Clinical activity was observed regardless of PD-L1 expression (43). In addition, approximately half of patients achieved an anti-tumor response within the first 2 months, and nearly 80% within the first 4 months (43). The study showed that patients with laCSCC receiving cemiplimab after more than one recurrence after surgical excision had less than half the probability of achieving a response if compared to patients receiving upfront immunotherapy (43). This makes it essential, in the case of lesions that are potentially resectable but for which a curative outcome cannot be reasonably expected with surgery (i.e., in the presence of major risk factors), a careful multidisciplinary evaluation considering cemiplimab as a first-line treatment.

Cemiplimab has shown benefits not only in terms of clinical activity and efficacy, but also in terms of safety and quality of life. In fact, the toxicity profile of cemiplimab is comparable to that observed with other PD-1 inhibitors. Only 5% of patients had to discontinue therapy due to an adverse event of grade 3 or higher (18). According to health-related quality of life data, cemiplimab led to a clinically relevant improvement in terms of both QLQ-C30 pain scale and QLQ-C30 global health status (44).

Regarding special populations such as organ transplant patients, limited data are available. A recent systematic review showed that among 57 transplanted patients who received an immune checkpoint inhibitor for advanced malignancies, 37% experienced organ rejection, and 14% died due to rejection (45). Most of the observed rejections were among kidney (40%), liver (35%), and heart (20%) transplant patients (45). The overall response rate was 30-40% for PD-1 inhibitors (45). In case of advanced CSCC, a careful multidisciplinary approach is required to assess the risk of organ rejection and the benefit of PD-1 inhibitor treatment. In addition, patients should be fully informed of the possible risks and benefits before starting treatment with immune checkpoint inhibitors. In addition, retrospective data of 12 patients with HIV infection and advanced malignancies treated with PD-1/PD-L1 inhibitor therapy showed objective responses without unexpected adverse events nor significant impact on HIV viremia (38). In another study, pembrolizumab showed to be safe in HIV-infected patients, in particular in maintaining CD4+ T-cell count and viral suppression (46).

TABLE 2 | The principal ongoing clinical studies recruiting patients with advanced or high risk CSCC.

Drug(s)	Name of clinical trial	Phase	NCT number	Status	Estimated completion date
Pembrolizumab	Neoadjuvant Study of PD-1 Inhibitor Pembrolizumab in PD-1 Naive Cutaneous Squamous Cell Carcinoma (CSCC)	2	NCT04808999	Not yet recruiting	October 2028
Atezolizumab	Neoadjuvant Atezolizumab in Surgically Resectable Advanced Cutaneous Squamous Cell Carcinoma	2	NCT04710498	Not yet recruiting	September 2024
Cemiplimab	Neoadjuvant Plus Adjuvant Treatment With Cemiplimab in Cutaneaous Squamous Cell Carcinoma	2	NCT04632433	Recruiting	February 2026
Nivolumab or Nivolumab plus Ipilimumab	Neoadjuvant Nivolumab or Nivolumab With Ipilimumab in Advanced Cutaneous Squamous Cell Carcinoma Prior to Surgery	2	NCT04620200	Recruiting	November 2024
Cemiplimab	Cemiplimab Before and After Surgery for the Treatment of High Risk Cutaneous Squamous Cell Cancer	1	NCT04428671	Recruiting	October 2030
Cemiplimab (47)	Cemiplimab in Treating Participants With Recurrent Stage III-IV Head and Neck Squamous Cell Cancer Before Surgery	2	NCT03565783	Recruiting	July 2021
Cemiplimab	Cemiplimab in AlloSCT/SOT Recipients With CSCC	1	NCT04339062	Recruiting	July 2022
Cemiplimab	A PD-1 Checkpoint Inhibitor (Cemiplimab) for High-Risk Localized, Locally Recurrent, or Regionally Advanced Skin Cancer	2	NCT04315701	Recruiting	January 2023
Nivolumab	Nivolumab for Treatment of Squamous Cell Carcinoma of the Skin	2	NCT04204837	Active, not recruiting	December 2023
Talimogene Laherparepvec and Panitumumab	Talimogene Laherparepvec and Panitumumab for the Treatment of Locally Advanced or Metastatic Squamous Cell Carcinoma of the Skin	1	NCT04163952	Recruiting	September 2024
IFx-Hu2.0 Vaccine	Immunotherapy With IFx-Hu2.0 Vaccine for Advanced MCC or CSCC	1	NCT04160065	Recruiting	June 2022
Cemiplimab	Study of Cemiplimab in Patients With Type of Skin Cancer Stage II to IV Cutaneous Squamous Cell Carcinoma	2	NCT04154943	Recruiting	December 2024
Cemiplimab with and without RP1	Study Evaluating Cemiplimab Alone and Combined With RP1 in Treating Advanced Squamous Skin Cancer	2	NCT04050436	Recruiting	March 2025
Cemiplimab	Study of Adjuvant Cemiplimab Versus Placebo After Surgery and Radiation Therapy in Patients With High Risk Cutaneous Squamous Cell Carcinoma	3	NCT03969004	Recruiting	February 2027
Avelumab with or without Cetuximab	Avelumab With or Without Cetuximab in Treating Patients With Advanced Skin Squamous Cell Cancer	2	NCT03944941	Recruiting	December 2023
Intralesional Cemiplimab	Pre-Operative Cemiplimab Administered Intralesionally for Patients With Recurrent Cutaneous Squamous Cell Carcinoma	1	NCT03889912	Active, not recruiting	February 2022
Nivolumab	Nivolumab in Patients With Advanced Cutaneous Squamous Cell Carcinoma	2	NCT03834233	Active, not recruiting	December 2022
Pembrolizumab	Pembrolizumab Versus Placebo Following Surgery and Radiation in Participants With Locally Advanced Cutaneous Squamous Cell Carcinoma (MK-3475-630/ KEYNOTE-630)	3	NCT03833167	Recruiting	September 2028
Avelumab plus Radiotherapy	The UNSCARRed Study: UNresctable Squamous Cell Carcinoma Treated With Avelumab and Radical Radiotherapy	2	NCT03737721	Recruiting	June 2023
Intratumoral Cavrotolimod With Pembrolizumab or Cemiplimab	Intratumoral Cavrotolimod Combined With Pembrolizumab or Cemiplimab in Patients With Merkel Cell Carcinoma, Cutaneous Squamous Cell Carcinoma, or Other Advanced Solid Tumors	1/2	NCT03684785	Recruiting	June 2023
Lenvatinib plus Cetuximab	Testing Lenvatinib and Cetuximab in Patients With Advanced Head and Neck Squamous Cell Carcinoma and Cutaneous Squamous Cell Carcinoma	1	NCT03524326	Recruiting	April 2023
Pembrolizumab with or without Cetuximab	Immunotherapy +/- EGFR Inhibitor In Advanced/Metastatic cSCC: Tackling Primary And Secondary Resistance (I-Tackle)	2	NCT03666325	Not yet recruiting	October 2022

AlloSCT/SOT, allogenic stem cell transplantation/solid organ transplantation; CSCC, cutaneous squamous cell carcinoma; MCC, merkel cell carcinoma; PD-1, Programmed Death-1.

FUTURE PERSPECTIVES

The ongoing clinical studies recruiting patients with advanced or high-risk CSCC are summarized in **Table 2**. In most trials a treatment regimen including a PD-1 inhibitor is being investigated, and especially in earlier settings, such as high-risk CSCC. Most significantly, in the R2810-ONC-1788 study (NCT03969004), patients with high-risk CSCC are randomized to receive cemiplimab for 1 year *versus* placebo after surgery and adjuvant radiation therapy. The primary endpoint is disease-free survival (DFS). Cemiplimab is also being investigated in the neoadjuvant setting. Specifically, Gross and colleagues presented at the European Society of Medical Oncology (ESMO) meeting 2019 data from a phase 2 study (NCT03565783) where 20 patients with stage III/IV (M0) (AJCC 8th edition) CSCC of the head and neck received 2 doses of preoperative cemiplimab achieving a 55% of pathological complete response (pCR) and a major pathology response (MPR) in 15% (47). There were no grade ≥ 3 adverse events (47).

Immunotherapy has led to pivotal changes in advanced CSCC both in terms of objective responses, survival, and improved

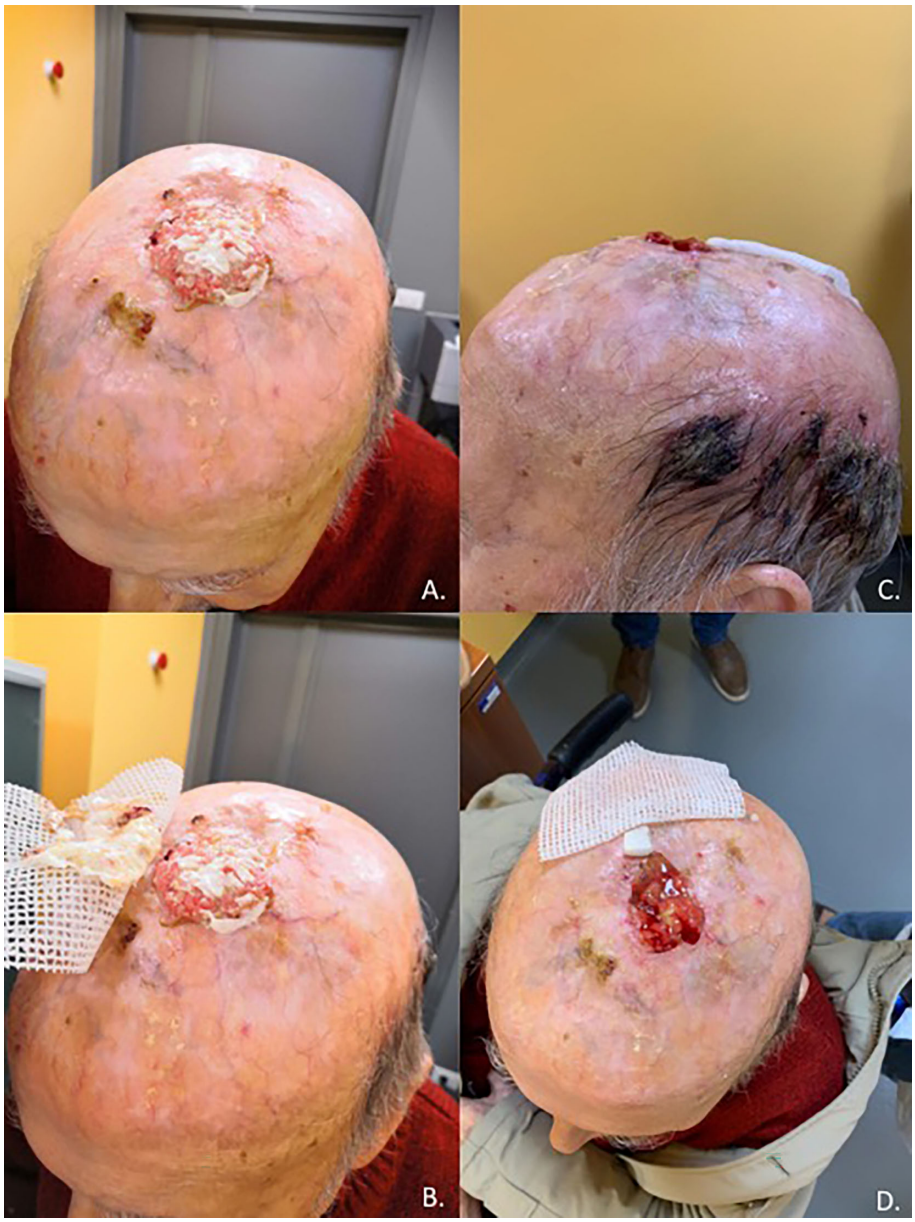

FIGURE 1 | Case report of a 92-year-old man with unresectable, non-eligible to curative radiotherapy, locally advanced CSCC invading the skullcap and leptomeningeal membrane **(A, B)** who achieved a rapid clinical response after one cycle of Cemiplimab **(C, D)**.

quality of life. However, patients with advanced CSCC receiving immunotherapy after more than one recurrence after surgical excision had less than half the probability of achieving an objective response (43). This could be related to primary or secondary resistance to immunotherapy (48). For this reason, clinical trials are ongoing with the aim of overcoming resistance to immunotherapy. In fact, the combination of PD-1 or PD-L1 inhibitors with other agents (such as radiotherapy, oncolytic viruses, or EGFR inhibitors) is being investigated to overcome primary or secondary resistance to immunotherapy, such as in the I-Tackle trial (NCT03666325) with the addition of cetuximab to pembrolizumab at primary or acquired resistance; or in the UNSCARRed study (NCT03737721) with the addition of radiotherapy to avelumab.

DISCUSSION AND CONCLUSIONS

Cutaneous squamous cell carcinoma is a common condition, although it remains rare in its advanced stages; high-risk cases require multidisciplinary care due to the complexity associated with both the disease and the often frail population (27). Before

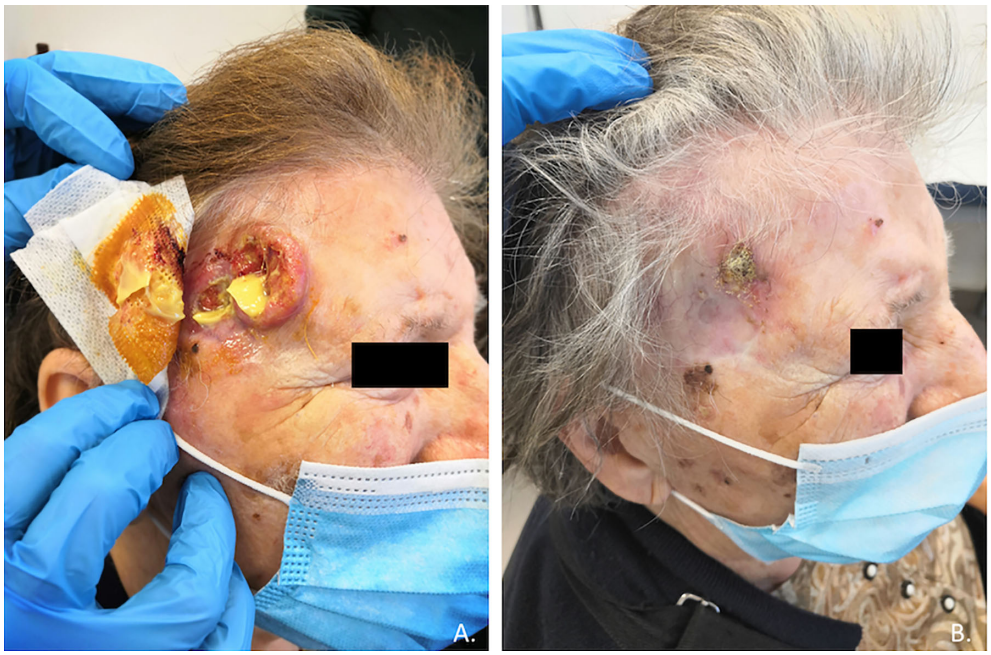

FIGURE 2 | Case report of a rapid clinical response, after only one course of therapy with cemiplimab, in an 83-year-old patient with locally advanced recurrence of cutaneous squamous carcinoma of the right temporal region **(A,B)**.

the introduction of immunotherapy in clinical practice, a standard of care for advanced CSCC was not clearly defined, and up to 60% of patients with advanced CSCC did not receive any systemic therapy at all, due to the low clinical activity and high risk of severe toxicities (21). Based on a strong preclinical rationale, clinical trials were conducted leading to the registration by the regulatory authorities of anti-PD-1 immunotherapy in patients with advanced CSCC (21). Cemiplimab was the first PD-1 inhibitor receiving an indication in CSCC after showing in a clinical trial rapid and durable responses in more than 40% of patients (in **Figures 1**, **2** we reported two clinical cases of rapid clinical response), with a favorable safety profile. In addition to that, cemiplimab led to an improvement in health-related quality of life with a reduction in cancer-related pain after a few cycles of therapy (18, 43, 44).

Anti-PD-1 drugs are the backbone of current clinical investigation in patients with CSCC. Specifically, several clinical trials with PD-1 inhibitors are currently underway investigating the activity, efficacy, and safety of adjuvant approaches in individuals with high-risk CSCC, and neoadjuvant approaches in patients with advanced CSCC. Based on the results of these studies, anti-PD-1 drugs may soon become standard of care in the adjuvant and neoadjuvant settings.

AUTHOR CONTRIBUTIONS

FS and PQ jointly supervised this work. All authors contributed to the article and approved the submitted version.

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REVIEW
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Merkel Cell Carcinoma: An Immunotherapy Fairy-Tale?

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Merkel cell carcinoma (MCC) is a rare, highly aggressive, neuroendocrine cutaneous tumor. The incidence of MCC is growing worldwide, and the disease-related mortality is about three-fold higher than melanoma. Since a few years ago, very little has been known about this disease, and chemotherapy has been the standard of care. Nowadays, new discoveries about the pathophysiology of this neoplasm and the introduction of immunotherapy allowed to completely rewrite the history of these patients. In this review, we provide a summary of the most important changes in the management of Merkel cell carcinoma, with a focus on immunotherapy and a landscape of future treatment strategies.

Keywords: merkel cell carcinoma, immunotherapy, merkel cell polyomavirus, advanced disease, anti-PD-1, neoadjuvant

INTRODUCTION

The history of Merkel cell carcinoma (MCC) therapy is studied with frustration and poor outcomes to treatments until the introduction of immunotherapy, which has radically changed the therapeutic paradigm of this disease.

The incidence of MCC is slowly but steadily growing worldwide. However, MCC is often misdiagnosed and part of this increase in incidence is probably due to the improvement of diagnostic skills, techniques, and the discovery of new biomarkers (1).

Overall, the highest incidence rate has been recorded in Australia, with 1.6 cases/100,000 (2).

In the US, a recently published epidemiological analysis based on the SEER-18 registry (1) counted 6,600 cases of MCCs diagnosed from 2000 to 2013, with an incidence rate rising from 0.5/100,000 in 2000 to 0.7/100,000 in 2013 and an incidence increase of 95.2% (from 334 cases in to 652), exceeding the 56.5% observed in melanoma. Combining these data with US census population data, the global number of new cases of MCC for 2013 is estimated to be 2,488, while the forecasts for 2020 and 2025 are 2,835 and 3,284–3,500 respectively.

In Europe, univocal data are lacking and the incidence of MCC is derived from smaller epidemiological studies. A population-based study published in 2019, including a population based in Northeast France (3), confirmed the increase in new diagnosis, from a rate of 0.05/100.000

in 1985–1989 to 0.22 in 2010–2013. Similarly, a Dutch study (4) showed a rise in the incidence rate for the period 1993–2016, increasing from 0.17 to 0.59. In these studies, the 5-year survival crude rate of MCC ranged between 38% (3) and 41% (2).

The clinical presentation is typically with a non-painful, solid, rapidly growing, and firm nodule, of red color or violaceous. Its surface can be ulcerated or not, covered by crusts, or surrounded by telangiectasias. The diameter at the time of diagnosis usually ranges from 1 to 2 cm (5) but can easily exceed 2 cm due to its rapid evolution. MCC arises frequently on UV-exposed areas (head and neck, limbs, arms), but it is important not to exclude its possible insurgence on non-exposed areas (6). MCC mostly affects Caucasian, older (median age of insurgence is 76 years), immunosuppressed, and hematological populations. All these characteristics and risk factors have been resumed in the acronymous "A.E.I.O.U." (Asymptomatic, Expanding rapidly, Immune-suppression, Older than 50 years, UV exposed sites), presented for the first time by Heath et al. in 2008 (5).

MCCs grow quickly and metastasize early, with 26%–36% of lesions having lymph node metastasis at the time of diagnosis and 6%–16% having synchronous distant metastasis (6–8). Overall, a large meta-analysis shows that almost 50% and 33% of patients ultimately develop local recurrence or distant metastases, respectively (9). Survival rates of MCC depend on the stage at presentation and range from 50.5% to 13.5% at 5 years of observation (6).

Origin of MCC

The histogenesis of MCC is still largely debated (10). Firstly described as a "trabecular carcinoma of the skin" by Toker et al. in 1972 (11), MCC took its name from some structural and immunohistochemical (IHC) features that share with Merkel cells (MCs), in particular the expression of ion channel Piezo 2 (12), cytokeratin 20 (CK20), chromogranin A, synaptophysin, and neuropeptides (13–17). However, the cytological and molecular similarity of a tumor cell with a normal cell cannot be considered, to date, a criterion for affirming its certain derivation; indeed, it has been demonstrated that cells undergo several phenotypic changes during oncogenesis, which can strongly modify their final differentiation profile (18). Accordingly, the acquisition of an MC-like phenotype, including neuroendocrine differentiation, during MCC oncogenesis could explain the similarities between MCs and MCCs (19). An example of this process could be the expression of atonal homolog 1 (ATOH1), a transcription factor shared by specific epithelial precursor of MCs (14) and MCC. Since ATOH1 is observed in MCC, its expression could explain the shared phenotype between MCs and MCCs (20). Interestingly, the expression of ATOH1 could be induced by the genetic ablation of Rb1 and the related Rb family protein p130 (21). Nowadays, the initial hypothesis of the MCC origin from MCs has been almost completely abandoned and several factors argue against the direct derivation from MCs. First, in other organs such as lung, strong data suggest that neuroendocrine carcinoma derives more from epithelial progenitors rather than a neuroendocrine cell (22, 23). Second, MCs are mainly post-mitotic cells and thus have low sensitivity to oncogenic stimuli as the expression of small T antigen (sT) that failed to induce cell proliferation or transformation in a transgenic mouse model (24). Third, MCs are most frequently present in the palm and sole in humans, whereas MCC occurs mainly in sun-exposed areas (head and neck, legs). Moreover, no infection of MCs by Merkel cell polyomavirus (MCPyV) has been reported (25). Finally, in an *in vitro* model, MCPyV pseudovirions could barely infect CK20-positive cells obtained from the fetal scalp (0.8%) (26), which argues against an efficient MCPyV infection triggering MCC oncogenesis in an already differentiated MC. Considering these findings, a non-MC could also be candidate for the ancestry of MCC, and an epithelial non-MC (27) as well as a fibroblastic (26) and B-cell (28) origin has been proposed.

Pathogenesis

Although many doubts have arisen regarding the cell of origin of MCC, in recent years several discoveries are helping to better define the pathogenesis of MCC, synthesized in **Figure 1**. Currently, the most credited hypothesis is that MCC may be the clinical outcome of two distinct pathogenetic and molecular diseases. In 2008, MCPyV, a member of the polyomavirus family, was discovered to be associated with MCC (30). MCPyV is a small, non-enveloped, double-stranded DNA virus, highly prevalent in the human population (more than 80% among subjects over 50 years old). The virus-related pathogenesis of MCC, illustrated in **Figure 1B**, requires two separate events. First, the circular double-stranded viral genome must be integrated into the host genome, perhaps after a DNA-damaging event. Second, the virus genome must be mutated, with loss of expression of the large T (LT) antigen and the expression of two neoantigens: small T (sT) and truncated large T (tLT). TLT antigen binds to and inactivates Rb, promoting cell-cycle progression and uncontrolled proliferation. ST antigen inhibits the proteasomal degradation. Both tLT and sT demonstrated to drive transformation in mammalian cells *in vitro*; however, numerous attempts to generate mouse models of MCC only partially emulated the disease. These data indicate that additional, as yet undetermined factors are required for induction of MCPyV-associated MCC (31–34). After the integration, host cells start to transcribe and express the MCPyV-related oncoproteins. This is an important phenomenon because the continuous expression of MCPyV oncoproteins is a required factor for survival of virus-positive MCC cells (35), but, at the same time, these persistently expressed non-self antigens elicit host immune recognition with the activation of T-cells and the production of humoral antibodies (36, 37). Interestingly, MCC-specific antibody titers correlate with tumor burden and, consequently, with the response to treatment (38, 39). Eighty percent of MCC in the northern hemisphere is due to the MCPyV viral infection. The remaining 20% seems to be the result of progressive DNA damage induced by UV (**Figure 1A**). Indeed, virus-negative MCC is the solid neoplasm with one of the highest tumor mutational burdens (including melanoma and NSCLC) (40). In most cases, these mutations can be inscribed in the so-called UV-signature mutations (29). The most common are in p53 (75%) and Rb (67%) and commonly result in loss of functional protein

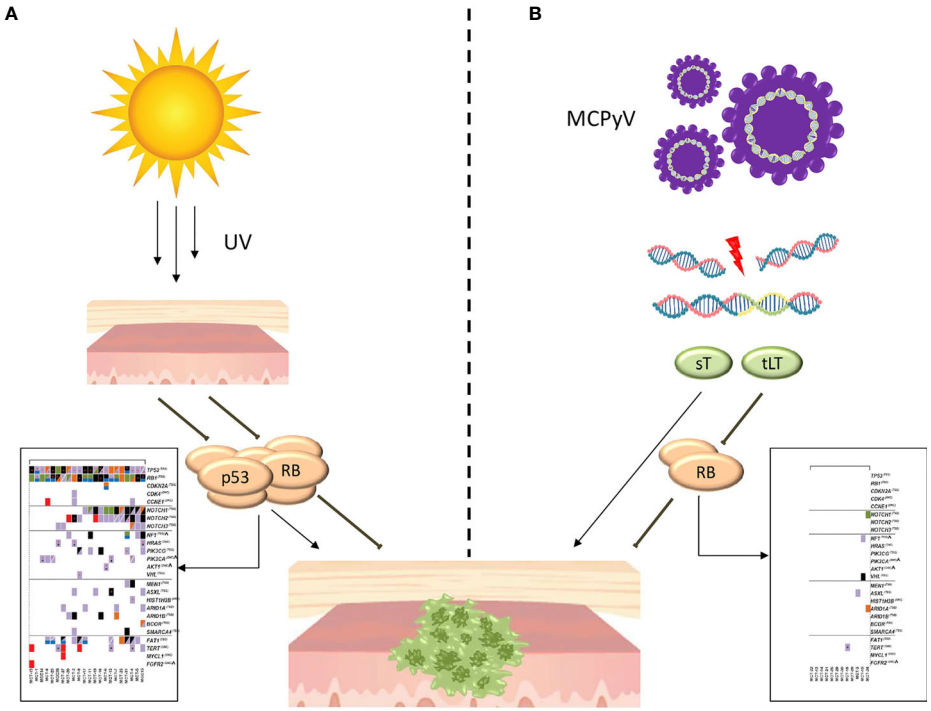

FIGURE 1 | Pathogenesis of MCC. **(A)** Pathogenesis of UV-induced MCC. The progressive DNA damage induced by UV leads to the accumulation of a large number of mutations, largely included in the so-called UV signature, with the most common in p53 and Rb. In the box (29) are reported cancer genes affected by mutation or copy number alterations in UV-induced MCC. **(B)** Pathogenesis of virus-induced MCC. The mutated viral genome is integrated into the host genome, with the expression of two neoantigens: small T (sT) and truncated large T (tLT). The TLT antigen binds to and inactivates Rb while sT antigen inhibits the proteasomal degradation. In the box (29) are reported cancer genes affected by mutation or copy number alterations in virus-induced MCC.

expression (41). In conclusion, two distinct pathogenic profiles of MCC have been described. Virus-positive tumor presents a low mutational burden, an antibody titer that correlates with tumor burden, a high PD-L1 expression, and a high TIL level. On the other hand, virus-negative MCC presents a high mutational burden with a median of 1121 mutation/esome, a variable PD-L1 expression and a variable TIL level. All these characteristics form the molecular and biological background that leads to the known sensitivity of this tumor to immunotherapy.

TREATMENT OF PRIMARY TUMOR

MCC being a rare disease, there is a lack of prospective clinical studies, and therefore the studies mostly derive from retrospective analyses.

Surgery is generally considered the first approach, especially in patients with local or regional disease (42–44). Resection margins for primary MCC are not well defined. Guidelines recommend 1- to 2-cm margins with the aim of removing microscopic satellite metastases (43).

Nonetheless, in a retrospective study published in 2018, it was found that a 1-cm margin did not increase the risk of local recurrence in respect to the 1–2-cm margin, and a more radical surgery did not have a significant impact in terms of disease-specific survival or overall survival, but increased the need for a graft or flap closure (45). However, the absence of a statistically significant difference could be explained with the practice to perform wider excision among the most aggressive-appearing lesions.

In another recent retrospective French trial (46), 214 patients were radically resected on the primary site. Among them, 58 (27.1%) had 0.5–1-cm margins and 156 (72.9%) had wider margins (> 1 cm). With a median follow-up of 50.7 months, 5-y OS was 76.8% and 76.2% respectively. Also in this case, there are several limits: the retrospective nature of the trial, the heterogeneous characteristics of the two groups of patients, and the use of radiotherapy as adjuvant treatment after surgery.

On the other hand, in a retrospective trial performed on 79 patients affected by stage I–II MCC, 1-y disease-free survival (DFS) was 51.3%, 71.4%, and 87.8%, while 3-y OS was 57.7%, 82.6%, and 100% among patients with margin < 1 cm, between 1 and 1.9 cm, and ≥2 cm, respectively (47).

Finally, in a recently published retrospective trial (48), 188 stage I–II MCCs were analyzed. A total of 48 patients were treated with surgery alone and, among them, 35 had narrow margins (≤1 cm) while 13 had margin > 1 cm. In the first group of patients, 7 (20%) developed local recurrence, while in the second group, 0 patients developed local recurrence. A group of patients underwent surgery plus RT: this group tended to present

higher-aggressiveness tumors or a higher-risk profile (e.g., immunosuppressed) but had less local recurrence than those who were treated with surgery alone (1% vs. 15%), regardless of surgical margins.

As a reasonable conclusion, we can assert that a radical surgery should be performed when possible and that narrow margins could be appropriate if combined with tumor-bed RT.

As we previously mentioned, because MCC is a very radiosensitive cancer, there is the opportunity of a subsequent step with adjuvant radiotherapy on the tumor bed. Indeed, RT demonstrated to improve not only locoregional tumor control but also overall survival in stages I and II, compared with surgery alone (49, 50). In a large, multicenter, retrospective cohort study, 6,156 stage I–II MCC patients who underwent local excision were analyzed (51). In this study, margins > 1 cm were associated with a statistically significant improvement of OS (HR 0.88), with a 5-y OS of 89.8% vs. 76.7% among patients who had local excision with closer margin (≤ 1 cm). In addition to that, radiotherapy induced a statistically significant increase in OS, regardless of surgical margins: patients with close margins who performed RT (HR, 0.81; CI, 0.74–0.89) obtained an OS rate comparable to patients who performed a wider local excision and no RT (HR, 0.80; CI, 0.71–0.89). A systematic review and meta-analysis specifically evaluated the impact of RT in terms of OS and DFS (50). A total of 17,179 cases were analyzed, finding a significant difference in OS (HR 0.8) and in DFS (HR 0.45) between RT and no-RT groups. At the same time, it was found out that local RT does not improve distant metastasis-free survival (DMFS).

RT should be performed as soon as possible after surgery (44), because delay seems to be associated with worse outcome (52). However, results of clinical trials are discordant about the correct timing of RT and in a large retrospective trial that counted 5,952 patients from the National Cancer Database (53); no difference in OS was seen between patients who underwent to RT within 4 weeks and up to 18 weeks.

Sometimes, radical excision may not be feasible, especially in the head/neck region and in elderly patients with poor performance status. In these cases, exclusive radiotherapy should be considered (54–56). In a retrospective trial published in 2021 (55), a total of 84 patients who were treated with either surgery with wide margins (2 cm) plus adjuvant RT (31, 36.9%) or RT alone (53, 63.1%) were analyzed. In these two groups, the local relapse rate was 13.7% in the RT group and 25.8% in the surgery plus RT group, without a statistically significant difference in terms of local or distant relapse and in OS.

SLNB and Treatment of Regional Lymph Node

In patients without clinically evident nodal disease, NCCN guidelines recommend to perform sentinel lymph node biopsy (SLNB) whenever feasible, no matter the size of the primary tumor (43, 44). The rate of positivity ranges between 11% and 57% and the size of tumor do not seem to correlate with SLN positivity (57–59). The pathological status of lymph nodes is very important to define the prognosis of a patient. A retrospective trial performed on 9,387 patients aimed to validate and refine the AJCC system (8^) showed a 5-y OS of 35.4% among 2,465 patients with nodal metastases (6). Moreover, a difference in terms of OS between patients with clinically negative and clinically positive lymph node metastases was found. Among patients without clinically evident but pathologically proven node metastases, 5-y OS was 39.4%, while for clinically detected lymph node metastases 5-y OS was 26.8%. Moreover, the difference in survival between patients with clinically negative and pathologically negative was 17.8% for T1 tumors (45% *vs.* 62.8%) and similar results were observed among T2, T3, and T4 tumors.

If the presence of micro-metastasis is confirmed, a nodal dissection and/or radiotherapy to the nodal basin is recommended (44). Adjuvant radiotherapy alone or adjuvant radiotherapy combined with a complete lymph node dissection was associated with improved OS in a large retrospective study that included 447 patients (60). The best therapeutic algorithm is still to be defined. Several retrospective studies tried to identify the best strategy. Perez *et al.* (61) in a retrospective single-institution study performed on 71 MCC patients, and Lee *et al.* (62) in a prospective study performed on 163 patients, and found no statistical difference between adjuvant RT, lymph node dissection alone, and radiotherapy with lymph node dissection, concluding that RT or complete lymph node dissection (CLND) could be equivalent. However, in 2020 Cramer et al. (60) published a very significant trial with 447 patients affected by T1–T4, cN0 pN1a, and M0 MCCs who underwent observation, CLND, RT, or CLND + RT. After 3 years of observation, OS was 50%, 52.9%, 67.9%, and 79.5%, respectively. In this trial, adjuvant RT significantly improved OS while CLND did not. Finally, another retrospective trial (63) performed on 72 patients and published in 2021 showed that RT improved OS. As in previously mentioned work, patients underwent observation, RT alone, CLND alone, or RT + CLND. In the same way, RT improved outcomes, especially when combined with CLND. As a conclusion, we can assert that in patients fit for surgery, CLND plus RT should be the treatment of choice, while in patients unfit for combination treatment, the choice should be RT alone. This allows, in selected cases, to obviate the lymph node dissection, and thus its complications, such as lymphedema, neurovascular injury, and surgical-site infections (64). Adjuvant irradiation of the lymphatic drainage area demonstrated to improve locoregional control and the 3-year disease-specific survival rate from 48% to 76% (49).

On the other hand, in case of negative SLNB, the therapeutic algorithm is still debated. In several trials, radiation treatment of the nodal basin was not recommended (65, 66), but guidelines suggest to consider it for high-risk patients.

If SLNB is not performed, elective surgery of at least the first draining lymph node level or radiotherapy is suggested (49).

To sum up and take into consideration the absence of a coded algorithm, the therapeutic approach of each case of MCC should be discussed by a multidisciplinary group consisting of at least an oncologist, a dermatologist, a surgeon, and a radiotherapist (67, 68).

SYSTEMIC THERAPY FOR ADVANCED PATIENTS

Traditionally, MCC is considered a chemosensitive tumor (69–73). However, chemotherapy (CT) has shown to induce a non-durable response, without a clear benefit in OS and with heavy toxicities (**Table 1**). Due to the rarity of the disease, no specific chemotherapeutic schemes have ever been developed, adopting all therapeutic strategies from small cell lung cancer (SCLC), a tumor that shares several characteristics with MCC.

Overall, data from a systematic review of literature that analyzed the benefit of CT in advanced MCC showed an ORR ranging from 20% to 61%, higher in the first line than in the second line, and a duration of response (DOR) shorter than 8 months (72). Voog et al. (69) published an analysis of the literature that analyzed data of 107 patients (29 locally advanced and 72 metastatic MCC) treated with several schemes of CT. Here, ORR was 69% among locally advanced and 57% among metastatic MCCs, with a high rate of toxic death in the first line (7.7%). Median OS was 24 months among locally advanced and 9 months among metastatic MCC, with an estimated 5-y OS of 35% and 17%, respectively. ORR in patients receiving second-line chemotherapy was 45%. In another retrospective study (71), 62 metastatic MCC patients were analyzed. All patients were treated with chemotherapeutic schemes, with platinum plus etoposide being the most common choice in the I line. In this analysis, ORR was 55%, with 13% of CR and 42% of PR, and disease control rate (DCR) was 61%. Median progression-free survival (PFS) was 94 days (3 months), and median OS 9.5 months. ORR in the second-line setting was 23% with a median PFS of 61 days (2 months). Finally, in a real-world study published in 2017 (73), data from 67 patients treated with CT in the first line and 20 patients treated in the II line were collected. In the I line group, ORR was 31.3% with a median PFS of 4.6 months and a median OS of 10.5 months. In the second-line group, ORR was 20% (CR = 0%) with a median PFS of 2.1 months and a median OS of 4.4 months.

In conclusion, we can affirm that CT could induce rapid and intense response in MCC patients, but response is not durable, in line with the ability of MCC to quickly develop resistance to CT. Moreover, CT has shown a high rate of toxic death, probably due to the population affected by MCC, often of old age and with severe comorbidities.

The therapeutic scenario in MCC radically changed with the introduction of immunotherapy.

MCC has long been considered a tumor linked, in some way, to the state of activation of the immune system (74). In particular, in support of this hypothesis there was the different incidence of MCC between the immunocompromised and immunocompetent population (3) and case reports of spontaneous regression of MCCs (75), likely due to a T-cell-mediated immune response. Moreover, increasing knowledge of pathogenesis of MCC has highlighted that both virus-induced MCC and UV-induced MCC had the biological rationale to respond to immunotherapy: in the first case, due to the infectious process (**Figure 1**), the production of oncoproteins, and the development of an active immune response; in the second case, due to the presence of a very high mutational burden. On this wave, and with high expectations, trials with immunotherapy in patients affected by MCC have begun to be conducted with the approval of three different agents, two PD-1 inhibitors and one PD-L1 inhibitor. Both these agents act to inhibit the link of the programmed death-ligand 1 (PD-L1) with its receptor, programmed cell death protein 1 (PD-1), normally involved in the suppression of the immune system.

Food and Drug Administration (FDA) approval of pembrolizumab, nivolumab, and avelumab took place on the basis of three phase II trials. Overall survival curves from studies with chemotherapy and immunotherapy (avelumab second line and pembrolizumab first line) are reported in **Figure 2**. Of note, the populations included in these three trials were substantially different in terms of stage and previous treatments, so the purpose of this extrapolation was to allow a historical and indirect comparison, whereas a direct comparison has never been made in clinical trials.

TABLE 1 | Clinical outcomes in selected chemotherapy studies for patients with Merkel cell carcinoma.

	Voog et al. (69)	Tai et al. (70)	Cowey et al. (73)	Iyer et al. (71)
Setting	Locally advanced (LA)/metastatic (MTS)	Locally advanced (LA)/metastatic (MTS)	Metastatic (MTS)	Metastatic (MTS)
Patients (N.)	69 LA 72 MTS	204	67 I line 20 II line	62 62 I line 30 II line
ORR I L	61% 69% LA 57% MTS	59%	31.3%	55%
mPFS I L	–	–	4.6 months	3.1 months
mOS I L	24 months LA 9 months MTS	21.5 months	10.2 months	9,5 months
5-y OS	35% LA 17% MTS	17%	24.5% (2-y OS)	–
ORR II L	45%	–	20%	23%
mPFS II L	–	–	2.1 months	2 months
mOS II L	–	–	4.4 months	5.7 months
Toxic death	7.7% (I line)	3.4%	–	0%

ORR, overall response rate; mPFS, median progression-free survival: 5-y, 5 years; mOS, median overall survival; I L, first line; II L, second line.

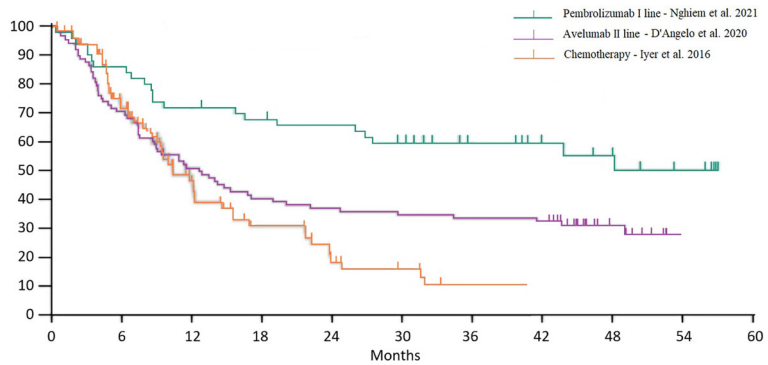

FIGURE 2 | Historical comparison between chemotherapy and immunotherapy overall survival curves.

The Immunotherapy Trials Network (CITN)-09/KEYNOTE-017 study has been a phase II, open-label, non-randomized, multicenter trial involving 50 patients affected by metastatic (m) (86%) or locally advanced (la) (14%) MCC not amenable to definitive surgery or radiotherapy (76). Eligible patients were treated with the anti-PD-1 pembrolizumab at a dosage of 2 mg/kg given intravenously every 3 weeks for up to 2 years or until the development of progressive disease (PD), unacceptable toxicity, or withdrawal of the consent. Patients who showed a progression of the disease were allowed to continue therapy beyond progression if they had a clinical benefit from the treatment. Twelve (24%) patients completed 2 years of treatment. The first analysis performed on 26 patients with a median follow-up of 33 weeks was published in 2016 (76). In this analysis, the ORR was 56%, with 4 CR and 10 PR. Neither PD-L1 expression (on tumor cells or on infiltrating immune cells) nor intratumoral CD8 T-cell infiltration nor viral status of MCC correlated significantly with clinical response to pembrolizumab. The subsequent update (77) considered a total of 50 patients with a median follow-up of 14.9 months. In this report, the ORR was 56%, with 12 CR and 16 PR. Median PFS was 16.8 months, and the estimated 24-mo PFS rate was 48.3%. Median OS had not been reached, while the estimated 24-mo OS was 68.7%. Again, PDL1 expression did not correlate with response and just a trend toward improved OS and PFS in patients with PD-L1 positivity greater than a 1% threshold on tumor cells was observed, but this did not reach statistical significance. On the wave of these results, on December 2018 the FDA granted accelerated approval of pembrolizumab for patients with locally advanced or metastatic MCC. The last update of this trial has been recently published and represents the longest observation of a cohort of patients treated with first-line anti-PD-1, with a median follow-up of 31.8 months (78). The ORR was 58%, with 15 patients achieving CR and 14 patients PR; median DOR was not reached. The majority of responses (90%) developed during the first 12 weeks from the start of treatment and after 3 years of observation 72.7% of responders maintained the response. Median PFS was 16.8 months, and estimated 3-year PFS was 39.1%; median OS was not reached at the time of the analysis, while estimated 3-year OS was 59.4%. When considering only the cohort of responders, 3-year estimated OS reached 89.5%, suggesting that ORR could be considered as an early predictor of OS. In this last update of this trial, factors associated with OS and ORR were analyzed. In detail, the degree of tumor burden reduction, the ability of completing the 2 years of treatment, and an ECOG PS of zero (0) correlated with OS. On the contrary, baseline tumor burden, age, gender, anatomic sites of metastases, tumor viral status, and PD-L status were not associated with ORR or OS. Interestingly, a lower neutrophil–lymphocyte ratio (NLR) during the first 3 months of treatment correlated with outcomes, but the same ratio evaluated at baseline or at any individual time point during the treatment was not statistically significant. Adverse events were substantially consistent with those observed in previous trials with pembrolizumab. Treatment-related adverse events (TRAEs) of any grade were reported in 98% of patients, with 30% of patients reporting grade 3–4 events. Eight patients (16%) discontinued treatment due to TRAEs, and one treatment-related death was reported.

Avelumab is a PD-L1 inhibitor that showed its efficacy in a multicenter, international, prospective, open-label, single-group, phase 2 trial named Javelin Merkel 200 (79). This trial enrolled patients diagnosed with stage IV MCC, refractory to at least a line of chemotherapy. Patient selection was not based on PD-L1 expression or Merkel cell polyomavirus status. Avelumab was given at 10 mg/kg by IV infusion every 2 weeks until disease progression or unacceptable toxicity was confirmed. A total of 88 patients were enrolled. At a median follow-up of 10.4 months, the ORR was 31.8% (28), with 8 CR, 20 PR, and 1 pseudoprogression. Responses were recorded at the first radiological evaluation in 79% of cases, with a median DOR not reached. Median PFS was 2.7 months while median OS was 11.3 months. On the wave of these early results, avelumab was approved by the FDA and EMA. Two subsequent updates were published (80, 81). In the last update, the median follow-up was 40.8 months. At this timepoint, ORR was 33% (29/88 patients), with 10 CR (11.4%). Avelumab seemed to perform better in patients with one previous chemotherapy line in respect to patients treated with two or more lines of chemotherapy (ORR

40.4% vs. 22.2%, respectively), while the sites of metastasis (visceral vs. non-visceral) did not appear to impact on ORR. Among patients whose tumors were assessable for PD-L1 expression (73), ORR was 36.8% in PD-L1-positive (57) and 18.8% in PD-L1 negative (16) patients. Regarding viral status, among 46 virus-positive and 31 virus-negative patients, the ORR was 28.3% and 35.5%, respectively. Such results were in line with a *post hoc* analysis published in the first report of the trial. At the time of the last analysis, responses were ongoing in 17 of 29 responders (58.6%) regardless of PD-L1 status, with 4 patients who maintained the response for more than 3 years. Median DOR was 40.5 months. PFS at 2 and 3 years of observation was 26% and 21%, respectively, while median OS was 12.6 months, with a 3- and 4-y OS of 32% and 31%, respectively. TRAEs of any grade occurred in 62 (70%) patients, with a particularly high rate of infusion reaction (17%) that induced to recommend the use of a premedication with H1-antihistamine and paracetamol 30–60 min before avelumab treatment; grade 3 TRAEs were reported in four (5%) of 88 patients. Two patients (2%) permanently discontinued treatment because of an adverse event. In this paper, exploratory biomarker analysis data were reported. Several factors were evaluated, but no single biomarker was consistently associated with a clinical benefit. Best outcomes were recorded among high TMB, virus-negative, or PD-L1-positive (or with a high level of TILs) patients that received just one prior systemic therapy.

Avelumab as a first-line treatment was evaluated in part B of Javelin Merkel 200 (82). Here, 39 stage IV chemo-naïve MCC patients were treated with avelumab upfront. Data from an interim analysis of this trial were reported in 2018, with a median follow-up of 5.1 months. At the time of the analysis, treatment was ongoing in 24 patients (61.5%), while 15 (38.5%) discontinued due to PD (7%–17.9%), adverse events (6%–15.4%), or death (2%–5.1%). Efficacy was evaluated in 29 patients with at least 3 months of follow-up, and in a subgroup of 14 patients with at least 6 months of follow-up. In the 3-month follow-up group, the ORR was 62.1%, with 4 (13.8%) CR and 14 (48.3%) PR, and a DCR of 72.4%. As observed in Javelin Merkel 200 part A and in KN017, 88.9% of responses were observed at the first radiological evaluation. Among responders, 14 (77.8%) patients had an ongoing response at the time of the analysis, with a median DOR not estimable. Median PFS was 9.1 months and the 3-month PFS was 67%. In the 6-month follow-up group, the ORR was 71.4% with 4 (28.6%) CR, 6 (42.9%) PR, and a DCR of 78.5%. Updated data with a median of 21.2 months of follow-up were presented in 2019 during the SITC congress (83). A total of 116 patients had been treated with avelumab, and, at the time of the analysis, treatment was ongoing in 26 patients (22.4%). The ORR was 39.7%, including 19 CR (16.4%) and 27 PR (23.3%), with slightly better results in the PD-L1-positive cohort in respect to the PD-L1 negative cohort (61.9% and 33.3%, respectively), and a median DOR of 18.2 months. Median PFS was 4.1 months with 6- and 12-month PFS rates of 41% and 31%, respectively. Median OS was 20.3 months, and the 12-month OS rate was 60%. In the PD-L1-positive and PD-L1-negative subgroups, 1-y OS rates were 71% and 56%, respectively. The SPEAR-Merkel study has been published in 2021 and reported clinical outcomes in patients affected by locally advanced or metastatic MCC treated with avelumab first line, in a real-world setting (84). A total of 36 patients were enrolled, 28 (32.1%) with laMCC and 19 (67.9%) with mMCC. Two-thirds of the overall 1L avelumab population (64.3%) discontinued 1L avelumab during the study period due to disease progression (33.3%), physician preference (27.8%), toxicity, or not documented (11.1% each). ORR was 64.3% (66.7% in laMCC and 63.2% in mMCC) with nine complete responses (three laMCC and six mMCC). The median DOR was 15.5 months, NR in patients with laMCC, and 9.6 months in patients with mMCC. The median PFS was 11.4 months, and the median OS was 20.2 months. Neither the median PFS nor the median OS was reached in patients with laMCC. In patients with mMCC, the median PFS was 10.0 months, and the median OS was 20.2 months. All results were consistent with data from the registration trial.

Data from the subsequent Expanded Access Program (EAP) program were published in August 2020 (85). In the EAP, patients who progressed after at least one line of chemotherapy and chemo-naïve patients who were ineligible for chemotherapy (evaluated case by case) were enrolled. Patients were not selected based on tumor PD-L1 expression or MCPyV status. A total of 494 patients were treated, including 15 who received treatment as a first line. Response data were available for 254 patients, and outcomes were provided for 240 patients. Results were substantially consistent with those from registration trials, with an ORR of 46.7%, including CR in 22.9%, PR in 23.8%, and a DCR of 71.2%. The safety profile was further confirmed, and avelumab showed a toxicity spectrum very similar to other anti-PD-1/PD-L1, except for infusion-related reactions, which occurred in nine patients. The relatively high number of infusion-related reaction deserves the recommendation to use a premedication with paracetamol and antihistaminic for at least the first four cycles of avelumab.

Finally, in July 2017 the results of the anti-PD-1 nivolumab were published (86). Nivolumab was evaluated among patients with five types of advanced virus-associated cancers who had received ≤2 prior therapies. At a median follow-up of 26 weeks, among 25 MCC patients who received treatment, 22 were evaluable for response, with an ORR of 68% and ongoing responses in 13 of 15 (87%) patients. Responses occurred in treatment-naive patients (71%), in patients with one to two prior systemic therapies (63%), and in both virus-positive and virus-negative tumors; 67% of responses occurred at ~8 weeks. At 3 months, PFS and OS rates were 82% and 92%, respectively.

The characteristics and results of all trials with immunotherapy for the treatment of advanced MCC are summarized in **Table 2**.

FUTURE DIRECTIONS FOR ADVANCED DISEASE

Future directions in MCC include several therapeutic strategies, such as immunotherapy, targeted therapies, and epigenetic

TABLE 2 | Summary of all clinical trials with immunotherapy for the treatment of locally advanced and/or metastatic MCC.

	KN 017 (78)	Javelin Merkel 200 (part B) (83)	Javelin Merkel 200 (part A) (81)	CM – 358 (86)
Drug	Pembrolizumab	Avelumab	Avelumab	Nivolumab
Line	I	I	≥II	≥II
				I
MCC status	Locally advanced/metastatic	Metastatic	Metastatic	–
				–
N. pts	50	116	88	8
				14
F.U. (mo)	31.8	21.2 mo	40.8 mo	26 weeks
ORR % (n)	58 (29)	39.7 (46)	33 (29)	63%
				71%
CR % (n)	30 (15)	16.4 (19)	11.4 (10)	0 (0)
				21 (3)
DCR % (n)	66 (33)	–	43.2 (38)	76 (6)
				71 (10)
PFS	m: 16.8 mo	1-y: 31%	m: 3 mo	3-mo: 82%
	3-y: 39.1%		3-y: 21%	
OS	m: NR	m: 20.3 mo	m: 12.6 mo	3-mo: 92%
	3-y: 59.4%	1-y: 60%	4-y 31%	

N.pts, number of patients; F.U., follow-up; ORR: overall response rate; CR, complete response; DCR, disease control rate; PFS, progression-free survival; OS, overall survival.

drugs, in both neoadjuvant, adjuvant, first-line, and subsequent line settings. Indeed, 50% of patients do not adequately respond to anti-PD-L1/anti-PD-1 monotherapy (treatment resistant, or relapsed) and second-line therapy in MCC is still uncoded. To answer this medical need and to give a therapeutic alternative to patients unfit for chemotherapy and absolute contraindication to immunotherapy, several trials with target therapy have been performed and others are currently ongoing. However, most trials with targeted therapies alone had disappointing results. A summary of all trials currently ongoing for advanced MCC is reported in **Table 3**.

MLN0128 is a second-generation TORC1/2 inhibitor that showed preclinical activity in MCC cell lines, decelerating tumor cell growth, diminishing cell proliferation, inducing apoptosis, and enhancing antitumor effect when combined with JQ1 (a bromodomain protein BRD4 inhibitor) (94). On this wave, a clinical trial with MLN0128 was performed (NCT02514824). The study never passed from phase I to phase II, and no efficacy data are available. From the few data reported, the study was closed due to a lack of efficacy and a slow recruitment (87).

Cabozantinib is a multiple-kinase inhibitor, including c-MET and VEGFR-2, commonly used in the treatment of several metastatic solid cancer. Cabozantinib (88) was evaluated in a prospective phase II trial (NCT02036476) that enrolled eight metastatic or locally advanced platinum-resistant MCC patients. The trial was closed prematurely due to poor tolerability and lack of activity of the study drug, which obtained a median PFS of 2.1 months and a median OS of 11.2 months. Notably, patients were not selected based on the presence of any mutation.

Oblimersen binds to human bcl-2 mRNA-stimulating apoptosis and is believed to facilitate non-apoptotic cell death by autophagy, to inhibit tumor angiogenesis, and to exert immunostimulatory effects. Preclinical studies (95) performed on MC-MA 11 MCC xenografts obtained encouraging results and provided the basis to a Simon two-stage phase II trial to evaluate oblimersen efficacy among MCC patients (89). A total of 12 patients were treated, but ORR was 0% and only 3 patients achieved a SD.

Imatinib was also evaluated as a potential treatment strategy in MCC. On the wave of the identification of c-Kit expression in this neoplasm, a clinical trial with imatinib mesylate was initiated (NCT00068783). Among 23 treated patients, ORR was 4% with 0 CR and 1 RP, and SD was achieved in 3 patients. Median PFS was 1 month with an estimated 6-mo PFS of 4%; estimated median OS and 1-y OS were 5 months and 17%, respectively (90).

Somatostatin analogues (SSAs) are commonly used in low- and medium-grade neuroendocrine tumors (NET), but several studies support their possible use in MCC therapy (96–98). Lanreotide has been evaluated in a phase II study (NCT02351128) on 35 patients (91). Among them, seven (20%) obtained a disease control form more than 3 months. Pasireotide had also been evaluated among melanoma and MCC patients in a phase I trial (NCT01652547). However, no data are available for the MCC cohort (92). In a recently published retrospective trial (96), 40 patients were evaluated for somatostatin receptor (SRS) expression. A total of 33 patients (85%) had some degree of SRS uptake, and 19 patients were treated with SSAs. Among them, seven had a response-evaluable target lesion and three (43%) experienced disease control, with a median PFS of 237 days. The major limit of this study is the confounding effect induced by radiotherapy, which made several lesions not radiologically evaluable according to RECIST. Interestingly, the degree of SRS expression did not correlate significantly with the efficacy endpoints.

Peptide receptor radionuclide therapy (PRRT) with (177) Lu-DOTATATE could be a potentially active therapy in MCC. Several case reports described objective responses in metastatic MCC patients (99, 100), and a phase II trial is currently ongoing (NCT04276597).

Combining targeted therapy and immunotherapy is known to be an interesting and promising strategy in several solid tumors (101, 102). In MCC, a number of clinical trials are ongoing to

TABLE 3 | Summary of all available trials for the treatment of locally advanced or metastatic MCC.

NCT	Phase	MCC stage	Drugs	N	Recruitment status	Study outcomes/primary objectives**	Ref.
NCT02514824	I/II	IV or recurrent	MLN0128	9	Completed	Negative. Lack of efficacy.	(87)
NCT02036476	II	IV or recurrent	Cabozantinib	8	Active, not recruiting	Negative. mPFS: 2,1 mo mOS: 11.2 mo Poor tolerability and lack of activity	(88)
NCT00079131	II	III–IV	Oblimersen	37	Completed	Negative ORR = 0%. SD = 3 patients.	(89)
NCT00068783	II	III–IV	Imatinib mesylate	40 (23)	Completed	CR = 0; PR = 1; ORR = 4%; SD = 3. mPFS = 1 mo; Estimated 6-mo PFS = 4%. mOS = 5 mo; Estimated 1-y OS = 17%.	(90)
NCT02351128	II	III–IV	Lanreotide	35	Completed	DCR 20% (7/35)	(91)
NCT01652547	I	IV	Pasireotide	10	Completed	Terminated early due to slow recruitment after 2 y from study initiation. No data on MCC cohort.	(92)
NCT03787602	II	III–IV	KRT-232 (MDM2 Antagonist)	46	Recruiting	ORR	
NCT04276597	II	III–IV	177Lu-DOTATOC	50	Recruiting	ORR	
NCT04261855	I/II	IV	Avelumab, radiation (EBRT), radiation (Lutetium-177 (177Lu)-DOTATATE)	65	Recruiting	PFS at 12 mo	
NCT02054884	II	IV	F16IL2, paclitaxel	13	Terminated (lack of enrollment)	ORR	
NCT04874831	II	IV	Avelumab, domatinostat	90	Not yet recruiting	ORR	
NCT04393753	II	III–IV	Avelumab, domatinostat	40	Recruiting	ORR	
NCT02035657	Proof of concept	III–IV	GLA-SE	10	Completed	Safety and feasibility	
NCT03783078	III	III–IV	Pembrolizumab	50	Active, not recruiting	ORR	
NCT04792073	II	III–IV	Avelumab, radiation	36	Recruiting	PFS at 12 mo	
NCT03599713	II	IV or recurrent	INCMGA00012	100	Recruiting	ORR	
NCT03988647	II	IV	Pembrolizumab, radiation	1	Active, not recruiting	ORR	
NCT03167164	I/II	IV	Avelumab, bevacizumab, capecitabine, Cisplatin, cyclophosphamide, 5-fluorouracil, leucovorin, nab-paclitaxel, omega-3-acid ethyl esters Radiation (stereotactic, body radiation therapy), ALT-803, ETBX-051, ETBX-061, GI-6301, haNK	0	Withdrawn (trial not initiated)	Safety and ORR	
NCT03853317	II	IV	Avelumab, N-803, haNK		Recruiting	ORR	
NCT02465957	II	III–IV	aNK (NK-92)	24	Active, not recruiting	PFS	
NCT03228667	II	III–IV	N-803, pembrolizumab, nivolumab, atezolizumab, avelumab, durvalumab, pembrolizumab, PD-L1 t-haNK	636	Recruiting	ORR	
NCT01913691	II	IV	Ipilimumab	0	Withdrawn	OS at 12 mo	
NCT01758458	I/II	IV or recurrent	Aldesleukin, MCPyV TAg-specific polyclonal autologous CD8-positive T cells, radiation, recombinant interferon beta	4	Terminated (A phase I/II study (NCT01758458) is now recruiting)	Safety and median time to new metastasis	
NCT01440816	II	NA	Tavokinogene telseplasmid (tavo)	15	Completed	lincresing in expression of IL-12	
NCT03071406	II	IV	Ipilimumab, nivolumab, radiation	50	Recruiting	ORR	
NCT04590781	I/II	III–IV	Pembrolizumab, XmAb18087	142	Not yet recruiting	Safety and ORR	
NCT01013779	II	II–III	Carboplatin, etoposide, radiotherapy	43	Active, not recruiting	Time to locoregional failure and safety	
NCT02584829	I/II		Avelumab, recombinant INF beta, radiation, MCPyV TAg-specific polyclonal autologous CD8-positive T cells	8	Active, not recruiting	ORR and safety	
NCT02819843	II	III–IV	TALIMOGENE LAHERPAREPVEC (TVEC), radiation (hypofractionated radiotherapy)	19	Active, not recruiting	ORR	
NCT00003549	II	III–IV	CMF regimen, cyclophosphamide, fluorouracil, methotrexate	80	Completed	Not avalable	
NCT04160065	I	III–IV	IFx-Hu2.0	20	Recruiting	Safety	

(Continued)

TABLE 3 | Continued

NCT	Phase	MCC stage	Drugs	N	Recruitment status	Study outcomes/primary objectives**	Ref.
NCT04853602	expanded access	III–IV	IFx-Hu2.0	-	Recruiting	Not available	
NCT03684785	I/II	III–IV	Cavrotolimod, pembrolizumab, cemiplimab	130	Recruiting	Safety	
NCT03304639	II	III–IV	Pembrolizumab, radiation (stereotactic body radiation therapy)	100	Active, not recruiting	PFS	
NCT00346385	I	IV	BB-10901	97	Completed	Safety	
NCT03901573	I/II	IV	NT-I7, atezolizumab		Recruiting	Ssafety	
NCT02978625	II	IV	Nivolumab, talimogene laherparepvec	68	Recruiting	ORR	
NCT03458117	I	III–IV	Talimogene laherparepvec (T-VEC)	20	Recruiting	Activation of biomarkers	
NCT00004922	II	IV	Irinotecan hydrochloride	31	completed	Not available	
NCT00003514	II	IV	Antineoplaston A10, antineoplaston AS2-1	0	Withdrawn	Not available	
NCT03747484	I/II	III–IV	Autologous MCPyV-specific HLA-A02-restricted TCR-transduced CD4+ and CD8+ T-cells FH-MCVA2TCR, avelumab, pembrolizumab, radiation	16	Recruiting	Safety and ORR	
NCT03816332	I	III–IV	Ipilimumab, nivolumab, prednisone, tacrolimus	16	Suspended (scheduled interim monitoring)	Safety	
NCT02831179	I	III–IV	Capecitabine, temozolomide, veliparib	0	Withdrawn (loss of funding support)	Maximum tolerated dose	
NCT03107663	I	III–IV	^{89}Zr-Df-IAB22M2C	15	Completed	Safety	
NCT01204476	I	III–IV	Cixutumumab, everolimus, octreotide acetate	27	Completed	mPFS: 43,6 weeks, mOS: 25,5 mo. No data on MCC cohort.	(93)
NCT03074513	II	III–IV	Atezolizumab, bevacizumab	164	Active, not recruiting	ORR	
NCT04234113	I	III–IV	SO-C101, pembrolizumab	96	Recruiting	DLT	
NCT03435640	I/II	III–IV	NKTR-262, bempegaldesleukin, nivolumab	64	Active, not recruiting	Safety	
NCT03629756	I	III–IV	Etrumadenant, zimberelimab	44	Active, not recruiting	Safety	
NCT04725331	I/II	III–IV	BT-001, pembrolizumab	48	Recruiting	Safety/ORR	
NCT02890368	I	IV or recurrent	TTI-621, PD-1/PD-L1 Inhibitor, pegylated interferon-α2a, T-Vec, radiation	56	Terminated	Safety	
NCT04246671	I/II	III–IV	TAEK-VAC-HerBy	45	Recruiting	DLT	
NCT03935893	II	III–IV	Tumor-infiltrating lymphocytes (TIL), fludarabine, cyclophosphamide	10	Recruiting	DLT	
NCT04272034	I	III–IV	INCB099318	100	Not yet recruiting	Safety	
NCT04242199	I	III–IV	INCB099280	140	Recruiting	Safety	
NCT04260802	I/II	III–IV	OC-001, anti-PD-1/anti-PD-L1	80	Recruiting	DLT	
NCT03841110	I	III–IV	FT500, nivolumab, pembrolizumab, atezolizumab, cyclophosphamide, fludarabine, IL-2	76	Recruiting	DLT	
NCT03652077	I	III–IV	INCAGN02390	40	Active, not recruiting	Safety	
NCT03538028	I	III–IV	INCAGN02385	22	Completed	Safety	
NCT02643303	I/II	III–IV	Durvalumab, tremelimumab, poly ICLC	102	Recruiting	PFS at 24 weeks	
NCT04187872	I	III–IV	LITT + pembrolizumab	16	Recruiting	Immune effect on blood	
NCT03212404	I	III–IV	CK-301 (cosibelimab)	500	Recruiting	DLT	
NCT01155258	I	III–IV	Temsirolimus, vinorelbine ditartrate	19	Completed	MDT	
NCT02479698	II	III–IV	Allogeneic BK-specific Cytotoxic T-lymphocytes	100	Recruiting	ORR	
NCT03589339	I	III–IV	NBTXR3	60	Recruiting	ORR	
NCT00002947	I	III–IV	Indium In 111 pentetreotide	35	Terminated	Not available	
NCT00655655	I	III–IV	Everolimus, vatalanib	96	Completed	MTD	

When published or presented, outcomes are reported with corresponding reference.

N, number of patients enrolled; Ref, reference; m, median; PFS, progression-free survival; OS, overall survival; ORR, overall response rate; CR, complete response; PR, partial response; SD, stable disease; DCR, disease control rate; DLT, dose-limiting toxicity; MTD, maximum dose tolerated.

***If no results are available, we indicate the primary objectives of the study.*

assess such combination strategy. One of the most promising agents to use in combination is domatinostat, an enzyme histone deacetylase inhibitor (HDAC) able to modulate the tumor microenvironment and to enhance antitumoral immunological response. In a phase I study performed on 24 pretreated patients, affected by several solid cancers, this oral molecule showed a favorable toxicity profile at 200 mg/BID, being able to induce 1 CR, 1 PR, and 18 SD (103). Combination between domatinostat and immunotherapy (pembrolizumab) has been subsequently evaluated in a phase II trial (104) that assessed the safety of this combination and the potentially ability of domatinostat to increase the antitumor activity of pembrolizumab. Currently,

two phase II trials with avelumab plus domatinostat are recruiting patients (NCT04874831; NCT04393753).

ADJUVANT AND NEOADJUVANT APPROACH

Adjuvant and neoadjuvant approaches are not a current clinical practice. However, several clinical trials are investigating treatments this setting, with interesting results (**Table 4**). The first ADMEC trial (NCT02196961) with adjuvant ipilimumab *versus* observation in resected MCC patients was closed after 22.3 months of follow-up due to a futility analysis showing lack of efficacy and a strong toxicity of ipilimumab (105). Data of the phase II ADMEC-O trial with adjuvant nivolumab (NCT02196961), the phase III ADAM trial (NCT03271372) with adjuvant avelumab, and the phase III STAMP study (NCT03712605) are still awaited. Notably, several clinical trials include very early stage MCC, like stages I and II (see **Table 4**).

A neoadjuvant approach was explored in CheckMate 358 (106), a phase I/II study that enrolled 39 patients affected by completely resectable MCC (stages IIA–IV). A total of 36 patients received 2 cycles of neoadjuvant nivolumab, followed by surgery. Pathological response (pR) and radiological response (rR) were correlated with clinical outcomes. All patients were evaluated for pR by study investigators, while a total of 26 patients were evaluated by central pathologic review, finding a pathological complete response rate (pCR) of 47.2% (n = 17) and 46.2% (n = 12), respectively; among patients evaluated centrally, the major pathological response (MPR) rate was 15.4% (n = 4). A total of 33 patients were radiologically evaluable, with an ORR of 54.4% (n = 18). Notably, radiographic response seemed to underestimate the degree of pR: indeed, among 11 rR < 30% (non-CR, non-PR), 5 had pCR; moreover, rCR has been significantly less than pCR. Median recurrence-free survival (RFS) and median OS were not reached at 20.3 months of follow up, while 24-month RFS and 24-month OS were 68.5% and 79.4% in the whole population, respectively. Both pR and rR correlated with RFS and OS. Indeed, 24-month RFS among patients that had a pCR/MPR by central review and among patients who obtained at least an rPR were 88.9% and 90.9%. In the same way, 24-month OS among patients who developed a pCR by central review, or at least an rPR, was 100.0%.

TABLE 4 | Summary of all available trials currently ongoing for the treatment of completely resected MCC with an adjuvant intent, or potentially resectable MCC with a noeadjuvant intent.

Trial	NCT	Phase	Stage MCC	Drugs	N	Recruitment status	Study out-comes	Ref.
Ipilimumab adjuvant ADMEC (DeCOG) Ph II, open, randomized vs. observation	NCT02196961	II	II–III–IV completely resected	Ipilimumab	40	Terminated	Negative. no difference in PFS	(105)
Adjuvant Therapy of Completely Resected Merkel Cell Carcinoma With Immune Checkpoint Blocking Antibodies vs. Observation (ADMEC-O)	NCT02196961	II	II–III–IV completely resected	Nivolumab	180	Active, not recruiting	No data	
Nivolumab and Radiation Therapy or Ipilimumab as Adjuvant Therapy in Treating Patients With Merkel Cell Cancer	NCT03798639	I	III completely resected	Nivoluamb, Radiation, Ipilimumab	7	Active, not recruiting	No data	
Adjuvant Avelumab in Merkel Cell Cancer (ADAM)	NCT03271372	III	III completely resected	Avelumab	100	Recruiting	No data	
Immunotherapy Adjuvant Trial in Patients With Stage I–III Merkel Cell Carcinoma (I-MAT)	NCT04291885	II	I, II, III completely resected	Avelumab	132	Recruiting	No data	
Pembrolizumab Compared to Standard of Care Observation in Treating Patients With Completely Resected Stage I-III Merkel Cell Cancer, STAMP Study	NCT03712605	III	I, II, III completely resected	Pembrolizumab, radiation	500	Recruiting	No data	
Neoadjuvant Nivolumab for Patients With Resectable Merkel Cell Carcinoma in the CheckMate 358 Trial	NCT02488759	I/II	IIA–IV resectable	Nivolumab	39	Active, not recruiting	24 mo-RFS pCR/MPR: 88.9%; 24 mo-RFS rPR/rCR: 90.9%; 24 mo-OS pCR/MPR: 100.0% and 88.9% 24 mo-OS rPR/rCR: 100%	(106)
Neoadjuvant Lenvatinib Plus Pembrolizumab in Merkel Cell Carcinoma	NCT04869137	II	II–III–IV resectable	Pembrolizumab, lenvatinib	26	Recruiting	No data	

N, number of patients; ref, reference; RFS, Relapse Free Survival, pCR/MPR, pathologic complete response/major pathologic response; OS, Overall Survival.

A neoadjuvant study with pembrolizumab plus lenvatinib (NCT04869137) is currently recruiting patients.

DISCUSSION

Treatment of MCC is an emerging issue in everyday clinical practice. If in the past years this tumor was considered as a sort of SCLC in terms of biological behavior and clinical management, today it has become an object of numerous studies. Indeed, until recently, standard treatment was based on chemotherapeutic schemes with disappointing results, with a median survival of 9–10 months (69–73). Currently, the standard of care for the treatment of this neoplasm is immunotherapy with avelumab (anti-PD-L1) which received FDA and EMA approval, and pembrolizumab and nivolumab which was approved for the same indication by the FDA only. First-line pembrolizumab in locally advanced and metastatic MCC achieved a median OS not reached at a median follow-up of 31.8 months, and a 3-y OS of 59.4% (78), while first-line avelumab in metastatic MCC showed a median OS of 20.2 months (83). In pretreated patients progressing to chemotherapy, avelumab showed a median OS of 12.6 month and a 4-y OS of 31% (81).

The fact that immunotherapy performs worse in the second-line setting rather than in the first line is likely to depend on the type of patient, classically fragile, elderly, and with severe comorbidities, whose conditions tend to a progressive worsening, and on the biology of this disease which is characteristically very aggressive. Therefore, in patients with no absolute contraindications to immunotherapy, upfront treatment with anti-PD-1/anti-PD-L1 agents is recommended. A high burden of disease and/or the presence of clinical symptoms do not contraindicate the initiation of upfront immunotherapy. Indeed, it has been shown that immunotherapy is able to induce rapid responses, most of them observed at the first radiological evaluation, lasting over time (78, 83). Starting the therapeutic strategy with a chemotherapy treatment has shown, in a retrospective study, to cause a substantial reduction of patients who will be able to receive second-line treatment, a reduction of the duration of the first line itself, and a reduction of the time to second-line initiation, due to the rapid progression observed in the course of chemotherapy (107).

Until today, no predictive factors for anti-PD-L1/PD-1 therapy are accepted, although tumor PD-L1 expression, virus status, and some other factors may correlate. Tumor PD-L1 expression (PD-L1 negative versus PD-L1 positive) seems to correlate with efficacy of immunotherapy, in line with results observed in other tumor types. However, no definite conclusions have been drawn.

The second line in MCC remains an unmet medical need.

Indeed, almost 50% of patients do not respond to anti-PD-L1/anti PD-1 and, at the time of the disease progression, few therapies are easily available other than chemotherapy. The motivation for this choice is twofold. First, chemotherapy has a high ORR and often these patients progress rapidly and with high disease burdens: chemotherapy allows us to reduce tumor burden, partially improving the patients' quality of life. Second, due to the rapid kinetic of this tumor, the survival of these patients in the absence of treatment (best supportive care) is extremely low and chemotherapy, although with known limits, allows us to obtain some advantages. Clinical practice involves the use of standard chemotherapy schemes such as platinum in combination with etoposide.

There are currently no recruiting trials for patients progressing from anti-PD-1/anti-PD-L1 therapy, and this is certainly a major limitation to the therapeutic prospects for patients under treatment. In our opinion, it would be appropriate to start second-line trials, for example to evaluate the effectiveness of the continuation of anti-PD-1 in association with standard chemotherapy. This approach has already given positive results in SCLC, a neoplasm that shares several characteristics with MCC in terms of clinical and biological behavior, tumor kinetic, and sensitivity to chemotherapy. Indeed, carbo/cis-platinum plus etoposide plus anti-PD-L1 as a first line of treatment has been evaluated in Caspian and Empower 133 trial (108, 109) and showed a good safety profile and improved efficacy in terms of OS and PFS in respect to chemotherapy alone. To date, a similar approach in MCC remains completely unexplored in the first and second lines.

Numerous trials are evaluating strategies with molecularly targeted drugs. After some disappointing results with cabozantinib (88) and oblimersen (89), new hopes are now placed in treatment with somatostatin analogues. Indeed, encouraging data from case reports and case series are currently available, as well as from a small phase II study with lanreotide, which showed a DCR of 20% (91, 92, 98). Larger and more standardized clinical trials will be needed to define the real benefit of these treatments.

As we reported before, immunotherapy provides a clinical benefit in approximately 50% of patients, with the aim to increase the percentage of responders, overcome the mechanisms of primary resistance, and prevent the development of secondary resistance, like MHC-I downregulation, low CD8 T cell response, and Th2 polarization of CD4 T cells (110, 111). One of the most promising agents is domatinostat, which showed a favorable toxicity profile in a phase I trial and promising results in combination with pembrolizumab in a phase II trials (104). Currently, two phase II trials with avelumab plus domatinostat are recruiting patients (NCT04874831; NCT04393753). The adjuvant/neoadjuvant approach is currently not part of everyday clinical practice, but it is an extremely promising field of research. The very positive results of the CM 358 study with nivolumab in the neoadjuvant setting (106) showed the great potential of this therapeutic strategy and numerous trials are being developed to define the role of a possible early treatment in MCC. In CM 358, the pathological complete response rate and the major pathological response rate were 46.2% and 15.4%, respectively. Notably, pathological complete response rates in neoadjuvant anti-PD-1 trials in NSCLC and in melanoma were 15% and 19%–25% (112, 113). In light of these preliminary results, there is high expectation for the currently ongoing trials with adjuvant nivolumab, adjuvant avelumab, and neoadjuvant pembrolizumab plus lenvatinib.

AUTHOR CONTRIBUTIONS

ET conceived the review focus, conducted the literature review, summarized the manuscript, analyzed the data, wrote the first draft, and finalized the manuscript. FS and PQ coordinated and supervised the review. ALd'A, GR, EC, AB, FC, FS, and PQ reviewed the literature and revised and made corrections to the manuscript. All authors contributed to the article and approved the submitted version.

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Cemiplimab in an Elderly Frail Population of Patients With Locally Advanced or Metastatic Cutaneous Squamous Cell Carcinoma: A Single-Center Real-Life Experience From Italy

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Background: Cutaneous squamous cell carcinoma (CSCC) is the second most common skin cancer whose incidence is growing parallel to the lengthening of the average lifespan. Cemiplimab, an antiPD-1 monoclonal antibody, is the first approved immunotherapy for patients with locally advanced CSCC (laCSCC) or metastatic CSCC (mCSCC) thanks to phase I and II studies showing high antitumor activity and good tolerability. Nevertheless, at present, very few data are available regarding cemiplimab in real-life experience and in frail, elderly, and immunosuppressed patients as well as regarding biomarkers able to predict response so as to guide therapeutic choices.

Patients and Methods: We built a retroprospective cohort study including 30 non-selected patients with laCSCC (25) and mCSCC (five) treated with cemiplimab from August 2019 to November 2020. Clinical outcomes, toxicity profile, and correlations with disease, patients, and peripheral blood parameters are explored.

Results: The median age was 81 years (range, 36–95), with 24 males and five patients having an immunosuppressive condition, while the frailty prevalence was 83% based on

index derived from age, Eastern Cooperative Oncology Group performance status, and Charlson Comorbidity Index. We reported 23 responses (76.7%) with nine complete responses (30%). A statistically significant higher response rate was observed in head and neck primary tumors and in patients with hemoglobin level >12 g/dl. No difference was observed with respect to frailty, median age, sex, and body mass index. The baseline low neuthophil/lymphocyte ratio and low platelet/lymphocyte ratio resulted to be also correlated with a better response. Moreover, lymphocyte, neutrophil, and monocyte behaviors had an opposite trend in responders and non-responders. An overall response was reported in four of five immunosuppressed patients. Seventeen patients (57.6%) have an ongoing response and are still alive. Six responders had interrupted treatment (two for toxicity and four for personal choice) but maintained their response. The treatment was well tolerated by the majority of patients. The most common adverse events were fatigue in seven patients (23.3%) and skin toxicity in 10 patients (33.3%), including pruritus in six patients, rash in three patients, and bullous erythema in one patient.

Conclusions: In our real-life experience, cemiplimab showed a high antitumor activity with acceptable safety profile similar to those in trials with selected patients. Moreover, its antitumor activity resulted to be not impaired in very elderly patients and in those with immunocompromised status.

Keywords: cemiplimab, advanced cutaneous squamous cell carcinoma, checkpoint inhibitors, elderly patients, immunocompromised patients

INTRODUCTION

After basal cell carcinoma, among non-melanoma skin cancers, the second most common cancer is cutaneous squamous cell carcinoma (CSCC), whose incidence rates are dramatically increasing over the last decades (1–4).

The risk of developing CSCC increases with age and could depend on the lifetime accumulation of ultraviolet (UV) radiation damage and the onset of immune suppression such as in patients with immunodeficiency virus infection, hematological neoplasms, or autoimmune diseases treated with immunosuppressive agents (5, 6).

The standard therapy for localized CSCC is surgery eventually associated to complementary radiotherapy, but in some cases this approach is not sufficient or not feasible due to locally advanced extent at onset (7). In addition, approximately 5% of patients are metastatic at diagnosis or develop metastases or inoperable local recurrence after complete excision. For these patients, until recently, the standard treatment was platinum-based chemotherapy, but it provided disappointing results and short duration of responses. Moreover, the heavily toxic profile of these drugs compromised the quality of life of patients, often elderly and with several significant comorbidities, requiring dose reductions or a definitive suspension of treatments (8–10). The epidermal growth factor receptor inhibitor cetuximab was also used as first-line single drug, but it showed limited efficacy in advanced CSCC (11).

Of note is that it has been demonstrated that CSCC is characterized by a high mutational load capable of inducing a high expression of tumor neoantigens, making this tumor suitable for immunotherapy (12–14). This therapy meets its biological rational also in the accumulation of immune inhibitory molecules such as programmed death-1 (PD-1) ligand in the microenvironment during tumor growth (15, 16).

Recently, cemiplimab, a monoclonal antibody against the PD-1 receptor, has been approved in the US and EU for patients with locally advanced CSCC (laCSCC) or metastatic CSCC (mCSCC) unfit for curative surgery or radiotherapy. In fact, phases I and II studies reported a significant antitumor activity of cemiplimab in about half of patients regardless of PDL1 expression or extent of total genetic mutation burden, with an acceptable toxicity profile (17). However, these studies recruited selected patients, with the exclusion of those with immunosuppressive status such as transplant recipients and patients with hematological diseases or relevant comorbidities and organ function alterations, as often seen in the elderly population. These clinical features are often found in the real-world population of advanced CSCC and collectively define frailty as a common vulnerability condition among older cancer patients which is associated to an increased risk for poor therapeutic outcomes (18, 19). Thus, due to the limited data still available in non-selected patients (14, 20–24), in this paper, we report our experience with cemiplimab in a frail population treated outside controlled clinical trials and including very elderly patients presenting with several co-morbidities and patients with immunosuppressive conditions requiring a careful assessment of the cost–benefit profile of treatment. Moreover, owing to the absence of biomarkers able to predict response that would guide the therapeutic choice, we investigated the correlations between therapy outcomes and both clinical and blood parameters. The role of simple blood parameters was

previously explored in a cemiplimab series (22) showing a predictive value of the absolute lymphocyte count and was established in various cancer settings (25) in which specific white cells and their ratios, like neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), were shown to mirror the complex interplay between thrombosis, inflammation, and immunosuppression (26, 27). Thus, we assessed the predictive role of blood count both as pre-treatment value and as longitudinal variations.

PATIENTS AND METHOD

Patients and Study Design

We built an observational cohort study by retrospectively reviewing the medical records of 30 consecutive patients with laCSCC or mCSCC treated at the "Giovanni Paolo II" National Cancer Institute of Bari, Italy, from August 2019 to January 2021. Among these patients, 19 began treatment within a compassionate use program made available by Sanofi-Regeneron Company until the official approval by the Italian Regulatory Agency in May 2020. Cemiplimab was administered at a flat dose of 350 mg every 21 days until disease progression or unacceptable toxicity.

The screened patients were 18 years or older with histologically confirmed laCSCC or mCSCC. The patients were evaluated if their medical records reported the Eastern Cooperative Oncology Group (ECOG) performance status (PS), a complete medical and therapeutic anamnesis, and at least one measurable target lesion, including visible CSCC lesions as documented by digital medical photography or any other evaluable lesion at radiological imaging according to Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1) (28).

Clinical evaluation from the multidisciplinary tumor board was required to confirm that the patients were unfit for surgery or radiotherapy. During the cemiplimab therapy, the addition of local treatment was allowed according to a subsequent board evaluation due to shrinkage, making the lesions suitable of these therapies or due to palliative intents. These patients were included in the analysis if they achieved a RECIST response to cemiplimab before the addition of local treatment. Disease staging was performed prior to treatment and included a total body CT scan for all patients. All patients underwent baseline laboratory tests to assess the main organ functions, including a complete blood cell count and a complete metabolic panel with serum creatinine, blood urea nitrogen, aspartate aminotransferase, alanine aminotransferase, and total bilirubin. The same tests were performed during treatment as standard laboratory care. Moreover, the TSH, fT3, fT4, ACTH, and cortisol levels were regularly sampled to detect any possible immune-related adverse event early. In our study, all patients treated with cemiplimab were included irrespective of the presence of alterations in bone marrow, renal, liver, cardiac, pulmonary, and endocrinological function. Chronic liver viral infections were allowed, provided that the patients were strictly monitored.

The patients were not included in the analysis if they were treated prior with anti-PD-1 or anti-PD-L1 therapy and had active concurrent malignancies other than cutaneous squamous cell carcinoma. However, enrollment was allowed for patients with stable hematological malignancies, adequately treated basal cell carcinoma of the skin, *in situ* carcinoma of the cervix, *in situ* ductal carcinoma of the breast, and low-risk early-stage prostate adenocarcinoma under active surveillance. All the patients included in the analysis signed a written informed consent as part of the study as previously approved by the ethics committee of the IRCCS Istituto Tumouri Giovanni Paolo II, Bari, Italy (Prot. 590/16 C.E.). Sixteen of our 30 patients were also included in an Italian multicenter study (24).

Procedures

The patients received cemiplimab intravenously over 30 min at a flat dose of 350 mg every 21 days until disease progression, unacceptable toxicity, withdrawal of consent, or at the discretion of the physician if continuing the treatment could put the patient at risk or if it was deemed in the best interest of the patient, considering a balance between the benefits and the risks of treatment.

The assessments of tumor response were performed every two cycles by photographs of the superficial lesions and every 3 months by CT or MRI scan of laCSCC, while for mCSCC tumor assessment this was performed every 3 months by radiological evaluation. All responses were confirmed at least 4 weeks after the criteria for response were initially met: all responses that were not confirmed at the following evaluation were considered stable diseases at the assessment of best overall response. Treatment beyond progression was allowed in case of clinical benefit at the discretion of the clinician.

All patients who received at least one dose of cemiplimab were assessed for safety. Toxicity assessments included reporting of laboratory monitoring, clinical parameters, and treatment-related adverse events graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0 (CTCAE v5.0). Treatment interruptions were allowed in case of grade 3 or higher treatment-related adverse events. The patients were considered for resumption of treatment once the treatment-related adverse event resolved to grade 1 or baseline. Otherwise, treatment was discontinued, and the patient was addressed to regular clinical and radiologic follow-up.

Standard peripheral blood parameters (total leukocyte count, neutrophils, lymphocytes, monocytes, hemoglobin, platelets, NLR, and PLR) were registered before treatment and after 1, 2, 3, and 6 months from the start of immunotherapy to verify correlations between these hematological features and clinical outcomes.

Medical data were reviewed to categorize the comorbidity of a patient by the modified Charlson Comorbidity Index (CCI) (29). A frailty index adopted in studies on a similar population (30–32) was set up by adding scores assigned to age, ECOG performance status, and CCI as defined in **Supplementary Table S1**. A score ≥2 defined the frail population.

Outcomes

We firstly assessed the best overall response, considering the proportion of patients with complete or partial response (overall response rate, ORR) and the proportion of non-progressing patients (disease control rate, DCR). We then evaluated the time between the start of treatment and the first date of recurrent or progressive disease or death from any cause (progression-free survival, PFS) and the time between the onset of complete or partial response and the first evidence of recurrent or progressive disease or death for any cause (duration of response, DOR). We also assessed overall the survival (OS); safety and tolerability were registered and graded as well according to the CTCAE 5.0 classification of adverse events. Finally, we performed a statistical analysis to assess the possible correlations between therapy outcomes and disease and patient characteristics and hematological parameters.

Data Collection

Clinical data from medical records were collected in an anonymized database including the characteristics of patients (sex, age at diagnosis and at metastatic disease, significant comorbidities, previous treatments, and PS), the features of the disease (primary tumor site, grade of differentiation, tumor size, disease free interval, and stage), clinical outcomes (response and duration, PFS, and OS), and adverse events. Peripheral blood tests were also collected and analyzed.

Statistical Analyses

The results are presented according to the intention-to-treat principle. The proportion of patients achieving an objective response, stable disease, or progressive disease was evaluated according to clinical or RECIST 1.1 criteria and analyzed in descriptive statistics.

The duration of response, PFS, and OS were estimated by the Kaplan–Meier method. For DOR, patients with complete or partial response without disease progression were censored at the time of their last valid tumor assessment. Similarly, patients without disease recurrence or progression and patients alive at their last tumor assessment were censored from PFS and OS, respectively.

The association between ORR and age, hemoglobin, total leukocytes, neutrophils, lymphocytes, monocytes, platelets, NLR, and PLR was measured on an interval scale and analyzed with the Mann–Whitney non-parametric test, whereas all other features in the ordinal and nominal scale were analyzed with the chi-square test.

The variations of blood count parameters were registered and compared between the responder and the non-responder subcohorts. Furthermore, basal NLR and PLR were dichotomized according to their median values as low or high; then, the combinations of their values were correlated with response. Results were considered statistically significant for p-values inferior to 0.05.

RESULTS

Patients' Population

The main baseline characteristics of the study population are summarized in **Table 1**. Briefly, the main features of our cohort were male sex (80%) and median age of 81 years (range, 36–95), with a prevalence of frailty of 83% according to the adopted index and a median CCI of 2 (range, 0–5). Mostly, there were laCSCC (83.3%) located at the head and neck region (23 patients, 76.7%) that had undergone at least one surgery for CSCC. Only 33% of patients had been previously treated with radiotherapy, while 3 patients underwent subsequent concomitant radiotherapy after completing six, two, and four cycles of cemiplimab with the palliative intent to treat painful and ulcerated lesions.

TABLE 1 | Patients' demographic characteristics.

Patients	30
Median age, years (range)	81 (36–95)
Sex	
Male	24 (80%)
famale	6 (20%)
ECOG performance status	
0	7 (23.3%)
1	17 (56.7%)
2	6 (20%)
Primary cutaneous squamous cell carcinoma (CSCC) site	
Head or neck	23 (76.7%)
Limbs	5 (16.7%)
Ubiquitous skin lesions	2 (6.7%)
Previous chemotherapy for CSCC	3 (10%)
Previous radiotherapy for CSCC	10 (33.3%)
Previous surgery for CSCC	
0–1 surgery	15 (50%)
2–4 surgeries	7 (23.3%)
More than five surgeries	8 (26.7%)
Histological differentiation of tumor	
Well differentiated	4 (13.3%)
Moderately differentiated	12 (40%)
Poorly differentiated	10 (33.3%)
Unknown	4 (13.3%)
Locally advanced CSCC	25 (83.3%)
Metastatic cutaneous CSCC	5 (16.7%)
Immunosuppressive conditions[a]	5 (16.7%)
Main comorbidities	
Cardiovascular	20 (66.7%)
Metabolic	5 (16.7%)
Respiratory	6 (20%)
Mental disorders	3 (10%)
Frailty score	
Not frail	5 (16.7%)
Frail	25 (83.3%)
Charlson Comorbidity Index	
0	6 (20%)
1	8 (26.7%)
2	7 (23.3%)
3	5 (16.7%)
4	2 (6.7%)
5	1 (3.3%)

Data are n (%), unless otherwise specified.

[a]*Three patients with lymphoproliferative disease and two patients receiving immunosuppressive therapy.*

Clinical Outcomes

All patients were evaluable for response and safety. An objective response was observed in 23 patients (76.7%, 95% CI: 57.7–90.1), including nine complete responses (30%) and 14 partial responses (46.7%). Moreover, one patient (3.3%) obtained a stable disease for 4 months. Globally, the DCR was 80% (95% CI, 61.4–92.3). Six patients reported progressive disease as best response (20%). The median

duration of response was not reached at data cutoff. At present, the longest duration of response is 22 months, and it is still ongoing. Clinical outcomes are summarized in **Table 2**. In a female with bilateral gross preauricular lesions, we observed a pseudoprogression of the right lesion with an initial increase in size followed by a progressively slow decrease to near-complete remission. In **Figure 1**, we reported some representative cases of responsive patients.

The median time to response was 2 months (range, 1–5). The main characteristics of tumor responses are shown in the swimming plot (**Figure 2A**) and waterfall plot (**Figure 2B**).

Regarding correlations between patient/disease features and therapeutic outcomes, we observed a higher ORR in head and neck primaries (87 *vs.* 42.9% of others, $p = 0.016$), in well differentiated histotypes (100%, 95% CI: 39.8–100 *vs.* 75% of moderately and 80% of poorly differentiated), in patients without comorbidity (100%, 95% CI: 54.07–100 *vs.* 70%), and in patients with no or one surgery than in those receiving more than one surgery (80%, 95% CI: 51.9–95.7 *vs.* 71.4 and 75% of two to four surgeries and five or more surgeries, respectively). A modest better response was also reported in patients older than the median age of 81 years (81.3%; 95% CI 54.4–96.0 *vs.* 71.4%, 95% CI: 41.9–91.6), in females (83.3%, 95% CI: 35.9–99.6 *vs.* 75% of male), and in ECOG 0–1 (79.2%, 95% CI: 57.8–92.9 *vs.* 66.7% of ECOG 2) as well as a slight increase in non-frail *vs.* frail (80 *vs.* 76%) and overweight

TABLE 2 | Assessment of tumor response (30 patients).

Response	*N* (%)	95% CI
Complete response, *n* (%)	9 (30)	13.6–46.4
Partial response, *n* (%)	14 (46.7)	28.8–64.5
Stable disease, *n* (%)	1 (3.3)	0.1–17.2
Progressive disease, *n* (%)	6 (20)	7.7–38.6
ORR, *n* (%)	23 (76.7)	57.7–90.1
DCR, *n* (%)	24 (80)	61.4–92.3
Observed duration of response ≥6 months, *n* (%)	18 (60)	
PFS, median (range)	16 (1–23)	
OS, median (range)	18 (1–23)	
Median observed time to response, months (range)	2 (1–5)	

ORR, overall response rate (defined as complete response + partial response); DCR, disease control rate (defined as complete response + partial response + stable disease); PFS, progression-free survival; OS, overall survival.

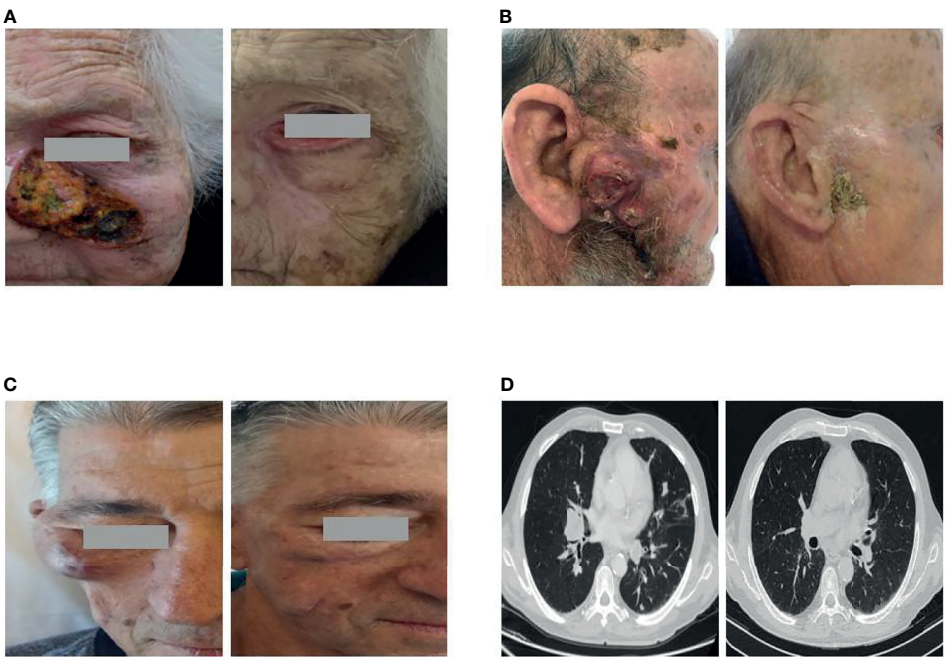

FIGURE 1 | Representative cases of patients obtaining a major response to cemiplimab. **(A)** An 88-year-old female with a large locally advanced cutaneous squamous cell carcinoma (laCSCC) of the left nasal-infraorbital region achieving a complete response. Neither had she received prior radiotherapy nor anticancer systemic therapy. **(B)** An 89-year-old man with a large laCSCC tumor of the right parotid region obtaining a complete response after 6 cycles of cemiplimab and concurrent radiotherapy. **(C, D)** A 67-year-old man with metastatic cutaneous squamous cell carcinoma in immunosuppressive therapy due to a previous kidney transplantation. The patient achieved a near-complete response both at the right zygomatic area and the metastatic lung lesions.

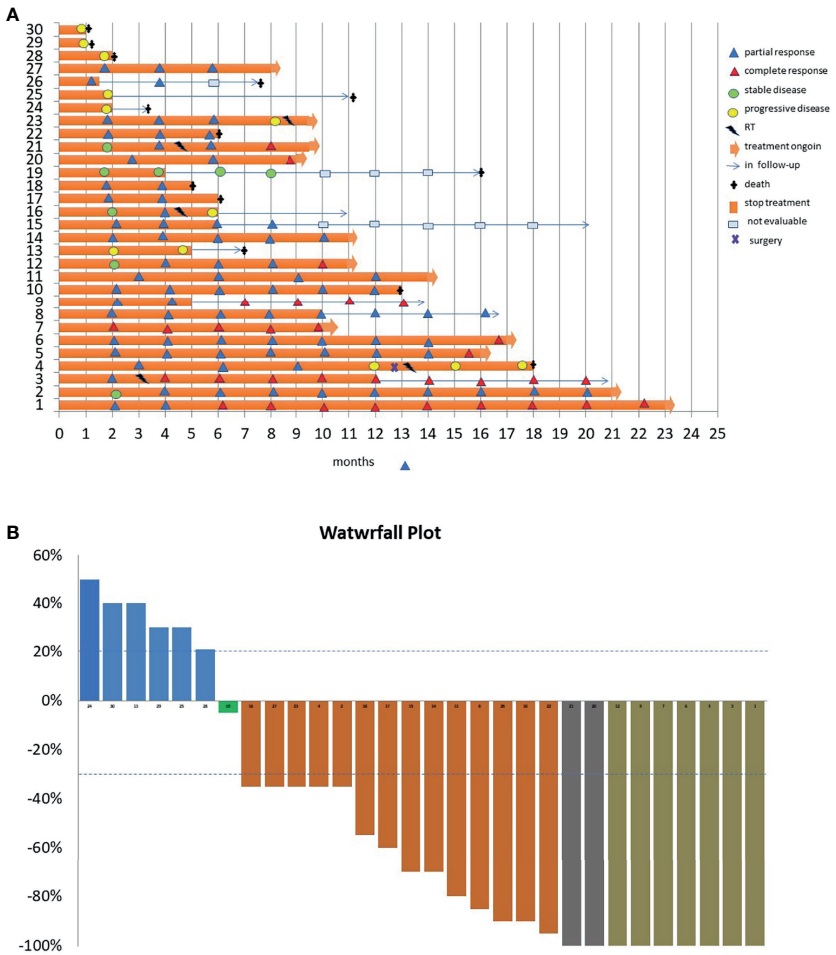

FIGURE 2 | **(A)** Swimming plot showing the time and duration of response (30 patients). Each horizontal line represents one patient. **(B)** Waterfall plot representing the rate of change in target cutaneous squamous cell carcinoma lesions from baseline during the cemiplimab course.

(BMI ≥25 kg/m^2) *vs.* non-overweight (BMI <25 kg/m^2) patients (80 *vs.* 75%) (**Figure 3**).

Among all patients, the median PFS was 16 months (1–23), and the median OS was 18 (1–23) at the data cutoff date of July 2021. With regard to PFS, 13 events were observed (including nine patients with progressive disease and four deaths). Regarding OS, 13 deaths were reported from enrollment to the data cutoff, providing a 57.6% 10-month OS (**Figure 4**).

Therapeutic Outcomes in Immunosuppressed Patients

Five patients (16.7%) had an immunosuppressive condition: three stable hematologic malignancies including two chronic lymphocytic leukemia and one idiopathic myelofibrosis previously treated with anti-JAK therapy for about 6 years, while two patients were on immunosuppressive therapy for renal organ transplantation and Crohn's disease, respectively. Among these five patients, we observed a RECIST response in four patients (80%), including one complete response in the patient with idiopathic myelofibrosis and two partial responses with a tumor shrinkage greater than 80% in the patients with solid organ transplantation and Crohn's disease. In these patients, the treatment with cemiplimab is ongoing, and the duration of response ranged from 8 to 22 months. Of the two patients with lymphoproliferative disease (B-cell lymphoma and chronic lymphocytic leukemia), one presented a rapid progression and the other progressed after a transient partial response lasting 6 months. Interestingly, no immune-related toxicity was reported in immunosuppressed patients; in particular, no worsening of pre-existing Crohn's disease as well as no evidence of graft rejection was observed in a kidney transplant patient who, until July 2021, received 20 cycles of cemiplimab achieving a near-complete response (**Figures 1C, D**) and continued immunosuppression with a combination of tacrolimus, sirolimus, and a low dose of steroids.

Hematological Parameters

The hemoglobin level was analyzed in all patients and correlated with clinical outcomes. The other hematological parameters were

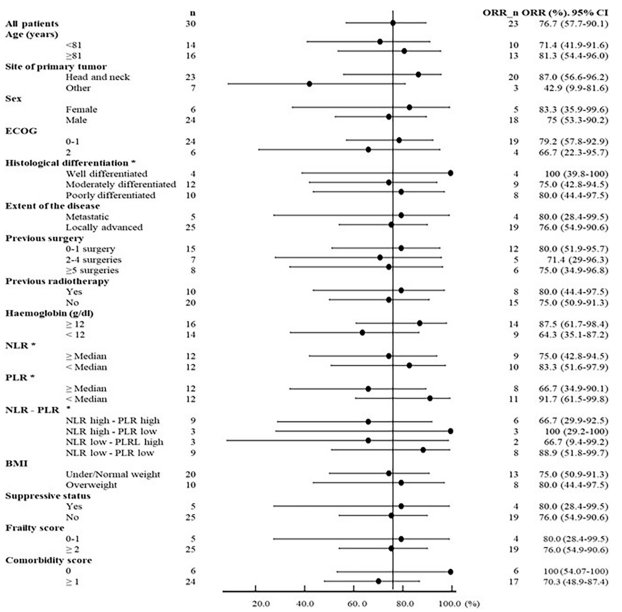

FIGURE 3 | Forest plot of response in selected subgroups of patients according to the main clinical and hematological characteristics. For hematologic parameters, we considered pre-therapy values: only 24 patients were considered eligible (three patients were excluded for concomitant hematological tumors, one for thalassemia major, and two patients for concomitant immunosuppressive therapy).

collected in only 24 patients (three patients were excluded for concomitant hematological tumors, one for thalassemia major, and two patients for concomitant immunosuppressive therapy).

We found a better response in patients with hemoglobin >12 g/dl (87.5%, 95% CI: 61.7–98.4, *vs.* 64.3% for hemoglobin <12 g/dl). However, when we considered this binary characterization, the association with the response to therapy was not significant (*p*-value of chi-square test equal to 0.134). On the contrary, we found a significant association when we considered the hemoglobin values measured on an interval scale (*p*-value of Wilcoxon–Mann–Whitney test equal to 0.042) (**Figure 5**).

Despite limitations related to the small number of patients, the combined scores of basal NLR and PLR after dichotomization of low PLR correlated to better ORR either in association with high NLR (100% correlation with 95% CI: 29.2–100) and, to a lesser extent, with low NLR (88.9% correlation with 95% CI: 51.8–99.7). Weaker correlations were observed for patients with high basal PLR in association either with low NLR (66.7% association with 95% CI: 9.4–99.2) or with high NLR (66.7% association with 95% CI: 29.9–92.5) (**Figure 3**).

We also evaluated these blood parameters before therapy and their changes over time. Due to the small number of patients evaluated, the results were not suitable for a statistical test and are reported only in a descriptive manner. The trends of the main parameters considered are summarized in **Figure 6**. Notably, the neutrophils and NLR progressively increased in non-responders compared to responders. Furthermore, the lymphocytes increased slowly during the course of therapy in the responders, while they decreased in the non-responders. The monocytes, already much higher at baseline in non-responders after an initial modest decrease, rapidly increased after 2 months of therapy. Finally, the platelets, already much higher in non-responders at baseline, decreased in both responders and non-responders during cemiplimab therapy. This behavior reflected that of the PLR.

Safety

Regarding the toxicity profile, the treatment was generally well tolerated by the majority of patients. The most common adverse events included skin toxicity in 10 patients (33.3%), with grade 2 pruritus in six patients, rash in three patients, grade 3 bullous erythema in one patient, and fatigue in seven patients (23.3%). Only three (10%) patients experienced severe grade 3/4 toxicity. A single

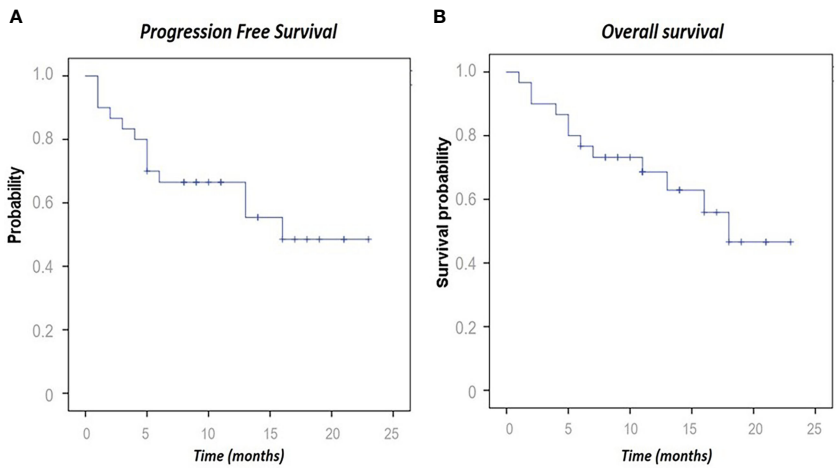

FIGURE 4 | Kaplan–Meier curves for progression-free survival **(A)** and overall survival **(B)**.

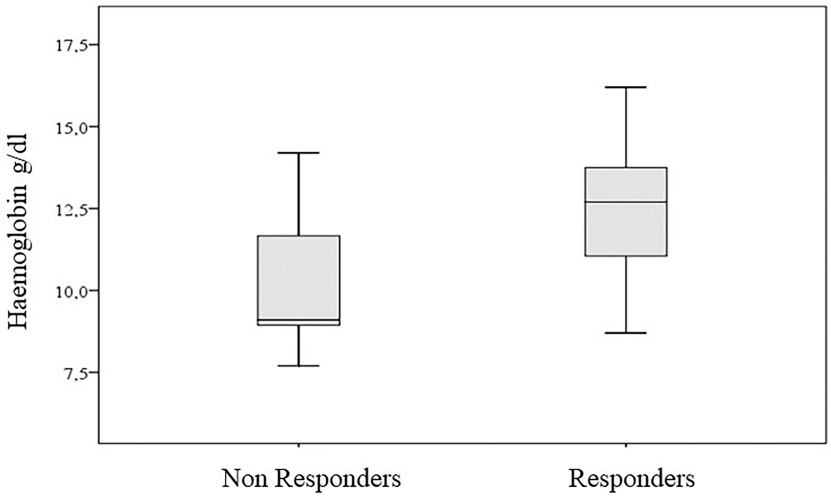

FIGURE 5 | Hemoglobin values according to clinical response.

grade 4 toxicity was observed after the completion of two cycles of treatment in a non-responsive patient with acute respiratory failure due to pneumonitis that required hospitalization and led to death. The treatment-related adverse events are summarized in **Table 3**.

Two patients discontinued the treatment for toxicity despite a response: one with complete response for grade 3 bullous erythema occurring after seven courses of therapy and one with partial response for grade 3–4 asthenia.

Three more patients discontinued treatment due to reasons other than side effects: one in complete response for own personal choice, one in partial response for pre-existing mental conditions compromising her compliance, and the last one in stable disease for rapid worsening of his ECOG (**Figure 2**). All these patients continue to have a response despite the end of the treatment. No additional toxicity on the irradiated lesions was reported in the three patients who underwent concomitant radiotherapy: these patients achieved a response, one of which was complete (**Figure 1**). Beyond eight deaths related to the progression of CSCC, five patients died due to unrelated cancer causes (one death for COVID-19 infection, one for myocardial ischemic attack, one for dementia complications, one for septic complication, and one for cirrhotic decompensation).

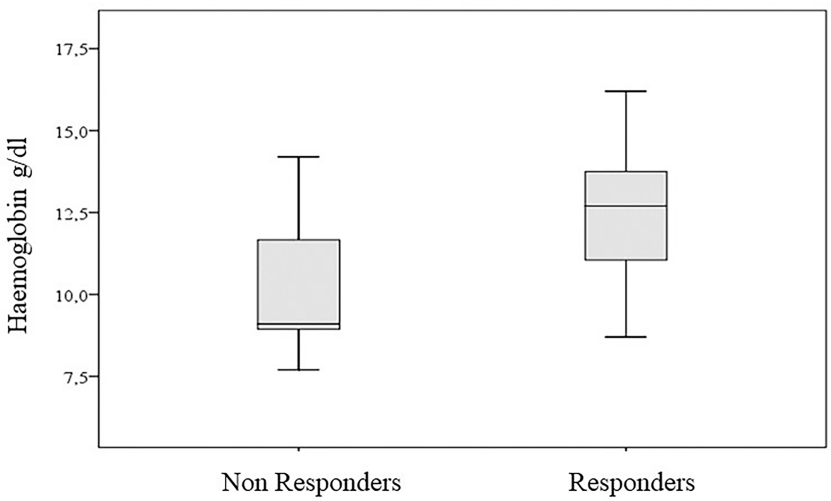

FIGURE 6 | Trends of the main blood parameters according to clinical response. Twenty-four patients were considered eligible (three patients were excluded for concomitant hematological tumors, one for thalassemia major, and two for concomitant immunosuppressive therapy). For non-responders, data from the 6-month sampling are not available.

TABLE 3 | Treatment-related adverse events (AEs).

Adverse event	Grades 1 and 2	Grade 3	Grade 4
Fatigue	6	1	0
Skin toxicity[a]	9	1	0
Respiratory failure	0	0	1
Interruption with definitive discontinuation due to AEs	0	2	0

Grades are defined as per the Common Terminology Criteria for Adverse Events, version 5.0.
AE, adverse events.
[a]*Pruritus in six patients, skin rash in three patients, and G3 bullous erythema in one patient.*

DISCUSSION

Advanced CSCC not amenable to curative surgery and radiotherapy is a severe condition almost always involving elderly and frail patients. In advanced stages, this skin cancer is a disfiguring, painful, and functionally limiting condition that requires a multidisciplinary management to ensure clinically substantial outcomes and preserve the quality of life.

Cemiplimab represented a paradigm shift in these settings, leading to remarkable 44 and 45% response rates associated to durable efficacy in 78 and 115 patients with laCSCC and mCSCC, respectively, according to the results of the phase 2 EMPOWER trials (17, 33).

However, after the approval of this PD-1 blocking agent, several clinical needs are still to be addressed. In particular, these randomized controlled trials underrepresented real-life patients with poor performance status and relevant comorbidities as pathologic or iatrogenic immunosuppressive conditions or organ function deterioration. All these conditions are frequently encountered for CSCC, often involving the elderly population, and delineate a clinical state of frailty characterized by decline across multiple physiological systems that places cancer patients at an increased risk of poor outcomes (18, 19).

In our observational study, we reported a population with a median age of 81 years, which is higher than that reported in controlled clinical trials (71 and 74 years in mCSCC and laCSCC, respectively) and in other real-world experiences (20–24), and a prevalence of frailty of 83%, which is greater than that reported in a metanalysis in generalized (18) and specific oncologic settings (19). Although there is no standard instrument to identify frailty, we set up a frail index based on a simple scoring system that accounts the main tool to assess vulnerability such as age, performance status, and comorbidity. This latest feature was a relevant trait of our population whose median CCI was 2 and accounted five patients with an immunosuppressed status. We observed an unexpected overall response rate of 76% with complete responses of 30%. Despite the poor profile of our patients, these results are better than those reported in controlled trials and initial real-world series showing 31 to 58% overall responses, respectively (14, 20–24). Our better results could likely be due to the prevalence of locally advanced over metastatic stage in our patient population and to the use of cemiplimab as first-line therapy in the majority of patients, which is notoriously associated to better response (34). What is worthy of consideration is that, compared to controlled clinical trials (17, 30) and other real-world series (20–24), our CSCC cohort has been less pretreated even with radiotherapy and surgery. Furthermore, accordingly with other authors, we observed a higher response rate associated with fewer surgical procedures (17) and for CSCC arising in the head and neck area (20–23). This last finding could reflect the influence of a higher degree of sun exposure on the mutational burden notoriously associated with a better response to immuno-checkpoint inhibitors (21, 22). Moreover, we were able to correlate a better response rate with the well-differentiated histological type.

Even with the limitations of the sample size, in the forest plot analysis, we did not find a correlation with response for other clinical features previously described as predictive markers, like male sex (35) and body mass index (36). We likewise found no differences in response between over or under the median age of 81 years. Our data, according with those from similar experiences in melanoma (37), clearly disproves the mistake that age-related impairment of the immune system hampers the effectiveness of PD-1 blockade. This evidence could explain the efficacy of cemiplimab also in the frail subgroup and add data to a poorly investigated issue on which trials are being planned (32). Of note is that we found an equivalent rate of response also in the subgroup of immunosuppressed patients. Other authors also reported responses in CSCC patients who have undergone kidney transplantation or with leukemia (14, 23, 24) as well as in patients treated with immunosuppressive drugs for an autoimmune disease (23, 24). In immunosuppressed patients, the likelihood of a response to PD-1 blockade has been demonstrated also in other cancers (38–40).

Interestingly, simple peripheral blood parameters appeared to be associated both to predicting and assessing response to cemiplimab early. Overall, we found a statistically significant association between pre-treatment hemoglobin levels and response using a threshold of 12 g/dl. As known, hemoglobin levels have a prognostic role in cancers (41) and are predictive for response to various anti-cancer therapies, especially when combined with albumin, lymphocyte, and platelet levels (42). It has been also reported that, regardless of its causes, hemoglobin levels could influence the activation status of T cells against cancer (43, 44). Other authors also reported an association between higher hemoglobin levels and better clinical outcomes both in CSCC and lung cancer patients treated with PD-1 inhibitor (24, 45).

Beyond hemoglobin, we focused on white blood cells whose role as an inflammatory index, influencing response to checkpoint, was established (25). In the baseline evaluation, the combinations of low N/L ratio and low PLT/L ratio appeared as predictors of response, also according with other authors who reported an association between pre-treatment absolute lymphocyte count and response (22). In the longitudinal

analysis, we found that the trend of lymphocytes, neutrophils, and monocytes appeared opposite in responder and non-responder patients. If confirmed in a larger population, these data could be relevant to monitor the treatment efficacy early and deserve to be investigated prospectively.

Regarding survival, even if treatments and follow-up are still ongoing in 17 patients, our study showed a trend in PFS and OS comparable to those of previous cemiplimab trials with long duration of response. The proportion of patients who had no disease progression at a median follow-up of 10 months was 57.6%.

The treatment was well tolerated by the majority of patients showing an overlapping toxicity profile with regards of clinical trials. The most common adverse events included skin toxicity and fatigue, with only three patients experiencing severe (grade 3/4) toxicity. Interestingly, three patients who achieved a response and with interrupted treatment due to toxicity or personal choice maintained the response. Moreover, in three patients treated with concomitant radiotherapy, we documented no additional toxicity. This combined therapeutic strategy deserves further investigations due to its interesting biological rational of a synergic action between radiations and immunotherapy with checkpoint inhibitors, as already demonstrated in different types of cancer (46–48).

CONCLUSION

In spite of the observational nature of our study and the limited number of patients enrolled, our experience adds evidence on the high antitumor activity of cemiplimab and its safe profile in a broad spectrum of non-selected patients. Moreover, our data offer the possibility of bridging the knowledge gap about cemiplimab performance in a very elderly and frail population.

Nevertheless, some open questions remain to be answered: the weight of the cost/benefit profile of the treatment in peculiar patients such as immunosuppressed patients, patients who have had a transplant, and patients with deteriorated performance status; the identification of biomarkers that could predict efficacy or early assess response to the treatment; the possibility to interrupted treatment at the achievement of a response; the potential combination with local therapy; and the long-term tolerability of the treatment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study was previously approved by the ethics committee of the IRCCS Istituto Tumouri Giovanni Paolo II, Bari, Italy (Prot. 590/16 C.E.), and written informed consent was obtained from all the patients enrolled in the study.

AUTHOR CONTRIBUTIONS

MG and SS conceptualized the study. IR, LF, DQ, AA, RF, EM, ER, FM, MT, FL, AN, SF, FMa, FF, AS, AAl, ST, and SS participated in data collection. AF and RM contributed to the methodology. SS, AF, DQ, AN, AA, FF, RF, LF, FM, MT, FL, EM, ER, SF, FMa, ST, AS, IR, AAl, RM, and MG participated in analysis, writing, and editing. MG supervised the study. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.686308/full#supplementary-material

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REVIEW
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Current Surgical Therapy of Locally Advanced cSCC: From Patient Selection to Microsurgical Tissue Transplant. Review

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Among the non-melanoma skin cancers (NMSC) the squamous cell carcinoma (SCC) is one of the most challenging for the surgeon. Local aggressiveness and a tendency to metastasize to regional lymph nodes characterize the biologic behavior. The variants locally advanced and metastatic require wide excision and node dissection. Such procedures can be extremely detrimental for patients. The limit of the surgery can be safely pushed forward with a multidisciplinary approach. The concept of skin oncoplastic surgery, the ablative procedures and the reconstructive options (skin graft, pedicled flap, microsurgical free flap) are discussed together with a literature review.

Keywords: locally advanced disease, microsurgery, non-melanoma skin cancer, oncoplastic, reconstructive surgery, SCC, skin oncoplastic surgery, squamous cell carcinoma

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INTRODUCTION

Definition of Locally Advanced Cutaneous SCC

In Caucasians, skin squamous cell carcinoma (cSCC) is the second most common type of skin cancer, accounting for approximately 20% of all non-melanoma (NMHC) skin cancers (1).

The definition of "locally advanced" cSCC (lacSCC) is ambiguous, it includes tumors that are not more amenable to surgery or radiotherapy, or those who require a multidisciplinary approach because of their size or clinical implications (2).

While the former cannot be successfully treated with surgery, the latter may have the last chance of cure through an aggressive surgical procedure.

Several parameters have been associated with higher risk of CCS development and subsequently worse prognosis.

Histological features include perineural invasion, poorly differentiated grade, acantholytic subtype, spindle or desmoplastic, and vertical tumor thickness > 2mm (3).

Instead, the clinical parameters are the location (ear, median face), diameter > 2 cm and the recently positive re-excision margin has been shown to be an independent risk factor.

Regarding of tumor size and thickness, respectively defined as the maximum diameter of the SCC and the maximum vertical distance between the tumor outer surface and the deeper cell nest, both of them are clearly related to increased risk of local recurrence and distant metastasis (4).

In contrast, primary tumor operability and tumor thickness of < 6mm were correlated with improved overall survival (5).

However, the two parameters mentioned above do not appear to accurately describe the salient features of a locally advanced SCC.

A large, thick and almost entirely exophytic SCC may have a remarkable size, but does not represent an insurmountable challenge for a dermatosurgeon qualified in plastic reconstruction techniques.

Conversely, a medium size tumor with an increased in-depth invasion, that spreads well beyond the subcutaneous fat layer, can require extremely aggressive resection with the sacrifice of functional structures like vessels, nerves and bone, thus causing disfiguring outcome and functional impairment.

So the Breslow measurement, expressing the mere cancer thickness, does not perfectly match with the anatomical tumor depth, so may not represent a valid parameter for defining an SCC "locally advanced" (6).

Limited to oral SCC, some studies correlated the tumor depth even with the risk of regional lymph node metastasis (7), advocating the necessity of elective regional dissection for tumors > 5 mm in thickness.

This argument solidifies anatomical depth as a predictive factor, on the basis of which a skin SCC might also be considered "advanced".

Therefore, in relation to the eligibility to surgery of an SCC, the concept of radial extension in the 2-dimensions plane must be replaced with the concept of a 3D space, including the anatomical depth in the evaluation of the real tumor magnitude.

Therefore, the SCC guidelines should include the assessment of growth in depth as well as the diameter of the tumor during the preoperative assessment.

Cutaneous squamous cell carcinoma represents the most common type of a rare female malignancy, the vulvar cancer (8).

Vulvar cancer is often associated with human papillomavirus (HPV) infection and usually affects young women, although HPV-independent SCC most likely affects older women.

The classification of vulvar cancer was revised by the International Federation of Gynecology and Obstetrics (FIGO) in 2014 (9).

The FIGO committee stated that the stromal invasion (defined as the measurement of the tumor from the epithelial–stromal junction of the adjacent most superficial dermal papilla to the deepest point of invasion) and the extension to adjacent anatomical structures play a role in pushing the stage in a higher level with worse prognosis.

So, an advanced vulvar cSCC could be defined by the size (> 2 cm), or if it extents to almost one among of the following urethra, anus and vagina. Recurring vulvar FCS can also be considered "advanced" if it poses a serious local management problem (10).

Squamous cell carcinomas may also occur on the surface of the male genitals.

In the AJCC Staging, 8th ed. 2018 (11), T1 corresponds to a tumor limited to the most superficial layers according to the anatomy of the region (gland, foreskin or shaft).

The perineural invasion contributes to T1 separation in a and b, and the vertical growth to deeper layers like corpus spongiosum and corpus cavernosum pushes the stage forward (from T1 to T2 and from T2 to T3, respectively), accordingly to an increased risk of metastasis and worse prognosis.

Thus, a penile cSCC, that extends deeper than the cutaneous envelope border, can be defined advanced despite the radial size.

Definition of Metastatic cSCC to Regional Lymph Nodes

In presence of regional nodal metastasis the cutaneous SCC is defined metastatic (mcSCC) (1), but the absence of distant metastasis still permits the operability in selected cases.

The association of any T with regional nodal involvement may be stage III or IVA according to AJCC 8th Ed. 2018 (12).

There is still debate about the appropriate role of surgery in the treatment of a regional metastatic cSCC. In a retrospective study Ch'ng et al. (13) report the grade of differentiation as the only primary tumor factor significantly associated with disease-specific survival.

Other parameters such as clear resection margins, tumor size and thickness, do not seem to have any real impact on the specific survival of the disease in the metastatic population.

Therefore, in mcSCC, surgery may be useful in controlling local disease rather than affecting overall survival, and lymph node exploration is an intrinsic component of the procedure.

PATIENT SELECTION

The Multidisciplinary Tumor Board Discussion

The initial presentation of a large (>2 cm) cSCC fixed to a deep plane invariably requires the surgeon to determine whether the tumor is operable or not.

That issue should not be addressed only by a dermatosurgeon, but would need the support of other specialists, due to anatomical structures to be resected and/or a complex reconstruction to be accomplished.

Another problem may be the recurring cSCC, which, after previous surgery with R1 o R2 margins, would still be considered recutable by a more extensive excision.

The multidisciplinary tumor board has proven to be effective in better cancer staging, and tumor management can differ in about 10% of cases, compared to what a single specialist would do (14).

However, the concrete impact of the multi-disciplinary approach on outcomes such as improved quality of life (QOL) or overall survival or disease-free survival has not yet been proven.

Undoubtedly the benefit of such preoperative evaluation is the possibility of management of the most complex clinical scenario (15), when the patient overall evaluation is required regarding surgery feasibility and the use of multimodal treatments.

Risk and Performance Assessment

A detailed clinical history review and a comprehensive physical assessment of the patient are mandatory prior to any difficult surgery.

Comorbidities, previous treatments, age and disability can have a significant impact on the final outcome of a surgical procedure in cancer patients.

The specialist has to keep in mind the potential side effects and complication due to these factors throughout the perioperative time, and recognize whether symptoms or organ dysfunction are imputable to cancer treatment or some other cause (16).

The most widely used perioperative score is the Eastern Cooperative Oncology Group/World Health Organization Performance Status (ECOG/WHO PS), that is employed both for short/medium term overall survival (17), and as a prognostic factor predicting extended length of stay after cancer surgery (18).

However, the ASA score appears to be a higher performing score with respect to 90-day postoperative survival (17).

Recently, some criticisms about ECOG have raised, pointing out that the one-dimensional nature of the tool and the assessment by the physician causing intrinsic subjectivity, make the score inadequate for oncologic tailored treatments (19).

Moreover, accurate discrimination between patients before and during wound healing appears to have a considerable impact on QOL and global outcomes (20).

In terms of functional impairment before surgery, the Barthel score is generally recorded at hospital admission.

A pre-existing functional disability at the time of diagnosis seems to have a significantly lower survival rate and indicates a need for interventions to improve prognosis (21).

The transition to the recording of the dimensions of fragility, multimorbidity and functional status was therefore recommended as part of standard clinical practice.

These results provide a valuable insight into global cancer treatment and encourage health professionals to plan for the early launch of rehabilitation programs to improve functional status.

The Imaging

Indications for radiology imaging of lacSCC are the need to detect invasion of adjacent/deep anatomical structures and the presence of regional/distant nodal involvement.

Computed tomography (CT) is the cornerstone of assessing the soft tissue extent of the tumor, bone invasion, and nodal metastases.

Pros of CT scans are the high definition of cortical bone surface, if bony invasion, and the detection of abnormal lymph nodes (not smaller than 1.0 cm in size), that can be precisely localized and identified as metastatic (22, 23).

The drawbacks are the need for iodinated contrast for better definition, which can cause or increase kidney failure in at-risk individuals.

Moreover, CT is less sensitive than magnetic resonance (MR) for intracranial diseases, perineural tumor spread, and soft tissue imaging such as muscle fascia or fat.

In selected scenarios, like temporal or orbital invasion, is often useful a combined preop study with CT and MR for optimal planning, due to the presence of different in density tissues and layers (22).

MR scans also allow fine assessment of the extent of tumor invasion in soft tissue (22, 23), while clear guidelines lack for radiologic imaging of patients with presumed perineural spread, it is generally agreed that high-resolution MR is the most sensitive imaging modality available (24).

The disadvantages of MR are incompatibility with implanted ferromagnetic devices and the need to stand still during the examination to avoid motion artifacts. This can prevent the acquisition of patients unable to remain immobile for essential tremors or Parkinson's disease, which is not uncommon in elderly patients.

Staging of lymph nodes can be performed in different modalities, undoubtedly ultrasound (US) is the least expensive, painless and non-invasive. US does not require immobilization of the patient, and has no risk of adverse reaction to contrast agents (23).

When suspicious lymph nodes are identified, a fine needle aspiration biopsy (FNAB) with US guidance can be used for sampling, given its higher sensitivity and specificity than conventional FNAB (23).

High-frequency US has been used for assessment of the size and extent of primary non-melanoma skin cancers, including depth invasion of the primary tumor (25, 26), but the need of special instrument and dedicated training precludes the systematic application.

The main disadvantage of the US is its intrinsic dependence on the operator, which can greatly influence the sensitivity and accuracy of the exam (23).

In occult metastasis detection the positron emission tomography (PET) plays a main role, the combination with CT is more sensitive in detecting nodal and distant tumor metastases than each modality separately (23).

Fields of application of PET-CT are detection of distant visceral metastases and occult adenopathy, it is successfully used in monitoring of tumor response to therapy, and surveillance of tumor recurrence.

In the latter scenario, this type of imaging is especially useful as it is able to detect local metabolically active relapse in areas with surgically modified anatomy (22).

The major drawback of PET CT is the false positives identified in areas of infection or acute/chronic inflammation that are not related to the neoplastic process. In addition, given the high metabolic demand of the brain, PET CT is not useful in assessing brain metastases, often requiring a separate MRI scan.

Timing of Surgery

Radiotherapy is an effective nonsurgical therapy available to patients with NMSC.

Cutaneous SCC is radiosensitive and most small cSCC treated with definitive RT exhibit complete remission and extremely low local recurrence (<5%).

Usually, younger patients are given hypofractional radiotherapy for consecutive days over a 4-5 week period to obtain the best long-term outcome.

In older patients instead, the preferred fraction size is higher in order to reduce the overall time of treatment within 2-3 weeks,

because of poor performance status that often contraindicates extended daily treatment (27).

Late cutaneous side effects following hypofractioned RT have been documented and potential skin necrosis should not be ruled out; therefore, a fractioned regimen is optimal to reduce this disadvantage.

In the presence of a locally advanced cSCC, deemed resectable, usually a wide excision to obtain R0 margins and subsequent reconstruction is preferable according to high risk of metastasis or debilitating disease progression within 3 months (28).

Following surgery, adjuvant radiation therapy should be avoided unless there is an extended N1 or N2 disease, or if there are "close" or R1 resection margins.

The combination of surgery and radiotherapy can be extremely effective in treatment of lacSCC developed in high risk areas for perineural invasion like ear, orbit and mid-face location (29, 30).

In a different scenario, the lacSCC can be considered unresectable in first instance, so definitive radiotherapy with curative or palliative intent may be administered.

An incomplete response to radiotherapy, or a tumor enlargement, may pose an indication to a salvage surgical procedure after irradiation.

The decision to implement multimodality treatment (postoperative radiotherapy) or salvage treatment (surgery after irradiation) is based on a careful multidisciplinary evaluation (31).

Aside from the well-established benefits in cancer treatment, it has been shown that preoperative radiotherapy increases the risk of postoperative complications (27).

Early radiation lesions consist of an acute inflammatory response and tissue vessel friability that can significantly affect the success of immediate surgery.

Conversely, the fibrosis process induced after RT can increase over time, negatively impacting the success rate of delayed reconstruction (32).

Previous irradiation may cause serious wound-healing problems, and immediate reconstructive procedure after tumor resection may be compromised as well by subsequent adjuvant radiotherapy.

For high rates of reconstruction failure when performed on an irradiated bed, post-operative radiotherapy has been suggested whenever possible (33).

When clinical circumstances require RT prior to surgery, the procedure appears more likely to be successful if carried out within 6 weeks, later the complication rate increases (33).

Another retrospective study on 217 free grafts in 199 patients compares the RT effects on tissues before and after surgery (34).

The conclusion is that the vascularization of the grafted bed decreases continuously according to the total dose and time after radiation treatment.

A time interval of 4 to 6 weeks following RT prior to surgery is then indicated to be preferable.

In a review of 2009 (35), about the effects of RT on microsurgical head and neck reconstruction, several confounding factors have been highlighted like dose of radiation, type of radiation, intensity modulated radiotherapy (IMRT) and variations in fractionation.

It has been hypothesized that all these variables may affect the outcome of reconstructive surgery, in addition to having an impact on oncology therapy.

Controversial studies have been conducted on the incidence of wound complications following concomitant chemo therapy (35).

As with radiation therapy, the timing of chemotherapy is a factor in the onset of complications.

Chemotherapy in the 2 weeks prior to or 1 week following surgery appears to cause more healing complications (36).

Although the effects of chemotherapy are transient, when added to radiation treatment, they tend to have a more severe impact on wound healing.

In a retrospective analysis (37) of 131 patients affected by advanced SCC of head and neck, 38 (29%) underwent 50 surgical procedures after chemoradiotherapy.

Complications were observed in 4 (11%) of the 38 patients and 5 (10%) of the 50 procedures.

Overall, the rates of major and minor complications across all interventions were 6% and 10% respectively.

Furuta et al. (38) instead reported major complications occurring in 8/34 (23.5%) of the group that received chemoradioteraphy before surgery, and 5 of the 8 (62.5%) required additional reconstruction surgery.

Recently Suzuki et al. (39) investigated the different rate of complications, surgical site infection (SSI), and survival in salvage surgery for patients treated by platinum-based chemoradiotherapy (Plat-CRT) or cetuximab-based bioradiotherapy (Cet-BRT).

They demonstrated that patients with Cet-BRT were significantly more associated with the presence of SSI ($P < 0.01$) and grades IIIb–V in the Clavien–Dindo classification ($P < 0.01$) used for rating the adverse event gravity.

Moreover, the results demonstrate the significant association between patients with Cet-BRT and older age in good agreement with results previously published by other authors.

All the studies mentioned above are characterised by limitations such as the study design and a small number of subjects.

Despite lack of robust statistical results and although the complications rate increase, there's agreement to provide anyway a surgical salvage operation to this group of patients, as a last chance, in presence of local recurrence after chemoradiotherapy protocols.

TUMOR RESECTION

The Limits of the Ablative Surgery

A lacSCC is a high risk tumor, so the trend is to widen the excision margins respect the low risk ones to decrease local recurrence rate.

It is also important to keep in mind that the metastatic potential of a primary cSCC is independent of the local treatment approach (40).

Recently, the European consensus group (41) suggested a range of 6-10 mm safety margins for cSCC with high risk factors, but pointed out how a specific recommendation on the clinical safety margins cannot be given, because of the lack of consistent reports supporting its independent prognostic value.

The margins width may vary in relation to tumor and patient characteristics, but the opportunity to reduce the extent of resection for aesthetic and functional issues is not clearly mentioned, unlike the specific deviations for special anatomic locations provided for primary site melanoma surgical therapy (42).

This can be explained by the more aggressive biological behaviour of cSCC at the primary site compared to melanoma and the consequent higher risk of recurrence.

In this respect, the current literature is inconsistent, given the lack of randomized trials, and it is not possible to provide conclusive results as regards the superiority of a determined surgical approach to the primary tumor (40).

Physical examination of the lesion with manual palpation and stretching with its surrounding area provides a quick assessment of the extent of involvement.

In spite of this, the actual extent of the lesion may still be vastly underestimated (43).

A not invasive preoperative method to plan more appropriate resection of soft tissue margins is the high frequency ultrasonography, that allows measurement of the 3-dimensional size of tumor with a relevant grade of precision (44).

The findings so far seem encouraging, but some limitations sound evident.

A primary, well defined, small in dimension tumor is objectively easy to examine with US, but in the presence of a large local recurrence surrounded by scarred tissue, that invades the deeper planes modifying the anatomical structures, the accuracy of such measurement appears less reliable.

When bone invasion is suspected, a preliminary study with computed tomography is the best support to calculate the entity of bone resection.

Often lacSCC requires detailed evaluation both the bone and the soft tissues, so the combination of CT scan and MR offers a wide spectrum of information that may allow a precise planning of resection.

This approach is extremely important in head and neck surgery, where imaging is not just used as a pre-operative assessment, but guides the operator throughout the procedure.

The impact of predetermination of excision margins on oncologic outcome has been carefully reported by Pu et al. (45), that compared the preoperative measurement of resection with pathology findings in computer assisted head and neck surgery.

As a rule, they adopted a distance of 15 mm from the bone invasion limit and a distance of 10 mm from the soft tissue involvement.

According to the NCCN Guidelines, surgical margins were classified as 'clear' (≥5 mm), 'close' (< 5 mm) and 'positive' (carcinoma *in situ* or invasive carcinoma at the margin of resection), in relation to the closest distance of resection margin extrapolated from the pathology reports.

More than 80% of the resection margins were clear of invasive tumors and all the bone margins were negative, so they concluded that predetermined surgical margins do not compromise oncological safety.

Main limitations were the small number of cases, the impossibility to determinate the "close" bony margin, due to the necessity of decalcification of the specimen, and the retrospective study design.

Intra-Operative Margins Assessment

Clinical circumstances and tumor characteristics can prevent fine preoperative planning, and even the most careful imaging has some limitations as well.

The intra-operative margin assessment may be an option to avoid these disadvantages, but each tissue requires a dedicated methodology.

In a remarkable review Rosenthal et al. (46) presented the available optical imaging strategies for intraoperative soft tissue margins assessment.

Optical imaging uses light emitted from a light source (xenon or laser) to magnify the unique properties of tissues with or without optically labelled targeting agents administered.

It allows for real-time feedback providing cancer-specific detection as opposed to peripheral tissue alterations associated with solid tumors.

However, use of these video-assisted surgical techniques necessitates of low ambient light environment and limits the surgeon's tactile feedback and 3-dimensional tumor visualization, critical in guiding oncology resections in open surgery.

In conventional surgery, a useful method for intra-operative assessment of soft tissue margins is the frozen section.

The surgeon performs the specimen collection, that is immediately processed by the pathologist through marking, freezing and cutting several sections of the specimen at variable distance (1 to 4 mm), then receives a feedback (47).

The question is how reliable is frozen section analysis (intraoperative) respect the standard protocol for formalin-fixed paraffin embedded tissue (postoperative).

A confounding factor is the specific frozen section processing, that can be the so-called "bread-loafing", thicker slices cut sequentially from the frozen specimen, or the complete circumferential and peripheral and deep margin assessment (CCPDMA), a more time-consuming procedure but with very thinner slices, and so more accurate (48).

Other limits are the sampling or interpretation errors of the specimen.

Due to that, the reports in literature are controversial finding a varying concordance of frozen section and definitive paraffin embedded examination ranging from 80% to 91% (49, 50), thus some have abandoned its use (51).

Factors that may contribute to increase the false negative rate are poorly differentiated subtype, lymphovascular invasion, and perineural invasion (50), frequent histology features in lacSCC.

Instead, there is currently no feasible practice for intraoperative bone margins assessment, due to time required for decalcification of the specimen.

Limitations to this approach lie in the necessity of concrete amount of both cortical and cancellous bone to be examined, the use of tools to obviate the irregularity and hardness of the bony slice, and the contamination by blood cells and bone dust.

The majority of reported results are satisfactory, but technical limitations precluded them from routine clinical application.

In a study of 2014 Nieberler et al. analyzed the intraoperative cytological assessment of the bone resection margins (ICAB) in patients with oral SCC, they attested the technique as reliable and suitable for routine clinical use (52).

In relation to the resection margin status defined by final histology, ICAB provided 80% sensitivity (95% CI, 28-99) and 97.5% specificity (95% CI, 86-99) with 95.5% accuracy.

The results are promising, but a dedicated technical device for brushing the bones and the correct timing of the operating room and pathology process are key to performing the intraoperative procedure.

The Anatomical Structures to be Saved

With the oncology goal of radical tumor resection, surgery planning must take into account the anatomical structures to be preserved for functional and aesthetic problems.

Randomly planned excision may be effective in the treatment of cancer, but may be detrimental to the patient's self-esteem, resulting in complaints and frustration.

The wide range of pre-operative exams allows in most cases, even the most complex ones, a realistic anticipation of which tissues should be replaced, repaired or saved.

Often the most challenging areas where lacSCC can develop are face and head region, hand and genitalia (53), so under these circumstances a precise reconstructive plan goes with the oncology procedure.

As mentioned before in this article, in literature there're not yet a clear indication when it is safe and recommendable to deviate from widen the resection margins in order to preserve as much as possible a very significant part of the body, and a frank and open discussion with the patient on pros and cons is mandatory.

A number of accounts concern about technical solutions to obviate to the impasse (54–56), but they are mainly case or retrospective reports, so it is impossible to draw any robust conclusions.

In a retrospective study on 179 male patients Prodromos et al. (57) found that a limited radical SCC excision with clear margins less than 5 mm did not appear to affect primary oncological control in a high-demanding area like the penile surface.

Local recurrence did not seem to have a negative impact on overall survival, while it was associated with lymphovascular invasion and higher tumor stage and grade.

En Bloc Resection Versus Micrographically Controlled Surgery: An Open Question

Two different approaches in the eradication of a locally advanced SCC are viable: an en bloc resection, elsewhere named wide large excision (WLE) or standard excision (SE), or a microscopically controlled surgery (MCS), usually referred as Mohs surgery (MMS).

As mentioned above, these two approaches differ not only in the technique of tumor excision, but also in the histological processing.

The first procedure is followed by a delayed specimen examination, usually prepared through the "bread-loafing" technique; the second requires an immediate analysis of multiple frozen slices (another variant, called 3D histology, introduces the paraffin embedded slice fixation).

As a result, the planning chosen by the surgeon affects the methodology adopted by the pathologist.

In consideration of the topic, the locally advanced cSCC, the practice may probably regard a large, thick and invasive tumor or/and relapsing, more than a primary, small and well define one; so the risks of not-free margins and local recurrence are much higher.

The European interdisciplinary consensus guideline on invasive cSCC has stated that cSCC with high-risk factors should be excised with a clinical safety margin of 6-10mm or by MMS/MCS (41).

This statement is based on a number of studies in favor of the superiority of MCS respect standard excision in accuracy and less rate of false negative margins.

One of the most quoted publications, by van Lee et al. (58), is a retrospective cohort study of 579 patients with cSCC treated with MMS or SE, where it is demonstrated a lower recurrence risk of cSCC of the head and neck after MMS (3%) than after SE (8%) during a median follow-up of 5 years.

The results are suggested to be correlated to smaller portion of the excision margin histologically reviewed with SE, so increasing the risk of a false negative result and, consequently, of an misdiagnosis of incomplete cSCC excision.

Several limitations affect that study though, the retrospective design and the impossibility to determine tumor features (depth growth, perineural/lymphovascular invasion and differentiation), risk stratification of patients and disease-specific deaths.

Chren et al. (59) conducted a prospective cohort study of 1174 consecutive patients with primary NMSC, the difference in recurrence rates between standard excision and Mohs surgery was 1.6% during a median follow-up time of 7.4 years.

The results indicated that the two treatments did not differ significantly in preventing local recurrence.

The literature seems unanimous on estimating the MCS superior to standard wide excision in preventing false negative margins and thus local recurrence but this may partially due to a patients selection bias.

Breuninger et al. (40) pointed out that the local recurrence higher rates for WLE and bread-loafing histology may be correlate to the intrinsic features of tumor, usually larger, thicker and higher-risk respect to the ones selected for MCS.

This observation is supported by the clinical practice, in presence of a large and invasive tumor a microscopically controlled surgery would take several hours to be accomplished,

given the size of the specimen and the number of margins to be processed.

To reduce the total duration of the procedure, the Mohs surgery lab must be close to the operating room (60), complete with basic equipment needs cryostat, staining equipment and a microscope.

Obviously, specialized lab staff are needed to process the samples.

More, when also bone invasion has to be intraoperatively defined, histology requires different strategies and tools (i.e., cytology) according to soft or hard tissue to be processed, if not, the overall diagnostic power of MCS will inevitably decrease.

Another issue related to adopting the MCS as a standard practice is whether the benefits are related to costs.

Some advocated the advantage of avoiding a potential second surgery for a local recurrence (61), others complained of inadequate reimbursement policies (62).

All these drawbacks make the MCS practicable only in a few selected cases, and not as a routine procedure.

A large, prospective, randomized trial focusing on the prognostic value of WLE and MCS is still missing, making it impossible to draw definitive conclusions.

Primary Site Management

A single, well defined, cutaneous SCC has been object of numerous studies and the surgical treatment is established in several national consensus groups (63–66), the European international guidelines on invasive squamous cell carcinoma of the skin (41, 67) provide an excellent update on the state of the art.Evidence-based recommendations with high strength of consensus are enunciated about the surgical treatment of SCC primary site and safety margins, although the latter has an inferior level of evidence, because the independent prognostic effect of high-risk factors has not been consistently reported.

A supposed deviation from that would be necessary when a lacSCC develops on a special location, such the preauricolar or periorbital regions (53), but a multidisciplinary surgical approach and a proper operative setting allow to observe the evidence-based guidelines in the majority of cases.

Several simultaneous cSCC or a single invasive cSCC surrounded by various actin keratoses can develop in a single area of the body, the called field of cancerization (68).

The proximity of distinct lesions, even of varying degrees of invasion and differentiation, may exclude the possibility of clear large margin resection.

In addition, there are conditions that predispose to the development of skin cancer, such as genetic alterations and induced immunosuppression; in the affected patients, the scalp, the H-zone and the dorsum of the hands are the most likely locations for other cSCCs in the future.

In these situations the surgery must address the entire cluster of multiple cSCC (41), not only the single locally advanced SCC.

On one side this radical approach permits to get free margins, even if close, and on the other ensures healthy surrounding tissue for a better wound healing.

Usually, excision requires reconstructive surgery, whose complexity may vary depending on the extent and depth of the sample, the segment of the body involved and the specific characteristics of the patient.

Patients can also benefit from multimodal treatment with preoperative or postoperative use of topical agents for local control of resection margins (69).

The size and the deep invasion of a large lacSCC (> 5 cm in diameter) may characterize an extreme case of surgical treatment.

The patient's good general conditions and the absence of distant metastases may make it possible to consider the feasibility of surgical therapy with radical intention, otherwise meaningless and extremely dangerous.

Literature harbors a wide range of reports documenting the successful treatment of giant squamous cell carcinoma of the skin (70–73), affecting the full-thickness skin envelope and involving the underlying parenchymal organs such as throat, larynx, lung, brain and so on.

A preoperative discussion, the most thorough ever, with the patient is mandatory on realistic expectations in terms of perioperative risk and overall survival.

Albeit technically feasible, extreme procedures can cause patient death for a number of reasons, in addiction the prognosis still remains poor within few months.

Compassionate motives, while commendable, should not influence a rational assessment of the patient.

Surgery may play a role even in the advanced and metastatic cSCC.

Whenever possible, palliative care should be offered without preconceived ideas in terms of opportunity and cost-saving policy.

Case-specific reasons may justify an aggressive and complex procedure for transient or partial recovery, which can greatly benefit the patient's quality of life over the remaining period (74).

LYMPH NODE MANAGEMENT

Sentinel Lymph Node Biopsy (SLNB)

Worldwide the sentinel node biopsy (SLNB) is largely employed for detection of occult lymph node metastasis of skin cancer.

The technique requires a dynamic lymphoscintigraphy within 24 hours before surgery with injection of a radioisotope at tumor primary site, that consents the intraoperative detection of the first lymph node draining the specific body area with a gamma probe. Alternatively, at the time of surgery a sub-cutaneous injection of blue dye at the primary tumor site will allow the detection of sentinel node (SLN) by staining (75).

The reason to perform SLNB is that earlier detection of occult nodal disease may increase survival or otherwise positively impact the local disease management.

In the AJCC staging system (12) the regional lymph node involvement is considered the worst prognostic factor in cSCC, so the surgical biopsy of sentinel node represents an important staging tool.

Several studies show that cSCC with nodal metastases is still curable, so beyond the staging goal, SLNB may have a curative intent (76).

SLNB has a high sensitivity and a negative predictive value for cSCC (sensitivity 79%, negative predictive value 96%) (77), thus more reliable than conventional imaging (CT and MRI).

At the time of diagnosis, the estimated prevalence of SLN involvement varies considerably in literature, ranging from 7.9% (78) to 21% (76).

The discrepancy may lie in the patients stratification by the risk, because the criteria used to define this parameter differ considerably among the studies and this is a serious limitation for the interpretation of results (78).

In a prospective observational study involving 653 consecutive patients (79), no regional metastasis was observed in patients with Breslow depths less than or equal to 2mm.

The prevalence of metastasis was attested at 4% for patients with a depth ranging from 2.10 to 6 mm and 16% for those with a depth of more than 6 mm.

After a the multivariate analysis, the Breslow depth was the most important predictor of regional metastasis together with tumor diameter and ear location.

In another review (78), no positive SLN was observed in patients with a depth of less than 2mm.

So, it seems that the probability of a positive SLNB increases with the Breslow thickness, especially if it's more than 6 mm and in association with tumor diameter of more than 2 cm, that are the meaningful features defining a locally advanced cSCC.

The question is whether early detection of occult lymph node metastasis through SLNB impacts the disease-free or the overall survival.

In a retrospective study focused on 720 locally invasive cSCC (thickness > 5 mm) (80), of which 150 underwent to SLNB, 90.9% of all patients developing locoregional metastases showed tumor-free sentinel lymph nodes.

Distant metastasis resulted in 1.58% of patients in the SLNB group and in 1.75% of patients in the observation group ($p = 0.898$).

Therefore, the results did not support any advantage in local disease control and overall survival in SLNB patients.

Given the serious limitations of the few studies available, no definitive conclusion may be made about the effective role of SLNB in advanced cSCC, further randomized trials are necessary, that compare control groups of patients with comparable high-risk tumors who do not undergo SLNB.

Regional Lymph Node Dissection

When node metastasis of lacSCC are detected with SLNB or during the preop examination and imaging, there is indication to remove the lymph nodes of the corresponding anatomical region.

The independent prognostic value of lymphadenectomy in relation to overall survival is uncertain, but its role in local disease control is evident.

To date, an elective neck dissection is intended to be radical but, same time, to spare the anatomical structures that do not harbor lymph nodes, and whose resection may cause severe functional impairment.

These structures to be preserved vary according to the different areas of the body.

For the head and neck, the lymph nodes are grouped in six levels according to Robbins (81), to which few other unusual lymph node locations can be added.

Removal of all six levels is not always required due to the lymphatic pathway of head and face drainage.

The selective (partial) lymphadenectomies are classified in base of which levels encompass, thus providing an effective treatment through a less demanding procedure.

The lymph nodes levels to be removed are chosen regard to the primary site of cSCC development, but the second and third level and often the fourth one are included in the resection most of the time, because the contiguity with the internal jugular vein, the anatomical terminal of all the lymphatic pathway.

Accessory nerve, sternocleidomastoid muscle and internal jugular vein are intended to be spared unless directly involved by tumor invasion (the so-called functional lymphadenectomy) (**Figure 1**).

In case of parotidectomy, as completion of tumor resection or neck dissection, the terminal branches of the facial nerve should be carefully dissected and spared whenever possible (82).

Intraoperatively is reasonable a change of surgical plan in consideration of macroscopic tumor invasion of one of the above mentioned structures (83).

In upper limb and upper trunk cancer surgery the corresponding lymph nodes are harbored in the axilla.

According to Berg (84), the armpit can be divided in three distinct levels.

The first, most superficial, is burden by the lateral edge of pectoralis major muscle and posteriorly by the edge of latissimus dorsi muscle, the second underlies beneath the pectoralis minor muscle and the third, the deepest, is in contiguity with the superior land mark the axillary vein, that follows up to the cross with the subclavian muscle tendon.

All three levels are generally included in resection, no selective lymphadenectomy is recommended, because of the proximity and continuity of the lymph nodes (41).

The axillary artery and vein, the brachial plexus, the long thoracic nerve of Bell and the thoracic pedicle of the latissimus dorsi should be saved from accidental damage.

No functional impairment is usually appreciable after the procedure, but some reported an occasional postoperative lymphedema affecting the upper limb in about 8-10% of cases.

Immediate physiotherapy and elastic arm dressing may help reduce discomfort.

Instead the groin lymph nodes are to be removed if the mcSCC developed at lower limb, lower trunk and genitalia.

They can be roughly divided into superficial and deep in terms of localization respecting the femoral vein.

The anatomical boundaries of groin are superiorly the inguinal ligament, laterally the edge of the sartorius muscle and medially the edge of the long abductor muscle (Scarpa's triangle).

Anatomically, the lymphatic drainage path follows the femoral vein, then the external iliac vein, so that even the external iliac fossa and the obturator fossa can be affected by nodal involvement.

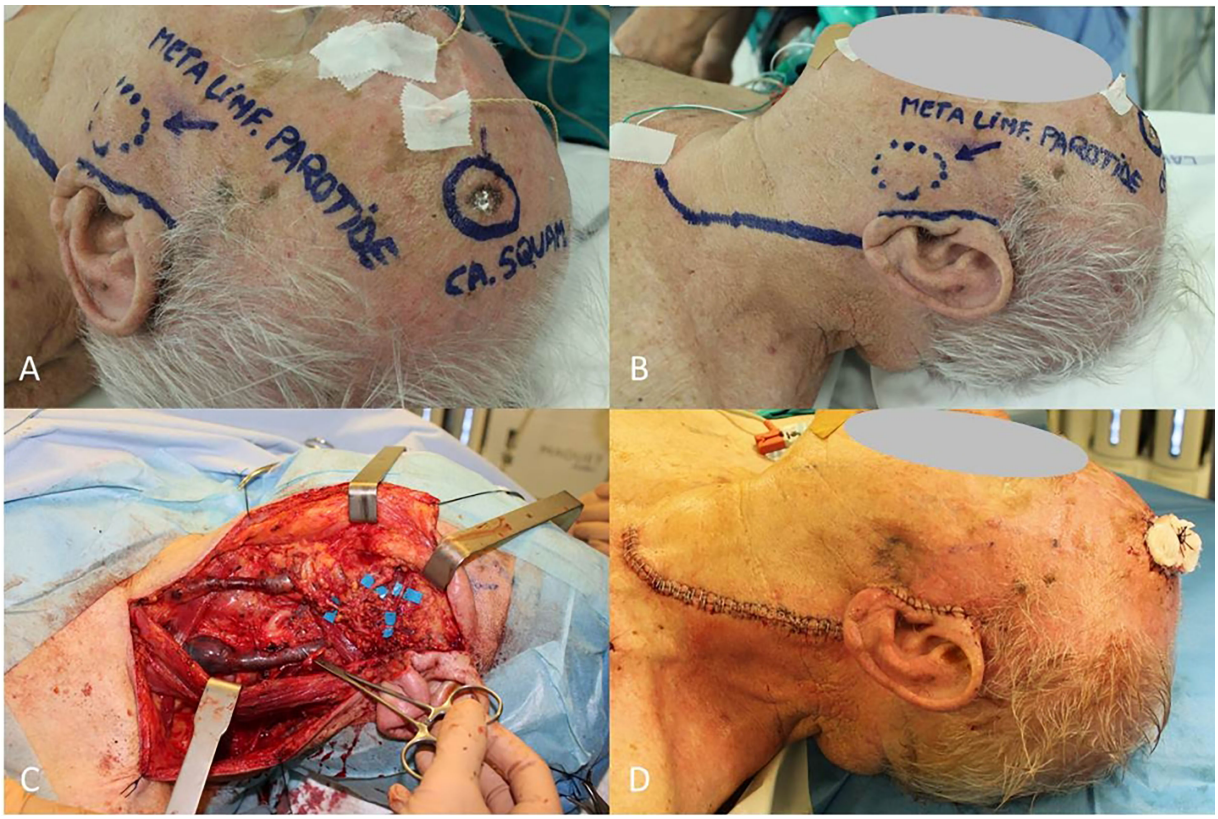

FIGURE 1 | **(A)** cSCC of frontotemporal region; **(B)** Nodal metastasis located in parotid; **(C)** Neck and parotid dissection complete with sparing of internal jugular vein, sternocleidomastoid muscle, accessory nerve and all the branches of facial nerve; **(D)** Final result.

Interestingly, contrary to the guidelines on the treatment of melanoma, there is no specific indication to extend lymph node removal to extraperitoneal level (41).

However, it is not infrequent facing with a regional metastatic cSCC (i.e., vulvar cancer) that involves all the groin region, whose eradication imposes an extension of lymphadenectomy of the abdominal nodes (**Figure 2**).

The structures to be electively spared are the femoral vessels and nerve, some advocate the great saphenous vein saving in order to reduce the probability of subsequent lower limb lymphedema, a side-effect much more frequent than in the upper limb.

The lateral femoral cutaneous nerve, a sensitive nerve, usually cannot be spared due to its subcutaneous course into the Scarpa's triangle, leaving a numbness area below the inguinal ligament.

Recently, the application of the laparoscopic approach to groin dissection has proven to be safe for oncology and has consistently reduced both complications mentioned above (85).

SCC Metastases to Special Locations

In addition to anatomically well-defined regional lymph nodes, other sites may harbour cSCC metastases (86–89). These unusual sites may be identified by imaging while staging or as an incidental report.

The surgical management is primarily driven by the necessity of histological diagnosis for correct tumor staging, then a radical excision may be accomplished as an isolated procedure or as the completion of the regional lymph node dissection.

Once again the multidisciplinary tumor board discussion can support the indication to surgery in case of an invasive procedure.

THE RECONSTRUCTIVE PLAN

The Oncoplastic Approach in Skin Cancer

Historically the plastic surgery has found the most brilliant application in head and neck reconstruction after cancer resection, due to the imperative necessity to provide an immediate repair of crucial anatomical structures.

With the progressive improvement of reconstructive methodology together with the increasing demand of better outcomes in terms of functional and aesthetic recovery, to date, a comprehensive skin cancer treatment should include adequate procedures to let the patient returning to a normal life.

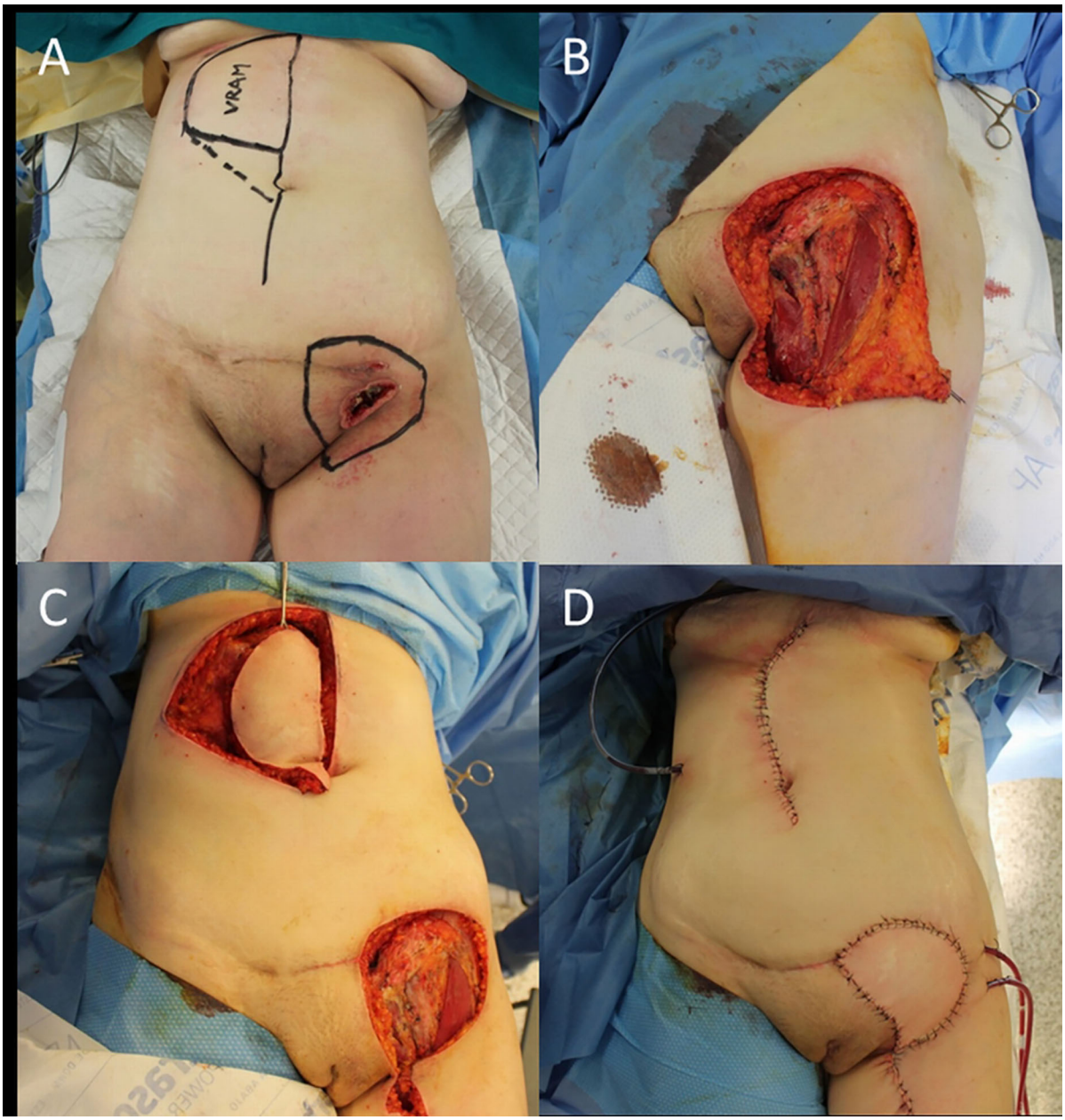

FIGURE 2 | **(A)** Recurrent cSCC of the vulva after surgery and radiotherapy, outlined the groin excision limits and the boundaries of the right vertical rectus abdominis muscle (VRAM) flap; **(B)** End of the wide excision and groin dissection; **(C)** Harvest of the VRAM flap; **(D)** Final result after flap rotation.

For a better planning of which solution would be the best choice to adopt, in other fields of tumor surgery the plastic surgeon is a component of the team that evaluates preoperatively the patient.

In breast cancer units, for example, from the very beginning of the entire care the patient undergoes to plastic preop assessment to early delineate the forthcoming procedure.

The term oncoplastic surgery indicates this special surgical approach to the issue, both oncologic and reconstructive, and the surgical techniques applied to (90).

The necessity of a "skin oncoplastic" approach is not yet be suggested, but the tremendous implications of an aggressive surgery, as required with a locally advanced cSCC, request a redefining of priorities and competences (91).

Timing of the Reconstruction

Theoretically, the optimal reconstructive procedure would immediately follow the skin cancer excision, to repair the damaged tissues or replace the missing ones.

That point of view is the most favored by plastic surgeons, in regard to better conditions of local residual tissues, less alterations due to inflammatory process and the frequent availability of vessels in the surgical field as source for microsurgical transplant.

An immediate reconstruction requires a fine preop planning, may lengthen of several hours the overall surgery time and may hinder an eventual second look for oncologic purpose because of skin flaps transposition.

The condition that indicates an immediate repair is the incompatibility of the wounds with life or with a reasonable postop recovery, in that case the reconstruction would be mandatory.

Another issue regards the clearance of resection margins, if the ablative extent has been maximum in relation to patient conditions, no matter if definitive histology would report R1 or close margins.

Instead, if the local conditions would permit a widening of resection and the clearance is uncertain, a delayed reconstructive procedure should be seriously considered (50).

The remaining tumor tissue at the edges of the resection will inevitably invalidate oncological and reconstructive procedures, promoting early local recurrence and preventing wound healing.

When free margins are questionable, the most complex reconstructions should be avoided in favor of the less demanding procedures (i.e., skin graft), that may allow temporary and suboptimal repair waiting for histology confirmation.

Today a number of engineered skin substitutes are available (92), mostly derived from porcine or bovine dermal tissue, that consent an immediate defect cover without sacrificing of the patient's skin.

Another interesting technologies are the vacuum-assisted closure devices (93), a sort of sealing dressing with a permanent aspiration system connected, that may protect the wound from contamination and prepare the surgical bed for definitive repair.

Thanks to these innovative solutions, a delayed reconstruction procedure can be planned safely with minimum patient discomfort and avoiding the problem of margin clearance.

Functional and Aesthetic Issues

Patients affected by a locally advanced cSCC reasonably will face with a great impairment in quality of life (QOL) as a result of the aggressive nature of their disease leading to extreme surgical procedures (94).

QOL is related to maintaining self-sufficiency, meaning re-establishment of daily activities and vocational rehabilitation, but it is also related to self-esteem.

Age and disease severity may negatively influence QOL, older patients reported significantly lower outcome than younger patients, and a clear reduction of QOL is considerable when patients with NMSC diagnoses are compared to those with actinic keratosis only (4 to 9%) (95).

The concepts of repair and of reconstruction may greatly differ in relation to the final outcome, because promoting the wound healing not necessary means for the patient a return to the preop physical and mental state.

Therefore, the simplest reconstruction will require less time to be accomplished, will be less heavy for the patient, but probably will not fully meet the needs after a complex tumor resection.

For example, the skin graft, probably the most largely used plastic surgery technique, is not free from concerns due to contraction, poor skin matching, and resulting deformity when applied in an aesthetically sensitive area (96).

Due to scars and unpleasant outcomes, most patients suffer some degree of psycho-social distress related to appearance, especially during the short-term post-operative period (97).

Advanced reconstruction skills are often necessary to improve the overall outcome, especially in topographically challenging areas, such as the face or upper extremities.

The correct use of the plastic surgeon's tools encompassed in the reconstructive ladder may be the key to better functional recovery and satisfactory result.

COMBINED PROCEDURES

Head and Neck

The head and neck area is by far the most common location for primary cSCC.

Historically, that was the first field of application of plastic reconstructive techniques after oncologic resections, due to the impossibility of amputation and the dramatic consequences of the second intention healing when it was achievable.

If the small SCC can be easily cut and repaired by a local cutaneous flap, the locally advanced one poses severe challenges in terms of functional impairment and aesthetic demands.

With the relative exception of the nose, more usually affected by basal cell carcinoma, the chronically sun-exposed areas, such as lips, forehead, ears and scalp, can be largely involved by tumor development requiring full-thickness excision of soft tissues and, not rarely, of the underlying bone surface.

For the forehead and the scalp usually the simplest technique, the skin graft, plays a significant role.

Consists in the harvest of a slice of dermal-epidermal layer of 0.4-0.5 mm in thickness with the use of a mechanical dermatome.

It is possible to take the graft manually with the aid of a blade, but in this case it will inevitably result in a greater thickness.

The biological concept is the possibility to transfer portions of skin to another side without vessel anastomosis, due to the limited amount of tissue cells transferred, that can be successfully supported by the underlying healthy tissue.

The major limit of the procedure consists in the necessity of a viable recipient bed, some tissues like bone, tendon, muscle fascia, and loose fatty tissue may not provide a sufficient blood supply to permit the graft survival.

Recently, a number of templates have been introduced in clinical practice to facilitate the skin grafting, acting as a scaffold for the regenerative tissue towards the graft.

The application limits the collateral shrinkage that usually affects the graft after maturation and permits grafting on uneven surfaces with sub-optimal perfusion.

Two models of regenerative templates are available, the first is expected to be immediately skin grafted, the second is covered by a temporary silicone patch and requires about 15 days before be grafting.

An example of procedure with wide large soft tissue excision and outer calvarian resection followed by two step reconstruction with skin substitute (template) and a skin graft is reported in **Figure 3**.

Instead, the surgical repair with a flap becomes necessary when the local conditions of the wound bed after resection will not allow direct or delayed grafting.

The flap has the great advantage of being independent from wound conditions thanks to its own blood supply.

A further benefit of respecting skin grafts is the quality of reconstruction that flaps can provide.

The wide large excision can leave a dead space to be filled, or may deprive bone of the essential soft tissue cover, in such situation only a reconstruction with a flap can be successful.

It requires a surgical dissection, so it is a time-consuming procedure and the area of the body from which it is harvested has to suffer considerable damage.

Undoubtedly, with proper flap selection and meticulous technique these disadvantages will be significantly reduced.

Flaps can be variably classified according to the tissue transferred (i.e., skin flap, muscle flap, bone flap), or on the basis of the specific blood supply (random flap, pedicled flap, island flap, perforator flap).

The first choice in the reconstructive procedures ladder will be a local flap, due to the proximity of the defect, and the low impact on the patient.

To replace a soft tissue defect usually a random skin flap is sufficient, but when the defect is too wide (i.e., a cancerization field) a multimodal flap-based reconstruction and skin grafts will get the result (**Figure 4**).

Another indication for performing flap-based reconstruction is the need for functional recovery when the whole anatomical subunit must be excised. For example, when a loaSCC arises in the lips a wide wedge resection will not permit the direct approximations of the three-layers structure of the lip, that will cause the inability in maintaining the bolus inside the oral cavity during eating. A pedicled local or regional cutaneous flap (**Figures 5**, **6**) , turned into the defect, will provide soft and elastic tissue that will act as a "bridge" to restore the oral boundaries and its sealing properties.

Moving from medial to lateral, the pre-auricolar region and the ear presents some of the most challenging problems to solve in case of a locally advanced SCC.

The presence of several different tissues and anatomical structures within a few centimeters (skin, muscle, bone,

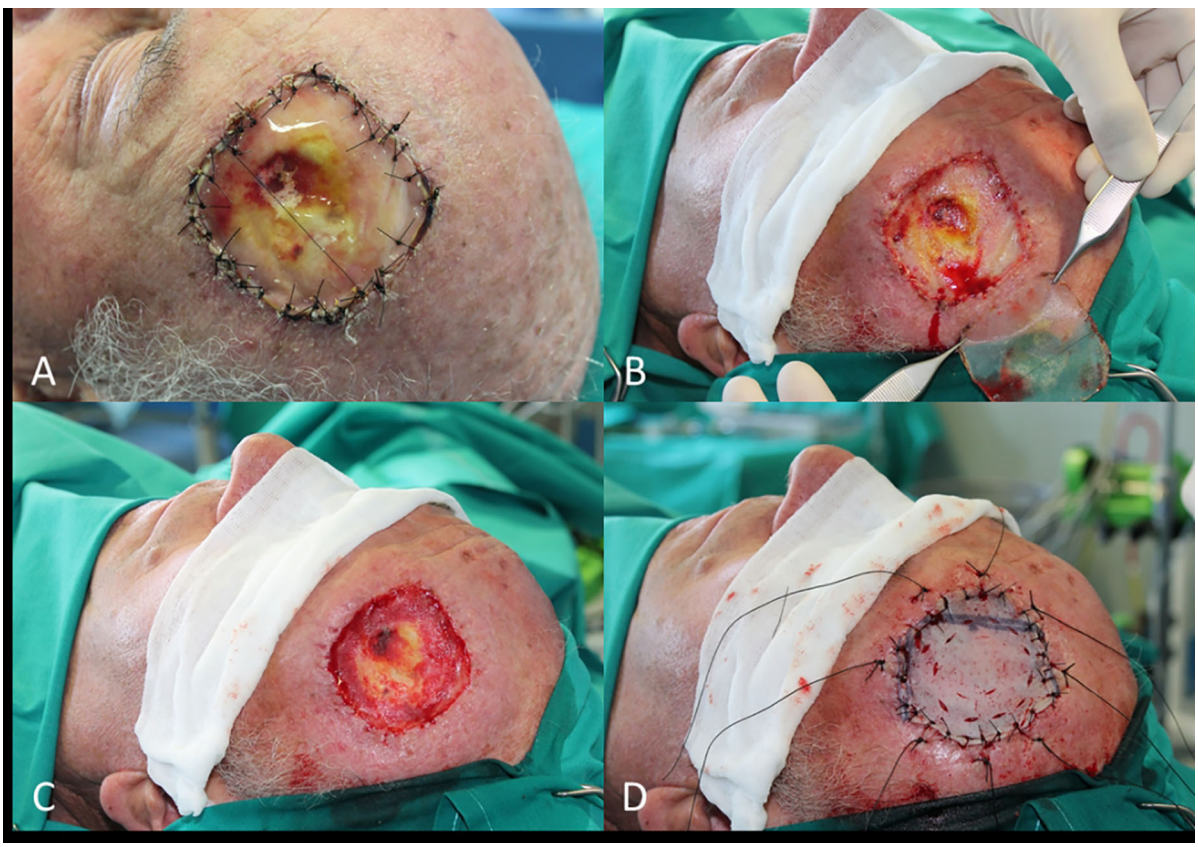

FIGURE 3 | **(A)** Reconstruction of scalp after WLE and regenerate template application; **(B)** Removal of silicone patch; **(C)** Wound bed debridement; **(D)** Final closure with skin graft.

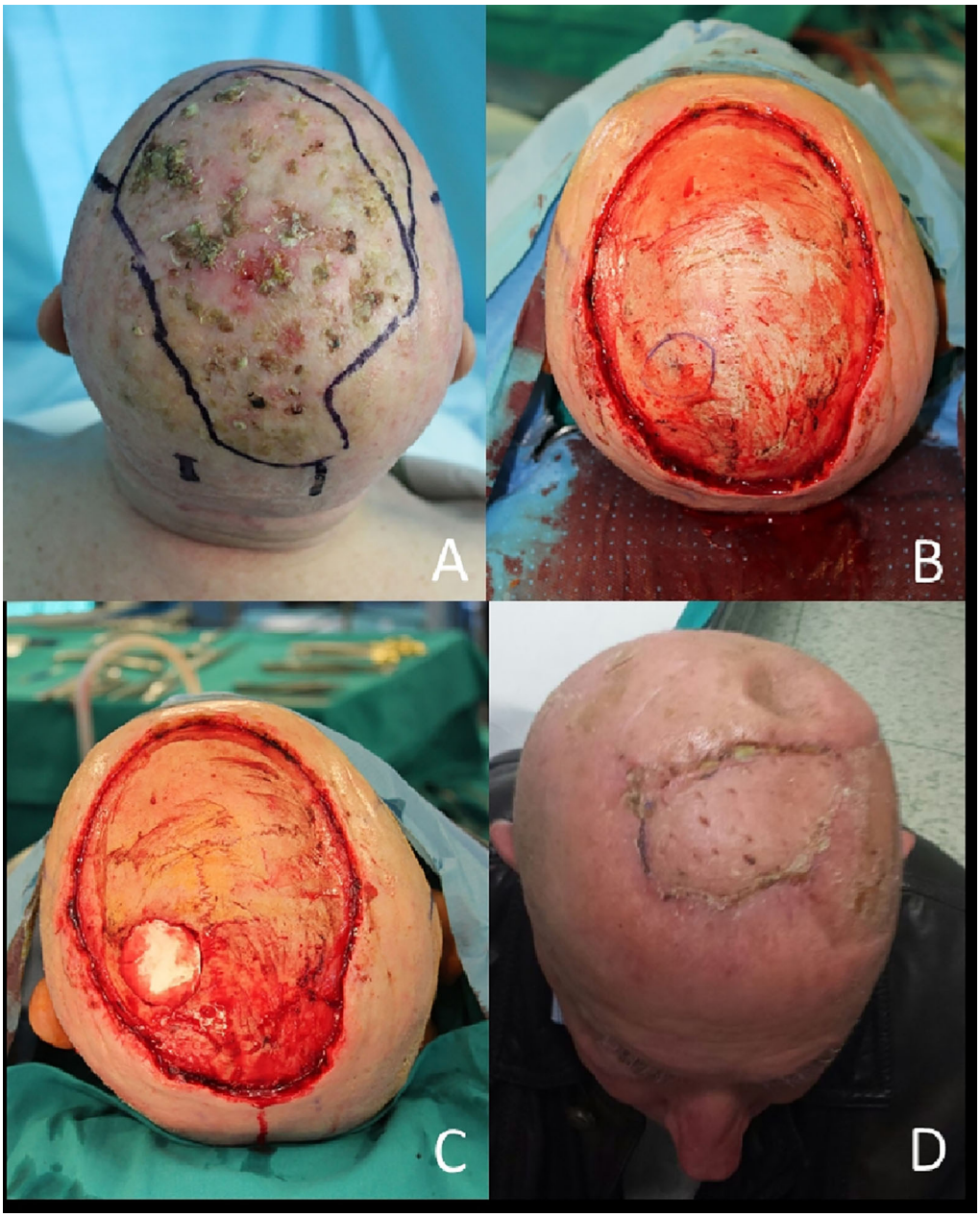

FIGURE 4 | **(A)** Field of cancerization on the scalp; **(B)** After en bloc resection, in blue outlined the tumor invasion of calvarian; **(C)** Bone resection complete; **(D)** Final result with local flap and skin grafts at 1 month.

parotid gland, facial nerve, outer and inner auditory canal) often require extremely wide resection due to the tumor quick in-depth grow.

The major pectoralis flap is a workhorse that has been employed for decades and still represents the "plan B" after failure of more sophisticated flaps or when the patient cannot sustain a time-consuming procedure (**Figure 7**).

More recently, the free flaps have replaced the pedicled ones, such as the major pectoralis flap, deltopectoral flap and Trapezius muscle flap.

Pedicled flaps are limited in the rotation by the length of the nourishing artery and concomitant vein, if the defect lies too distally from the blood supply another solution must be identified.

The ultimate, most complicated, technique of plastic surgery is the microsurgical free flap, which theoretically can provide healthy tissue in any part of the body.

The basis of microsurgical transplantation is the transfer of a part of the body (skin, muscle, bone, nerve or a combination of) by a vascular microanastomosis performed under a magnification microscope.

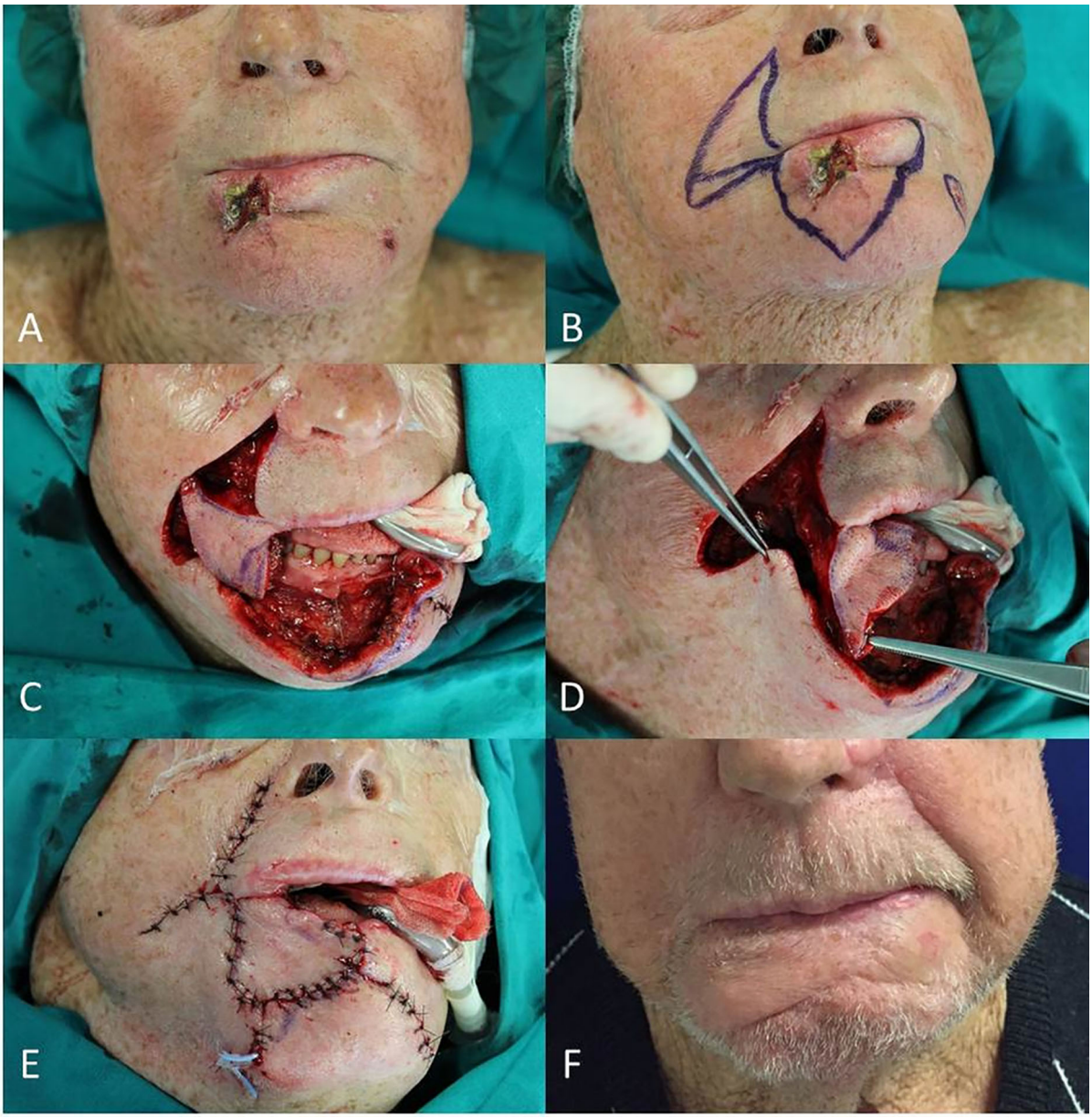

FIGURE 5 | **(A)** Locally advanced SCC of the inferior lip; **(B)** Planning of the wide wedge excision and the Estlander flap from the upper lip; **(C)** Soft and hard tissues removed; **(D)** Setting of the flap pedicled on the superior labial artery; **(E)** End of surgery; **(F)** Follow up at 6 months after right commissuroplasty.

Free flaps have been harvested from every part of the entire body surface, like the upper limb (i.e., radial or Chinese flap, **Figure 8**), the back (i.e., latissimus dorsi free flap), the trunk (i.e., deep inferior epigastric artery perforator flap - DIEP and superficial circumflex iliac artery perforator flap - SCIP), the thigh (i.e., anterolateral thigh flap – ALT, **Figure 9**), and the leg (i.e., medial sural artery perforator flap - MSAP).

Microsurgery has proven to be an efficient and reliable tool, making it the preferred choice for many oncology reconstructions (98).

The advantage of the free flap technique is the possibility to choose the tissue to transfer in the base of the necessity of the single case, replacing the missing tissue with one analogous with the same proprieties and characteristics.

These complex reconstructions require several hours, a dedicated operating room setting and high qualified personnel.

The overall quality of the outcomes with free flaps are largely superior than with conventional techniques (graft and random local flaps), the functional recovery is higher and faster, and the reconstruction aesthetic, whenever possible in these cases, is much better.

In most cases the microsurgical transplant provides the only real chance to perform extremely large and aggressive oncologic

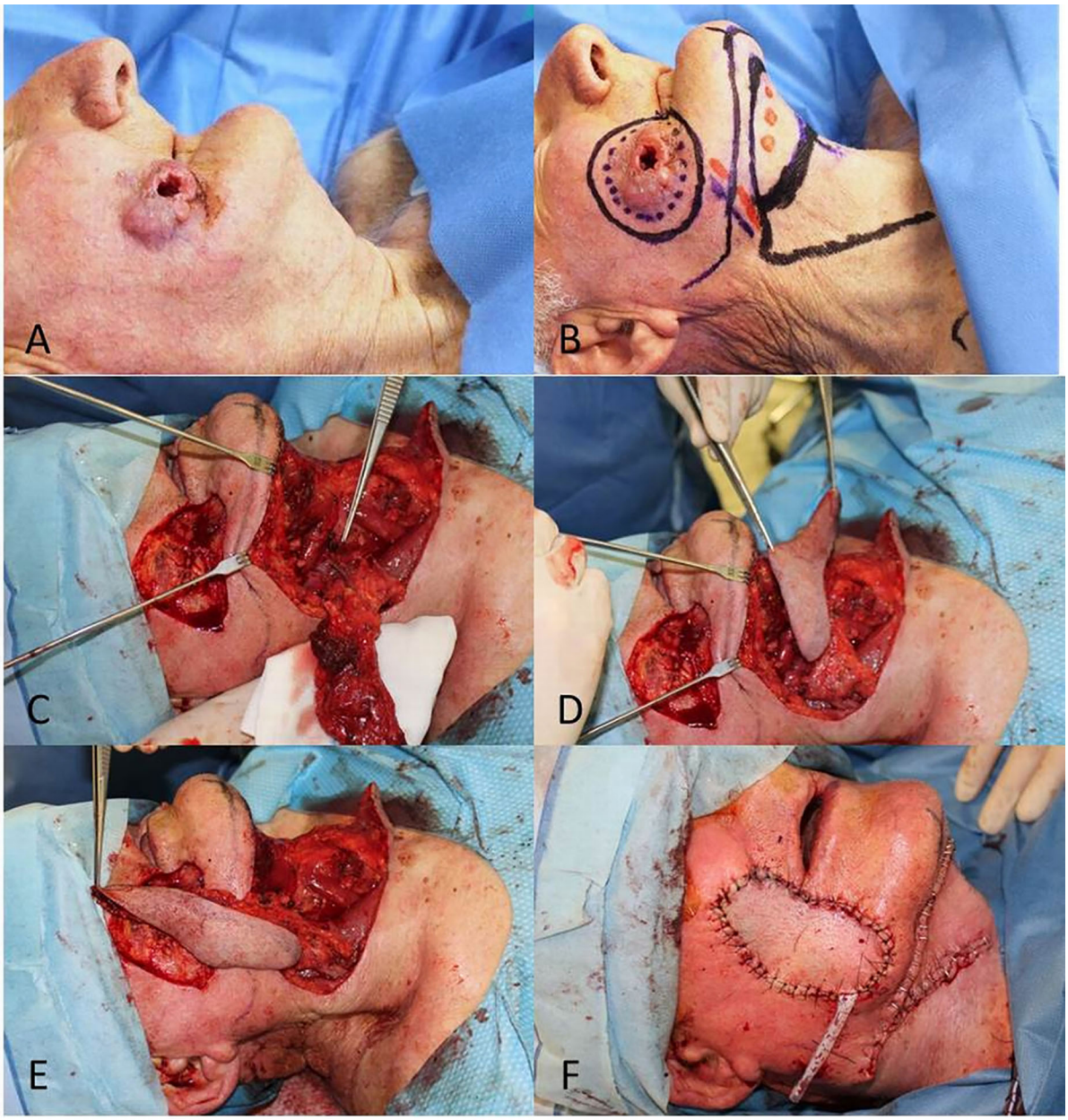

FIGURE 6 | **(A)** Locally advanced SCC of the cheek; **(B)** Planning of the full-thickness excision and the submental flap; **(C, D)** Excision complete and dissection of the flap based on the submental vessels; **(E)** Advancement of the flap to the defect; **(F)** Final result.

resections, otherwise impossible, therefore, has to be intended a part of the comprehensive tumor treatment.

Trunk

The trunk is a less common site of loaSCC development, and the conventional repair with direct tissue approximation after extensive subcutaneous undermining is straightforward most of the time.

When instead the anatomical region has to be necessarily restored, like in the case of a radical vulvectomy for loaSCC of genitalia, the surgeon has to turn to a flap-based reconstruction.

The progression of anatomical studies on the soft tissue vascular supply has led to the development of skin flaps based on a single vessel coming from the underlying layer, the so-called perforator flaps.

The versatility and minimum sacrifice associated with these flaps extended the scope.

Dissection can be much more tedious, because of the need to save other functional structures during the harvest of flaps.

In the reconstruction of female genitalia the use of perforator flaps has replaced in many cases the need to harvest a muscle flap (**Figure 10**).

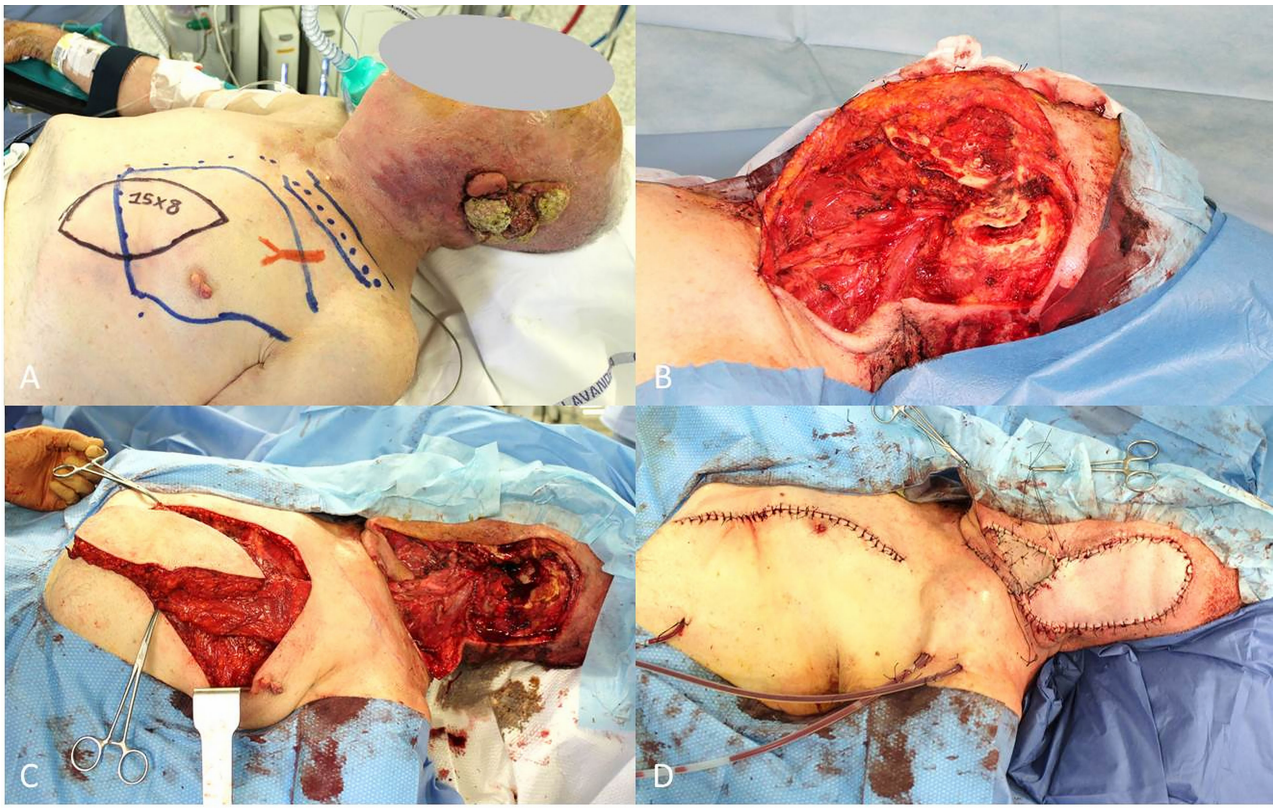

FIGURE 7 | **(A)** Locally advanced SCC of the ear and planning of pectoralis major muscle flap; **(B)** End of petrosectomy and neck dissection; **(C)** Pectoralis muscle flap harvested; **(D)** Final result after flap rotation.

A muscle flap, in which an entire muscle belly is harvested from its native bed and rotate to cover a defect, still plays a role when no other easier solution is available.

The transferring of a muscle flap with a portion of overlying skin is defined musculocutaneous flap, it permits to fill a deep dead space and to replace the skin cover at the same time (**Figure 11**).

Upper Limb and Hand

The upper extremities are not often affected by loaSCCs, on the contrary the hand, due to the permanent sun exposure, may be suffering from a rapid-grow SCC, that rapidly impairs the function and causes acute pain due to direct involvement of nerves.

Of course the finger amputation still plays a role, but, in the presence of thumb or multiple digit involvement by tumor, the conservation of a minimal function of grasp is an issue to be addressed.

In these selected cases a distant pedicled flap reconstruction should be considered, that may be accomplished through the sacrifice of a major upper limb vessel (i.e., radial flap) or with a less demanding flap like the posterior interosseous flap (**Figure 12**).

Lower Limb and Foot

The lower limb, in particular the anterior surface of the leg, is often the growth site of cSCCs, many of which can be easily removed and repaired with a skin graft.

The presence of a loaSCC instead invariably requires partial or total resection of a bony tibial tract, making direct grafting impossible.

In this circumstance it is necessary to use a flap, which may be a pedicled flap (i.e., gastrocnemius flap, pedicled MSAP flap, reverse ALT flap), or it will be necessary a microsurgical transplant of a distant healthy tissue to cover the lower limb defect (i.e., latissimus dorsi free flap – **Figure 13**, ALT flap, gracilis free flap).

As for the hand, the amputation is a procedure that has to be considered, especially when it can be safely performed leaving undisturbed about 15cm of the proximal tibial shaft, the minimum length required to be prosthesized.

LIMITS OF A SURGICAL APPROACH

Even the most aggressive excision can cause inadequate surgical margins to guarantee a long-term disease-free period.

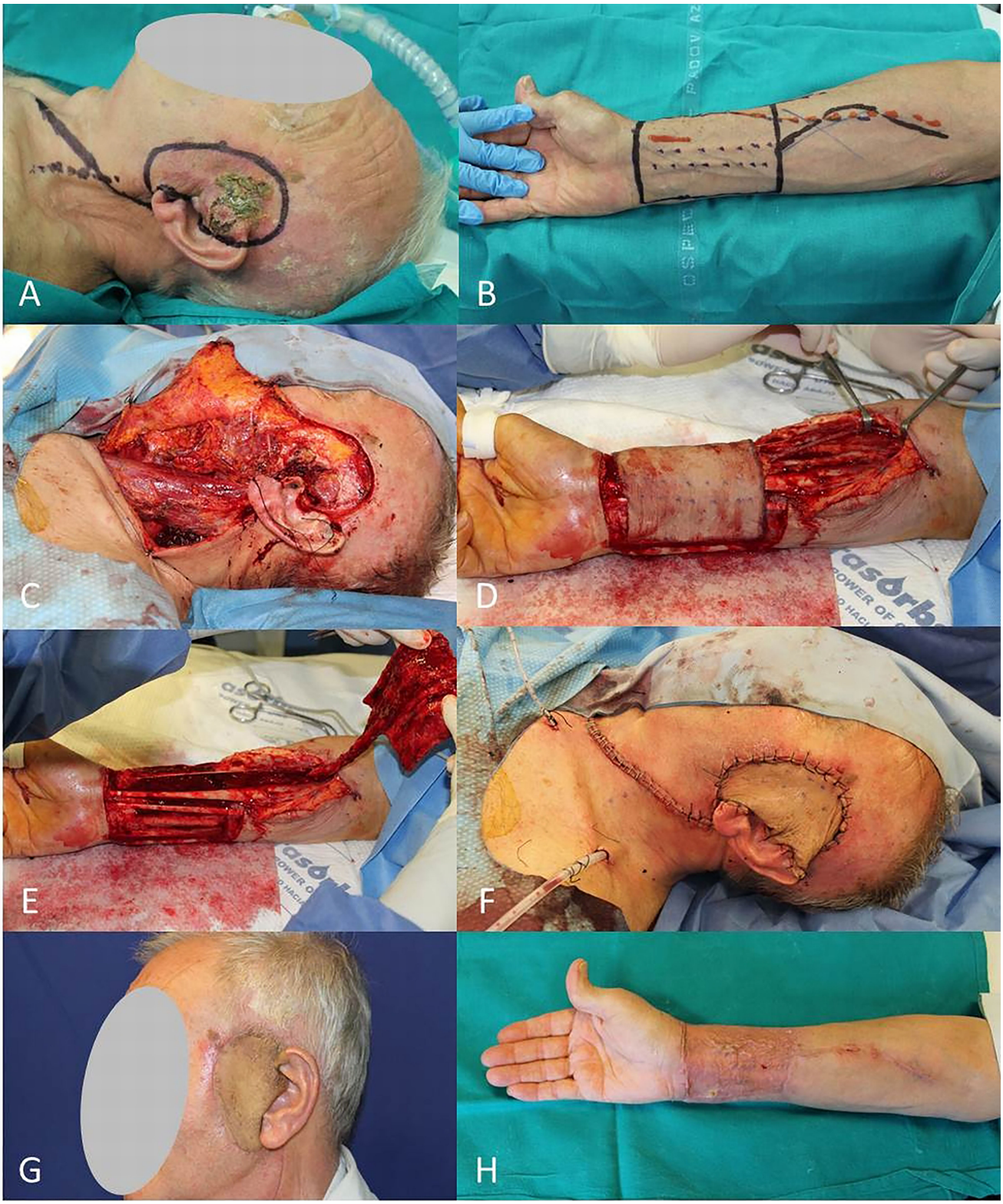

FIGURE 8 | **(A)** SCC of the preauricolar area; **(B)** Radial antebrachial free flap planning; **(C)** End of WLE and neck dissection; **(D, E)** Harvest of radial flap; **(F)** Immediate final result; **(G, H)** Free radial flap and donor site after 2 months.

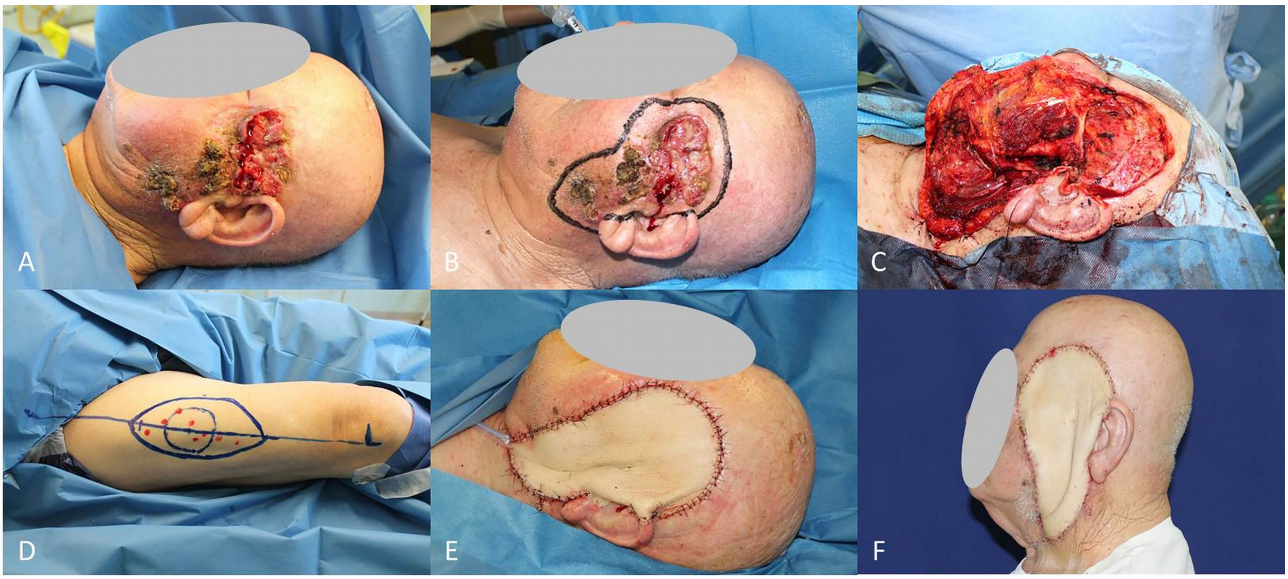

FIGURE 9 | **(A)** Locally advanced SCC of left midface; **(B)** Planning of the wide excision; **(C)** Soft and hard tissues removed, lymphadenectomy completed; **(D)** Planning of the ALT flap harvest; **(E)** Immediate result after the ALT flap insetting; **(F)** Final result at 30 days follow-up.

The histology features that usually characterize a locally advanced cSCC (size, depth, PNI) have been identified as a risk factor for future recurrence (99).

A history of recurrence, in turn, has been identified as a risk factor for future relapse and poor survival (100).

Therefore, a recurrent cSCC not only represents a local problem, but a high-risk CSC variant that needs to be excised as widely as possible.

A re-excision, even when possible, can be a challenge for the reconstructive surgeon, considering the less available options and scar tissue present.

A second repair with skin graft will permit an early detection of local relapse but functionally and aesthetically can be unsatisfactory.

Another strategy would be delaying the reconstructive procedure, to assess margin status before closing the wound, as suggested above (50).

On the other hand, it is not unusual for recurring cSCC to be caused by multiple suboptimal resections, or close-margin resections, instead of large excision.

This may be due to the impracticability of combined procedures in outpatient settings requiring a tertiary hospital facility.

In this case, after a comprehensive examination of the patient, a more complex procedure may be indicated with the aim of radical resection.

Thus, a review of all reconstructive options is the key to achieve the goal.

It is sometimes the reconstruction procedure that requires a surgical revision due to surgical site infection (SSI) or necrosis of the skin/flaps and dehiscence of the wound.

Replace a necrotic flap with a new healthy one may be challenging, but sometimes is the only reliable "plan B" for salvage procedure (101).

What is not possible in head and neck, it is feasible on the other hand in the extremities.

In presence of a locally advanced cSCC the amputation represents always a choice (102).

In the age of microsurgery may sound inappropriate considering amputation as an option, but older patients affected by numerous comorbidities may be not eligible candidates for such demanding surgery.

More, a reconstructive procedure of a single ray of the hand or a part of the foot may be not compensated by a concrete advantage in term of functional recovery and better outcome, so a frank discussion with patient about realistic pros and cons appears mandatory.

A much less discussed collateral effect of a complex surgery is the impact of anesthetics on immunosuppression in the short-term (103).

A several hours surgery may cause patient debilitation, and anesthetics seem to play a role in transient immunosuppression increase, thus promoting the widespread of cancer not adequately counteracted by the immune system (104).

Again, a careful preoperative evaluation with a multidisciplinary tumor board discussion is revealed as essential for selecting the eligible patients and appropriate treatment.

CONCLUSIONS

The treatment of choice for primary squamous cell carcinoma is surgery, but the locally advanced variant poses a great challenge to obtain free resection margins.

Wide local resection and complex reconstruction are necessary in most cases to fulfil both oncologic and functional requests,

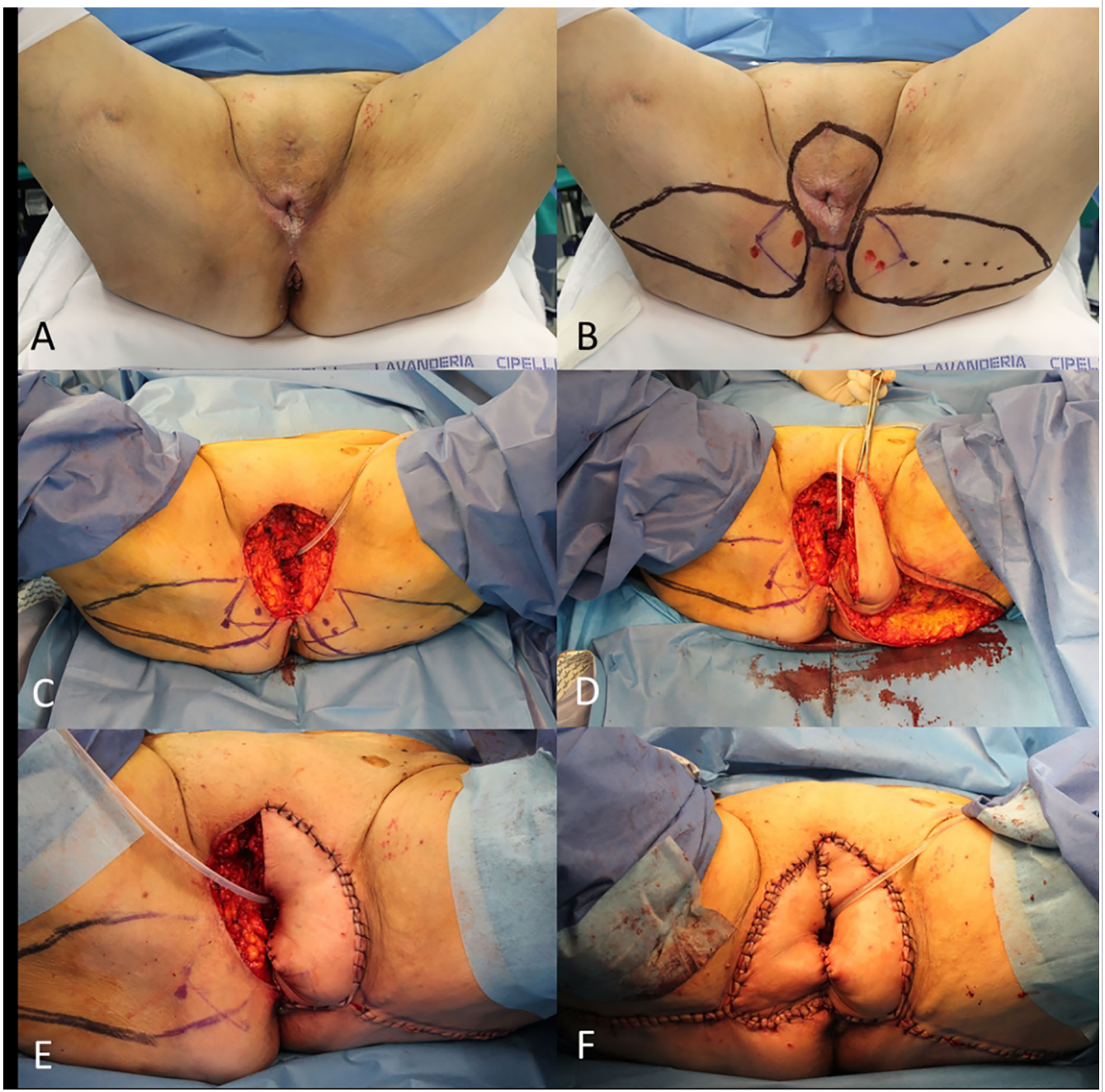

FIGURE 10 | **(A)** Recurrent cSCC of the vulva; **(B)** Planning of the radical vulvectomy and bilateral perforator (lotus) flaps; **(C)** End of the vulvectomy; **(D)** Rotation of the left perforator flap; **(E)** Left lotus flap insetting; **(F)** Final result.

although free-disease and overall survival remain uncertain, the patient quality of life may improve considerably.

Risks and advantages for patients undergoing such extreme procedures should be carefully discussed within a multidisciplinary tumor board for better defining patient selection and treatment. In metastatic cSCC and in non-responsive to chemoradiotherapy patients the surgical approach may still play a role in better local control of disease and a salvage procedure.

These challenging procedures are better addressed by a surgical team skilled in plastic surgery techniques in order to provide the best reconstructive options, included microsurgical free flaps.

The preliminary results of the new therapies (i.e., immunotherapy) appear to be very promising, but the relationship with surgery, in terms of timing of administration, is still under investigation.

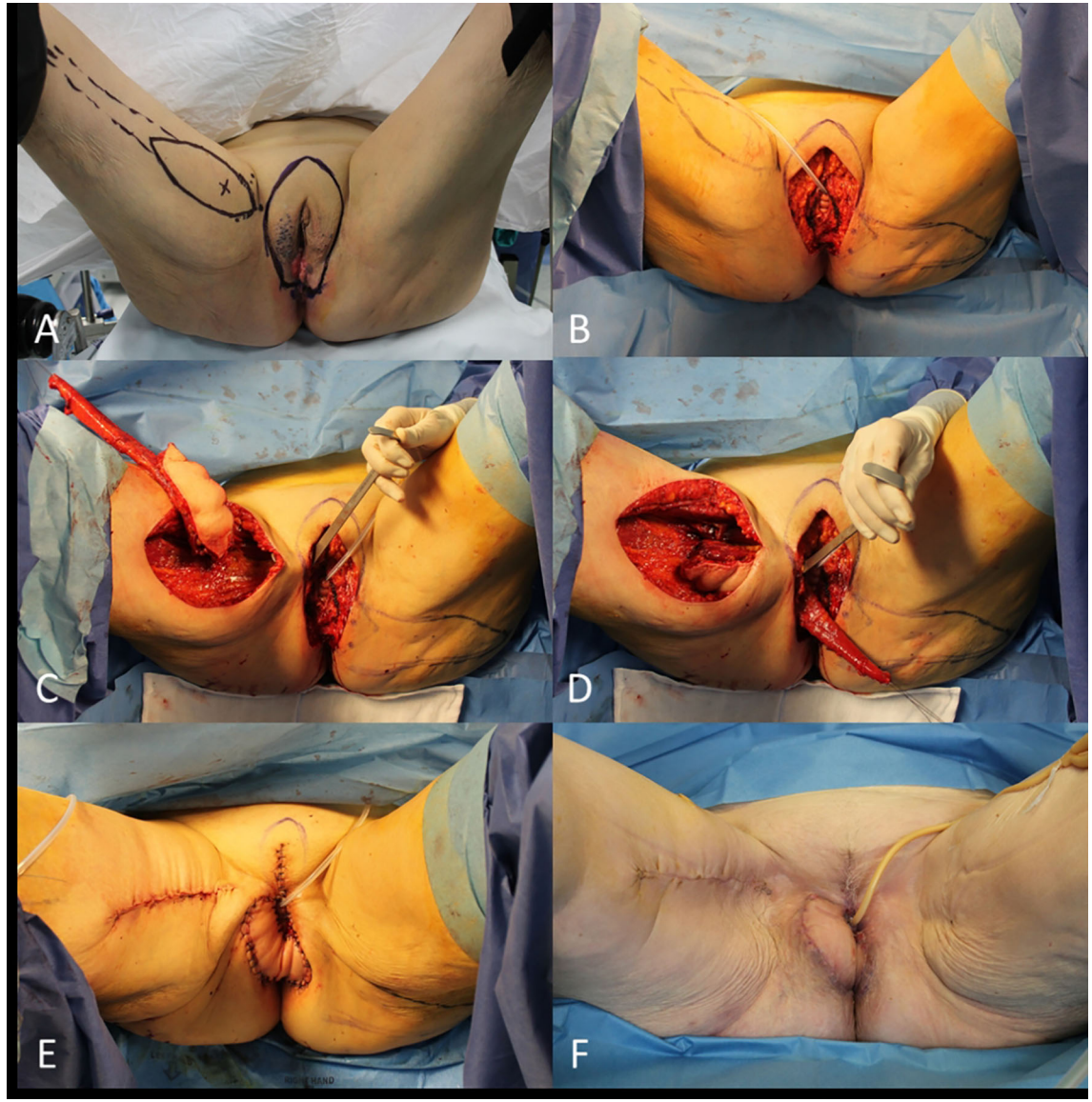

FIGURE 11 | **(A)** Recurrent cSCC of the vulva and planning of gracilis musculocutaneous flap; **(B)** End of the radical vulvectomy; **(C)** Flap dissection; **(D)** Flap transferring; **(E)** Immediate final result; **(F)** Final result at 1 month.

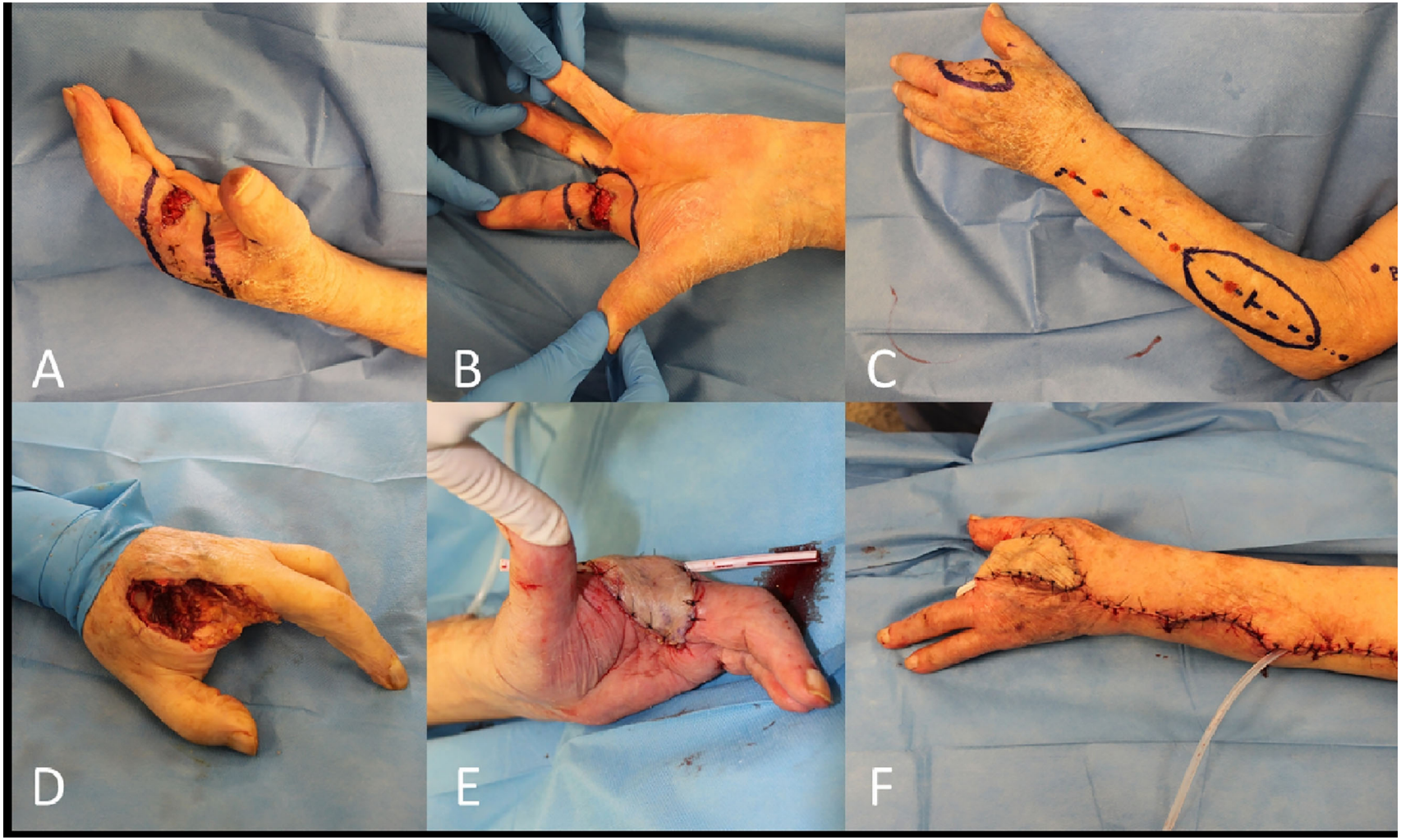

FIGURE 12 | **(A, B)** Recurrent and locally advanced cSCC of the base of the long finger (second amputated previously); **(C)** Posterior interosseous fascial flap planned; **(D)** End of the third hand ray amputation; **(E, F)** Final result after rotation of the flap.

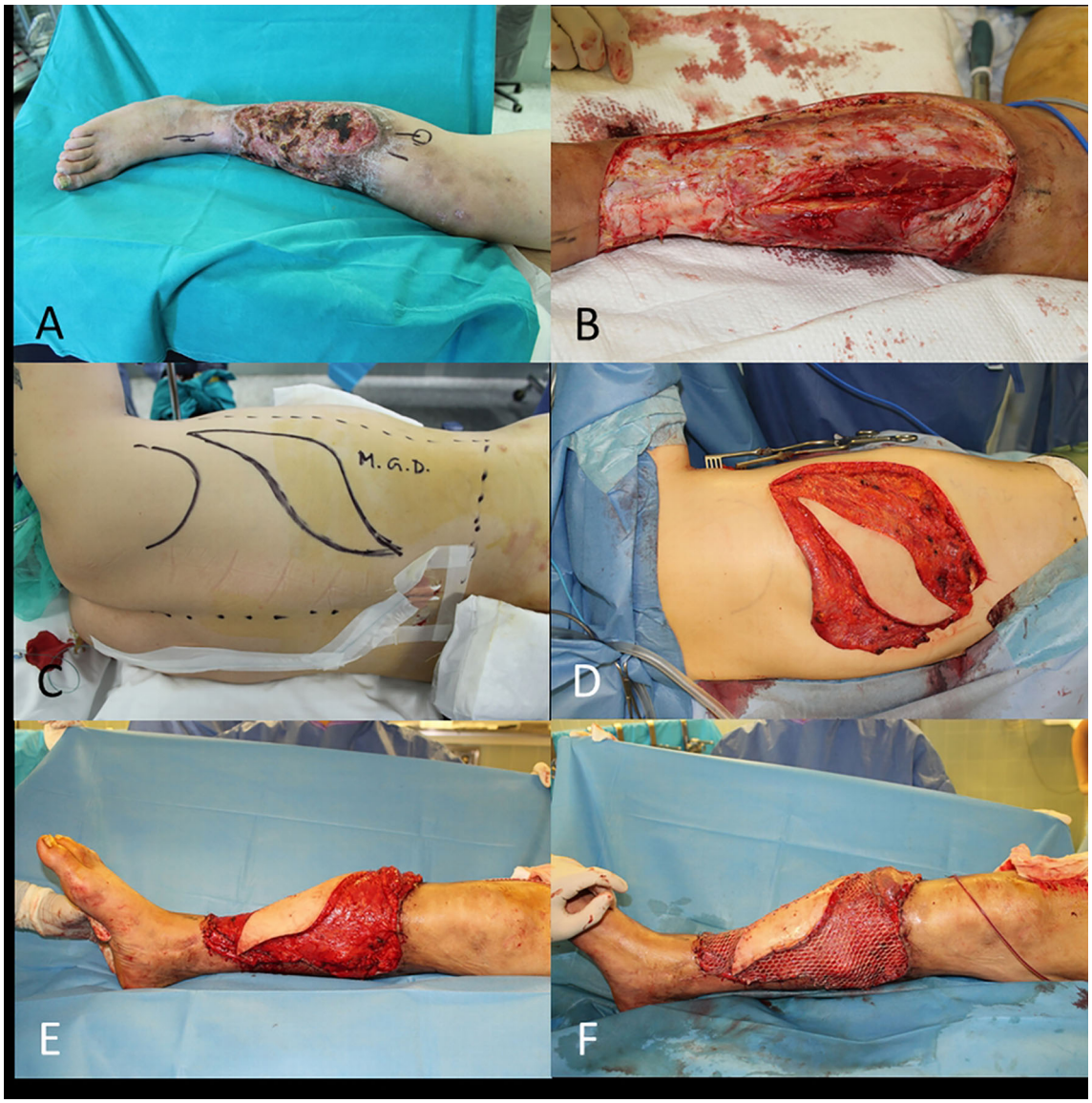

FIGURE 13 | **(A)** SCC of the leg; **(B)** End of WLE with bone exposure; **(C)** Free latissimus dorsi flap planned; **(D)** Flap ready for transfer; **(E)** End of microsurgical transplant; **(F)** Final result with skin grafts.

Further randomized trials are necessary to better define tumor independent factors that impact on overall survival and to compare different multimodal treatment strategies.

RESEARCH LIMITS AND BIAS

The literature review was conducted with an on-line research through PubMed® database, inclusion criteria have been data publishing since 2000, articles pertinent to the topic, and full-text available.

For any chapter we performed a dedicated database research combining the terms "cutaneous Squamous Cell Carcinoma", "cSCC", "locally advanced cutaneous SCC", "lacSCC", "NMSC surgery" with Boolean term "AND" with the specific topic of the sub-section (i.e., imaging, resection margins, radiotherapy, etc.).

Priority was given to studies with a higher level of evidence (systematic review, prospective design) but the majority of the studies presented a retrospective design.

Due to the limited number of available data inherent to some specific aspects of the research field, we included selected case reports and opinion papers in relation to their uniqueness and the marked adherence to the topic.

Besides the research limits some publishing bias has to be mentioned, as the small size of data considered in some articles, the discrepancy in number between study and control groups, and lack of systematic of some reviews.

The above reasons have precluded a robust statistical data analysis, and further randomized trials are strongly recommended before drawing any definitive conclusion.

AUTHOR CONTRIBUTIONS

TB, concept and design, data acquisition and analysis, drafting, accountability for all aspects of the work. GA, data acquisition, drafting, accountability for all aspects of the work. PT, concept and design, accountability for all aspects of the work. GM, drafting, accountability for all aspects of the work. AL, concept and design, accountability for all aspects of the work. BB, data analysis, drafting, accountability for all aspects of the work. VV, data analysis, drafting, accountability for all aspects of the work. FB, concept and design, drafting, accountability for all aspects of the work. All authors contributed to the article and approved the submitted version.

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The Role of microRNA in Pathogenesis, Diagnosis, Different Variants, Treatment and Prognosis of Mycosis Fungoides

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Mycosis fungoides (MF) is the most common type of cutaneous T-cell lymphoma (CTCL), accounting for approximately 50% of all CTCLs. Although various molecular changes in MF have been described in existing studies, no obvious disease-specific changes have been found thus far. microRNAs (miRs) are short, noncoding RNA molecules that play roles in the post-transcriptional regulation of oncogenes and tumor suppressor genes in various diseases. Recently, there has been rapidly expanding experimental evidence for the role of miRs in the progression, early diagnosis, prognosis prediction for MF. Efforts to improve early diagnosis and develop personalized therapy options have become more important in recent years. Here, we provide an overview and update of recent advances regarding miRs associated with MF. Furthermore, we provide insights into future opportunities for miR-based therapies.

Keywords: microRNA, Mycosis fungoides, pathogenesis, diagnosis, treatment, prognosis

INTRODUCTION

Mycosis fungoides (MF) is the most common type of cutaneous T-cell lymphoma (CTCL), accounting for approximately 50% of all CTCLs. The clinical manifestations usually present with erythematous patches and plaques with an indolent course and may slowly progress to tumors (1). Similar to inflammatory dermatosis, the lesions of MF patients in the stable stage can last for decades with a favorable prognosis. The period between the onset of skin lesions and diagnosis can vary from several months to years, with some patients' diagnoses delayed by more than four decades (2). However, in a proportion of early MF patients, the disease progresses rapidly and enters a more advanced stage with visceral spread, requiring more aggressive treatment regimens (3). Unfortunately, it is currently difficult to distinguish inflammatory dermatosis from early MF and identify patients with favorable or poor prognosis before treatment. Therefore, new approaches are needed to improve the accuracy of the early diagnosis and predict the prognosis of MF.

Although various molecular changes in MF have been described in existing studies, including chromosomal, genomic, and gene expression aberrations; no obvious disease-specific changes have been found thus far (4). MF is a clonal disorder with specific T-cell receptor (TCR) gene rearrangement. In addition to clinical and histological findings, TCR clonality testing is a helpful adjunct diagnostic method (5). However, the sensitivity of TCR rearrangement detection varies greatly according to different clinical stages, methods of assay and primer design (6–8). Therefore, there is still an urgent need to explore more specific and sensitive biomarkers to help us better understand and manage MF. MicroRNAs (miRs) are short noncoding RNA molecules that play roles in post-transcriptional regulation by binding to RNA-induced silencing complexes and controlling physiological and pathological processes in various diseases (9). In addition, miRs play important roles in tumorigenesis and function as oncomiRs or tumor suppressors by regulating the levels of oncogenes or antioncogenes (10–12). The miR expression profiles in MF have been studied extensively and have shown a high correlation with disease progression, prognosis, and response to treatment (**Table 1**) (11–42). In this review, the function and molecular mechanism of miRs in the progression, diagnosis, variants, prognosis, and treatment of MF are discussed in detail (**Figure 1**).

THE FUNCTION OF miR AND CLINICAL APPLICATION IN TUMOR

miR is a short, noncoding RNA molecule that can regulate mRNA expression at the post-transcriptional level and is widely found in viruses, plants and animals. miR is broadly involved in a variety of physiological and pathological processes, and dysregulated miR expression is related to cancer initiation and progression (10). It does not encode functional proteins but can degrade or inhibit protein translation by means of complementary pairing with target mRNA and eventually inhibit specific gene expression (43).

In recent years, many studies have shown that miR is involved in the occurrence and development of tumors and has a broad clinical application space in the diagnosis and treatment of human cancers and hematological malignancies. Specifically,

TABLE 1 | miR expression profiles of available studies in MF.

MiR ID	Expression	Functional role	Reference
miR-155	upregulate	diagnosis, progression, different variant (FMF), prognosis prediction, treatment	(11, 13–28)
miR-203	downregulate	diagnosis	(16, 19, 24)
miR-205	downregulate	diagnosis(racial differences)	(19, 24)
miR-92a	upregulate	progression, differential diagnosis, different variant(FMF, TMF)	(15, 23)
miR-93-5p	upregulate	different variant(FMF,TMF)	(13, 29)
miR-93	upregulate	progression, differential Diagnosis, different variant(FMF),treatment	(12, 15, 17, 24, 29–31)
	downregulate	early diagnosis	(32)
miR-19b	upregulate	different variant(FMF)	(23)
miR-34a	upregulate	different variant(FMF)	(13)
miR-223	upregulate	different variant(FMF), treatment response prediction	(13, 33)
miR-191	upregulate	treatment response prediction	(33)
miR-342	upregulate	treatment response prediction	(33)
miR-181	upregulate	progression	(30)
miR-181a	upregulate	diagnosis, progression, different variant(FMF,TMF)	(13, 30, 34, 35)
miR-181b	upregulate	different variant(TMF)	(13)
miR-338-3p	upregulate	prognosis prediction	(36)
miR-148a-3p	upregulate	prognosis prediction	(36)
miR-106b	upregulate	progression, prognosis prediction	(14, 36, 37)
miR-106b-5p	upregulate	progression, prognosis prediction	(36)
let-7a	downregulate	prognosis prediction	(15)
miR-17~92	upregulate	progression, different variant(unilesional MF)	(38)
miR-243	upregulate	diagnosis	(16)
miR-22	downregulate	progression	(39)
miR-200b	upregulate	diagnosis, good prognosis	(16)
miR-146a	upregulate	diagnosis	(34)
miR-222	not mentioned	diagnosis	(34)
miR-26a	not mentioned	diagnosis	(34)
miR-142-3p	upregulate	diagnosis	(16)
miR-130b	upregulate	differential diagnosis	(16)
miR-195-5p	downregulate	progression	(37, 40)
miR-122	upregulate	progression, treatment	(41)
miR-15a	downregulate	progression, prognosis prediction	(17)
miR-16	upregulate	progression, clinical course prediction	(15, 32)
	downregulate	diagnosis, progression, clinical course prediction	(17)
miR-21	upregulate	differentia diagnosis, clinical course prediction	(14)

FMF, Folliculotropic mycosis fungoides; TMF, mycosis fungoides with larger cell transformation.

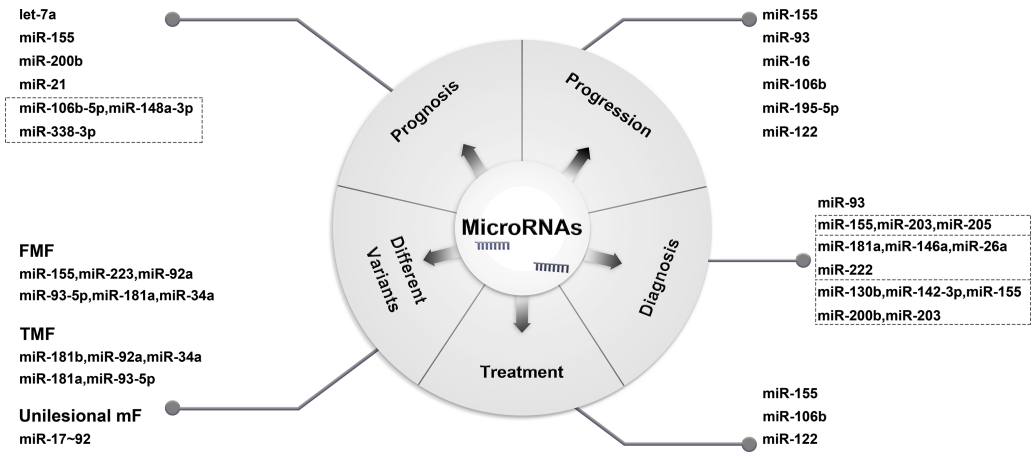

FIGURE 1 | Functions of miR in MF.

miR in serum or tumor tissue samples can be used as potential tumor markers for early diagnosis, such as breast cancer and B-cell lymphoma (44–46). In addition, studies have shown that miR can be used as a specific molecular target for targeted therapy (9, 47). Currently, miR mimics and miRNA inhibitors in the preclinical phase of drug development have shown potential as novel therapeutic drugs in tumor-treating fields.

miR AND CUTANEOUS T-CELL LYMPHOMA (CTCL)

CTCL comprises a heterogeneous group of disorders with variable clinical presentations, histological features, and prognoses. Growing evidence demonstrates that miR is involved in the development and progression of CTCL (11, 16, 39, 48–50). There are significant similarities and differences in the expression of miR among different variants of CTCL. For instance, miR-155, which was first identified as abnormally expressed in CTCLs, can be used as an oncogenic driver to promote tumor growth in both MF and anaplastic lymphoma kinase (ALK)-negative anaplastic large-cell lymphoma (ALCL) (18, 51). There exists a significant difference in the miR expression profile between tumoral MF, erythrodermic MF and the more aggressive leukemic variant of CTCL—Sezary syndrome (SS)—such as the miR-155, miR-21, miR-93, miR-195–5p, and miR-17/92 (14, 15, 52, 53). Additional, Dercer expression have been served as an molecular marker in MF and might be of clinical relevance in MF, lymphomatoid papulosis and primary cutaneous CD4-positive small/medium T-cell lymphoma (54). To date, the mechanism of miR dysregulation in CTCL has not been fully elucidated and recognizing the abnormal expression of miR among different subgroups of CTCL variants, especially MF, is particularly important for elucidating the pathogenesis, early diagnosis, and identification of new therapeutic targets of CTCL.

miR AND MF

miR Could Be a Gene Regulator in the Pathogenesis and Progression of MF

The molecular pathogenesis of MF remains limited. miR may function as an oncogenic or tumor suppressor and contribute to the pathogenesis and progression of MF through interactions with specific target genes.

miR-155 acts as an oncogenic miR and is overexpressed in multiple solid tumors and B-cell lymphoma (55–57). Currently, it is one of the most intensively studied miRs in MF. Significant upregulation of miR-155 and miR-92a in tumoral MF was first observed by Van Kester et al. in 2011 (15). Subsequent studies further identified the overexpression of miR-155 in both early and advanced MF, and the expression level of miR-155 in biopsy samples increased with increasing clinical stage (17, 18, 26). *In vivo* and *in vitro* experiments confirmed that miR-155 plays an important role in the development of MF and contributes to tumor growth by decreasing G2/M arrest and apoptosis (20). Additionally, previous studies indicated that the JAK/STAT5 pathway can promote the expression of miR-155 and promote the proliferation, growth, and survival of malignant MF cell lines *in vitro* (11). Interestingly, microbes have also been implicated in disease progression in CTCL (58, 59). A recent study showed that S. aureus and its enterotoxins might enhance miR-155 expression and promote disease progression by stimulating the expression of post-transcriptional regulators of malignant T cells (21).

Moreover, miR-93 has been described as an oncogene miR that can prevent apoptosis and promote tumor cell survival in various cancers (12, 31). miR-93 is overexpressed in advanced MF compared with inflammatory dermatosis and functions in the progression of MF (15, 17, 24, 30). Gluud et al. found that miR-93 can interfere with the expression of tumor suppressor cyclin-dependent kinase inhibitor 1 (p21) in MF tumor T cell lines. In turn, the expression of the p21 protein was significantly increased in cells (MF2059 and MF3675) transfected with a miR-

93–5p inhibitor, resulting in a 20–30% decrease in the proliferation of malignant T cell lines (29). In addition, Katona et al. observed a trend toward a loss of PTEN expression with histological progression of MF (60). The tumor suppressor gene PTEN is a known target of the miR-93–5p and miR-181 families (61, 62). However, contrary to the findings of previous studies, a recent study on miR-93 found that the expression of miR-93 was significantly downregulated in both early and advanced MF compared with normal and eczema cases (32). Therefore, further studies with larger cohorts of MF patients are needed to explore the role of miR-93 in the progression of MF.

In addition to miR-155 and miR-93, miR-16 dysregulation may also play a certain role in MF progression. Maj et al. found that the decrease in miR-15a and miR-16 is related to the development of advanced MF (17). This finding is consistent with the results of most previous studies regarding miR-16 as a tumor suppressor in various tumors, including pituitary adenomas and chronic lymphocytic leukemia (CLL) (63, 64). However, van Kester et al. observed that miR-16 in tumoral MF was upregulated compared with inflammatory dermatosis (15). Similarly, additional evidence suggests that miR-16 was significantly upregulated in advanced MF compared with patients at early stage and could be used to predict aggressive clinical course (32). Thus, more studies are needed to explore the specific expression and biological function of miR-16 in MF.

Recent studies have indicated that miRs not only play an important role in MF tumors but also in the tumor microenvironment (TME). miR-106b expression was observed in dermal T lymphocytes in skin lesions from patients with MF, and the expression level increased as the disease progressed. miR-106b can promote tumor proliferation *in vitro* by inhibiting the tumor suppressor p21 and thioredoxin-interacting protein (37). In addition, the local expression of miR-106b in stromal cells indicated that miR-106b may play potential roles in the MF TME. Microenvironment-mediated changes in miR expression in tumor cells mediating progression have also been highlighted. Research has shown that cancer-associated fibroblasts (CAFs) can protect MF cells from doxorubicin-induced cell death and promote migration through the secretion of CXCL12 (65). However, the role of miRs in MF progression between tumor cells and matrix components in the TME has not been elucidated. The value of the TME in exploring the pathogenesis of MF deserves further investigation.

In addition to the abovementioned oncogenic miRs, the cumulative inhibition of multiple tumor suppressor miRs may lead to the downregulation of multiple signaling pathways driving the disease progression of MF (66). miR195–5p may play a role as a tumor suppressor in MF, and its inhibitory effect is related to disease progression (37). The upregulation of miR-195–5p inhibits cell cycle arrest through the downregulation of ADP-ribosylation factor-like protein 2 (ARL2), and low expression of miR-195–5p in MF skin lesions may promote disease progression (40). In addition, the role of miR-22 as a tumor suppressor in numerous solid tumors is widely accepted, and low miR-22 expression is associated with advanced stage and metastasis (67). *In vitro* studies have shown that miR-22 is significantly downregulated in malignant CTCL T cell lines (MyLa2059), and Jak3/STAT pathway-mediated inhibition of miR-22 may play a key role in CTCL pathogenesis and progression (39).

miR Can Serve as Diagnostic Biomarkers in MF

Early clinical and pathological diagnosis of MF remains a challenge because of its clinicopathological similarity to benign inflammatory disorders, which may also exhibit clonal TCR rearrangement in some conditions (68, 69). Also, the lack of specific molecular markers that can reliably differentiate the malignant T-cells in MF from the reactive T cells in benign inflammatory disorders. Once entering the advanced stage, the median survival of patients with MF is only 1–5 years, with a 5-year survival rate of less than 15% (70). Therefore, the search for diagnostic molecules is still needed for early diagnosis and then timely treatment.

With the deepening of research, there is growing evidence to support the biomarker potential of miRs for the diagnosis of cancer. miRs that have been confirmed to be related to the early diagnosis and differential diagnosis of MF include miR-93, miR-146a, 146b-5p, miR-342–3p, miR-16, miR-181, miR-203, and miR-205 (24, 30, 34, 71). Specifically, miR-93 not only plays a role in the pathogenesis of MF but can also be used as a specific biomarker for the diagnosis of MF. The significant downregulation of miR-93 can be used for the early diagnosis of early challenging cases (32).

Although some single miRs do not have independent diagnostic value in MF, the specific combinations of miRs may achieve good diagnostic ability. As mentioned before, miR-155 plays an important role in the pathogenesis and progression of MF. However, given that higher miR-155 expression was also observed in T-cell-rich benign inflammatory dermatoses compared with early MF and folliculotropic MF (FMF), it cannot be used as a separate biomarker to distinguish early MF from benign inflammation dermatosis (18, 27). Interestingly, the combination of miR-155 with specific miRs has been proven to be of great value in differential diagnosis and early diagnosis in multiple studies. Ralfkiaer et al. developed a three-miR classifier composed of miR-155, miR-203, and miR-205 that can distinguish CTCL from benign inflammatory dermatosis with an accuracy of more than 90% (24). The strength of this classifier was also confirmed in subsequent independent cohort studies (71). Moreover, in view of the stability of miRs in serum or plasma, Dusilkova et al. established a plasma multiple miR classifier based on the upregulation of miR-155 and downregulation of miR-203/miR-205 to detect CTCL with 100% specificity and 94% sensitivity, making routine clinical monitoring possible in the future (19). In addition, other investigations have suggested that a four-miR classifier composed of miR-181a, miR-146a, miR-222, and miR-26a could discriminate tumoral MF from benign inflammatory disease (34). Moreover, miR-181a and miR-146a may be used as specific biomarkers of MF and are significantly upregulated in both early and advanced MF (30, 34, 36). Importantly, there may

be racial differences in the application scope of this classifier. An additional 5-miR diagnostic classifier, including miR-130b, miR-142–3p, miR-155, miR-200b, miR-243, and miR-203, was established for the diagnosis and prognosis of CTCL in a study of an Asian population (16). Taken together, the diagnostic classifier combined with multiple miRs has better diagnostic intensity and accuracy and is expected to be a valuable adjunct in future clinical work.

Is miR Differentially Expressed in Different Variants of MF?

According to its clinicopathologic characteristics, MF can be divided into several variants (72). Folliculotropic MF (FMF) is a rare variant of MF with distinct clinicopathological features. The clinical course and treatment response varies according to different stage of disease (early- and advanced-stage) (73, 74). Specifically, patients with FMF presenting with only patches and/or follicular papules (early-stage) had a favorable prognosis with a 5-year overall survival (OS) of 92%, while patients with FMF presenting with tumors and/or nodules (advanced-stage) had a 5-year OS of 50%. Additionally, a small number of MF patients may undergo large cell transformation (TMF), which is characterized by an aggressive clinical course and refraction to systemic therapies including anthracyclines, bexarotene, methotrexate with a median survival of 18.4 to 24 months (75–77). However, the molecular background of FMF and TMF has not been fully elucidated. Marosvári D et al. showed for the first time in 2015 that miR-93–5p, miR-181a, and miR-34a were significantly upregulated in FMF and TMF. Overexpression of miR-155 and miR-223 was also observed in FMF (13). Additionally, Garaicoa et al. compared the miR expression profile among tumoral MF, FMF, and TMF and found that the expression levels of miR-19b, miR-92a, and miR-155 in FMF and TMF were higher than those in tumoral MF (23). In recent years, some scholars have proposed that according to different clinicopathological features, FMF can be categorized in early stage with indolent clinical course and advanced/tumoral stage requiring aggressive treatment (74, 78). Atzmony et al. found that there was a significant difference in miR-155 expression between early and tumoral FMF, but there was no significant difference in miR-155 expression between early FMF and MF or between tumoral FMF and MF (27). To some extent, this finding confirms that there might be two different stages of FMF, but the relationship between MF and FMF in different stages still needs further research and exploration to provide a theoretical basis for personalized treatment of different stages and subtypes of MF. In addition, the study showed that miR-181b and miR-93–5p were highly expressed in the TMF, while the level of miR-155 was not significantly increased, indicating that miR-181b and miR-93–5p may play a role in the pathogenesis of the TMF, while the regulation of miR-155-related gene expression may not be involved in large cell transformation (13).

In some special cases, it is difficult to distinguish erythroderma MF (eMF) from Sézary Syndrome (SS) clinically and histologically. Due to the differences in treatment recommendations and prognosis, it is necessary to distinguish between eMF and SS. Rittig et al. found that there was a significant difference in the miR expression profile between eMF and SS. In particular, the expression levels of miR-106b, miR-155, and miR-21 in eMF were significantly lower than those in SS (14).

Unilesional MF is characterized by a solitary erythematous patch or plaque located on the trunk and upper extremities clinically and is histologically indistinguishable from typical MF. Unlike early MF, unilesional MF can maintain a benign clinical course without any treatment, and there is no obvious recurrence after treatment. Studies have shown that the miR expression profile of unilesional MF is different from that of early MF. The former has a high level of miR-17~92 members and is accompanied by Th1 skewing (38). The antitumor activity of miR-17~92 has been confirmed in numerous previous studies (79, 80). These findings suggest that there is a strong reactive T cell immune response in unilesional MF, which may explain the locality of the disease.

Existing miR-Based Therapeutics and Potential Treatment Options in MF

Local and systemic therapies available for MF have reduced tumor burden and improved quality of life. However, classic regimens based on anthracycline or nucleoside analogs can only obtain a short-lived response and have had limited impact on the survival of patients with advanced MF (81, 82). Therefore, advances in MF treatment research are focused on identifying new pharmacological targets. Current clinical trials show that miR-based treatment seems to be feasible (83). Given the important role of miR-155 in MF, Moyal et al. found that miR-155 promotes tumor growth in xenografted MF mice by reducing apoptosis. Anti-miR-155 can be used as monotherapy or in combination with apoptosis therapy and cell cycle checkpoint inhibitors to improve the effectiveness of MF therapy (20). Indeed, clinical trials were conducted to test the therapeutic efficacy of a miR-155-targeting nucleic acid modification inhibitor called cobomarsen (MRG-106) in MF in 2018 (26). Cobomarsen can reduce the expression of multiple gene pathways related to cell survival by blocking miR-155 and finally reduce cell proliferation and activate apoptosis. Therefore, it can be suggested that cobomarsen can potentially be used as a therapeutic agent for MF (26). Surprisingly, active antimicrobial therapy can inhibit the activity of MF by affecting the expression of miR-155 (22, 25). Indeed, Duvich et al. reported that the combination of antibiotics, a germicidal whirlpool bath system, and steroids has a significant effect on patients with SS. *In vitro* experiments demonstrated that active antibiotic therapy may inhibit malignant T cell proliferation in advanced CTCL by inhibiting the staphylococcal enterotoxin (SE)-mediated bystander T cell response (35). Recent studies have further shown that this inhibitory effect may be achieved by partially reversing SE-induced pathological processes involving STAT5 and miR-155 (21). Notably, *Staphylococcus aureus* is also present to a greater extent in skin lesions in patients with MF than in patients with non-lesional or healthy skin. miR-155 may be associated with secondary skin infection in patients with MF.

Additionally, the involvement of other infectious agents in MF, such as *Borrelia burgdorferi, Chlamydophila*, have been described (84). A previous study by Tothova et al. found that *Borrelia* might exert its causative role in MF through a chronic type-1 immune response with interferon-γ production (85). Besides, evidence of type C pneumonia in MF biopsies suggests an association between *Chlamydophila* infection and MF development (86). However, further studies are needed to assess the efficacy of anti-*Borrelia* therapy in treating *Borrelia*-positive MF and verify the correlation between *Chlamydophila* and MF in larger cohorts. In view of the important role of antimicrobial therapy and miRs in MF, the combination of miR-based therapy and antimicrobial therapy may produce synergistic therapeutic effects and improve the therapeutic efficacy of patients with MF, especially for advanced MF with large-area involvement.

Interestingly, miR-106b was previously known to provide the strongest prognostic prediction of a high risk of progression (87). Given that miR-106b is also highly expressed in the early stage of MF, the development of miR-106b inhibitors applied in the early stage of the disease may prevent or delay disease progression (36). In addition, inhibition of miR-93 resulted in decreased proliferation of malignant T cell lines. The effect of this reduction in proliferation was similar to that observed following inhibition of miR-155 (11, 29, 88). Thus, anti-miR-93 could be a valuable therapeutic agent for patients with MF. Furthermore, researchers found that miR-122 was overexpressed in advanced MF, reducing sensitivity to chemotherapy-induced apoptosis through signal transduction circuits involved in Akt activation and p53 inhibition. This finding provides a new idea for the use of chemotherapy sensitizers in advanced MF (41). In addition to the development of the abovementioned miR-based therapeutic drugs, the miR expression spectrum can also predict treatment response. Studies have shown that MF patients with rapidly elevated levels of miR-223, miR-191, and miR-342 after extracorporeal blood collection are more likely to show good clinical responses after 6–12 months of treatment (42).

miR May Play an Important Role in Predicting Prognosis in MF

miRs not only contribute to increasing the accuracy of diagnosis but can also be used to predict prognosis. Several studies have emphasized that miRs can be used as a potential prognostic biomarker in MF. For instance, the miRs of the let-7 family are downregulated in advanced and metastatic MF. The prognosis of patients with low expression of let-7a was worse than that of other patients (15). Of note, although multiple studies have shown that miR-155 can be used as a prognostic indicator of CTCL, its prognostic value in MF and the relationship between the intensity of miR-155 expression and disease stage are discrepant (16, 17, 26). Shen et al. found that miR-155 was associated with worse clinical outcomes in an Asian population with a total of 158 CTCLs (including MF, cutaneous anaplastic large cell lymphoma, peripheral T cell lymphoma and NK/T cell lymphoma). However, excluding other types of CTCL than MF, the expression of miR-155 had no significant correlation with the overall survival of patients with different stages of MF (16). The inconsistency in the above findings may be attributed to the different ethnic backgrounds of the patients and varied numbers of patients with different disease stages enrolled in the above studies. Comparatively, miR-200b is significantly associated with the overall survival of MF patients regardless of disease stage (16). High miR-200b expression implied favorable prognosis. Taken together, there may be differences in prognostic markers among subgroups of CTCLs, and it is necessary to develop specific prognostic biomarkers for MF patients.

Early identification of patients at a higher risk of progression may facilitate more individualized treatment of these patients. Therefore, it is worth drawing attention to the prognostic stratification of patients with early MF. Lindahl et al. developed and verified a 3-miR prognostic classifier based on miR-106b-5p, miR-148a-3p, and miR-338–3p, which can successfully divide patients into high- and low-risk groups for disease progression. This 3-miR classifier may be an effective tool to predict the progression of early MF at the time of diagnosis and has important prognostic value (36). Among them, miR-106b is the most powerful prognostic marker of disease progression in MF. As the disease progresses, miR-106b can regulate the expression of the tumor suppressor genes p21 and thioredoxin-interacting protein (TXNIP) and promote the proliferation of tumor cells in MF (37). Additionally, there were also differences in the expression levels of miR-16, miR-93, and miR-106a between progressive and nonprogressive patients (30). Of note, there may also be racial differences in the application scope of this classifier. Shen et al. verified that miR-155 and miR-200b can be used as effective predictors of clinical outcome in Asian populations (16). Therefore, it is necessary to develop and validate prognostic miR classifiers among populations with different racial backgrounds.

Future Perspectives of Exosomal miR-Based Therapeutics in MF

In addition to the tumor cells themselves, the malignant tumor phenotype can also transmit genetic information, including miRs, to other cells in the tumor microenvironment through exosomes, which can promote proliferation, angiogenesis, metastasis, and drug resistance. Moyal et al. first identified miR-155 and miR-1246 in exosomes derived from MF cell lines in 2021. It was confirmed that exomiR-155 can promote the motility of MF cell lines *in vitro*. In addition, exomiR-1246 expression levels appeared to be correlated with MF staging, and were significantly higher in plaque/tumor MF patients than in healthy population (28). Moreover, exosomal miRs can affect extracellular matrix (ECM) and immune system activation and recruitment to promote tumor cell survival. Furthermore, exosomal miRs can be used as promising noninvasive biomarkers and potential targeting factors and delivery vehicles for tumor diagnosis and treatment. Therefore, exosomal miR-based therapeutics are a potential feasible candidate therapy for MF.

CONCLUSION

MF is the most common cutaneous T-cell lymphoma. The main problem in the diagnosis and treatment of MF is the inefficient methods of early diagnosis and short-lived response to classical chemotherapy. The emergence of the role of miR in cancer progression has prompted us to elucidate the prospects of miR as new therapeutic target. Several studies have identified them as potential diagnostic and prognostic biomarkers in MF. The identification of these new gene regulatory targets opens up a new field for the diagnosis, treatment and prognosis of MF. However, aberrant miR expression have been detected in a variety inflammatory disease and malignancies, and disease-specific miR remains as one of the most challenging issues. Additionally, the functions of partial miRs in MF remain controversial and further investigation and validation in larger cohorts are needed. Also, miR-based therapeutics in CTCL is still in budding stages, the reliable and targeted site-specific delivery of miR might be a major obstacle to the use of miR-based therapy. Thus, it is urgently needed to overcome the above obstacles before development of novel miR-based therapeutics strategy.

AUTHOR CONTRIBUTIONS

PFW designed and wrote this article. YX made a contribution to revision. LW designed the whole project and revised the final manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Immune Checkpoint Inhibition in Non-Melanoma Skin Cancer: A Review of Current Evidence

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Immuno-oncology is a rapidly evolving field with growing relevance in the treatment of numerous malignancies. The prior study of immunotherapy in dermatologic oncology has largely focused on cutaneous melanoma. However, recent focus has shifted to the use of immunotherapy to treat non-melanoma skin cancers (NMSCs), such as basal cell carcinoma (BCC), cutaneous squamous cell carcinoma (cSCC), and Merkel cell carcinoma (MCC). NMSCs represent the most ubiquitous cancers globally and, while they have a lower propensity to develop into advanced disease than cutaneous melanoma, their absolute mortality burden has recently surpassed that of melanoma. Patients with advanced NMSC are now benefiting from the successes of immunotherapy, including checkpoint inhibition with anti-CTLA-4 and anti-PD-1 monoclonal antibodies. In this review, we discuss the existing clinical evidence for immunotherapy in the treatment of NMSCs, with an emphasis on checkpoint inhibitor therapies. We highlight key studies in the field and provide up-to-date clinical evidence regarding ongoing clinical trials, as well as future study directions. Our review demonstrates that checkpoint inhibitors are positioned to provide unparalleled results in the previously challenging landscape of advanced NMSC treatment.

Keywords: non-melanoma skin cancer (NMSC), immunotherapy, squamous cell carcinoma (SCC), basal cell carcimoma (BCC), merkel cell carcinoma (MCC), immune checkpoint inhibition (ICI)

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INTRODUCTION

Recent advances in the field of immuno-oncology have translated into breakthrough treatments for many solid and hematological malignancies. The study of immunotherapy in dermatologic oncology has largely focused on cutaneous melanoma, a disease that is more likely to metastasize and become life-threatening as compared to other non-melanoma skin cancers (NMSCs) such as basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC). Indeed, patients with advanced cutaneous melanoma were some of the first to significantly benefit from studies of checkpoint inhibition with anti-CTLA-4 and anti-PD-1 monoclonal antibodies. Despite a lower propensity to develop into advanced disease, NMSCs still remain a significant burden on the

healthcare system (1–11). Indeed, NMSCs are the most prevalent cancer globally and the absolute number of deaths each year attributed to BCCs and cSCCs in the US is now greater than that of melanoma.

Patients with advanced NMSC are now benefiting from the successes of immunotherapy previously observed in melanoma. Like cutaneous melanoma, NMSCs are generally characterized by UV damage, which translates into a high tumor mutational burden (TMB). High TMB is associated with the formation of neoantigens, the putative targets of immune cells that recognize and eradicate neoplastic cells. As such, immunotherapeutic strategies used in the treatment of melanoma that energize the immune system against these numerous tumor antigens, as in the case of checkpoint inhibitors or oncolytic viral immunotherapies, would also be predicted to be effective treatments for NMSCs (7, 12). In some cases, these therapies have demonstrated efficacy and are already being applied in the clinic.

In this review, we will discuss the existing clinical evidence for immunotherapy in the treatment of NMSCs, with an emphasis on checkpoint inhibitor therapies. We also discuss possible reasons for heterogeneity of responses among NMSC, ongoing clinical trials, and future study directions for immunotherapy as a therapeutic approach for NMSC.

EPIDEMIOLOGY

NMSCs are the most ubiquitous cancers in the world, estimated to account for over 30% of cancer diagnoses each year (1). However, accurate estimates are limited as many national tumor registries do not routinely assess highly prevalent NMSCs and epidemiologic models frequently fail to consider NMSC incidence in non-white populations. In addition, an assessment of the global burden of disease is challenging due to the need for more numerous population-based studies. While acknowledging the limitations of the epidemiological models available, current studies still point to the significant and growing public health burden NMSCs pose. One model estimates that in the US 5.4 million total NMSCs were diagnosed in 2012 (2). Additional models suggest that 2 million BCCs and 700,000 cSCCs were diagnosed in the US in 2012, whereas 2,488 MCCs were reported in 2013 (see **Figure 1**) (3–5). Globally, the incidence of NMSCs has continued to increase, rising 33% from 2007 to 2017 (1). In the US, the Rochester Epidemiology Project reported a 145% and 263% increase in the incidence of BCCs and cSCCs, respectively, between 1976 to 1984 and 2000 to 2010 (6).

Cumulative UV exposure is considered the chief risk factor in NMSC development (7). Accordingly, the rising global life expectancy and associated increase in total years of UV-exposure are posited as the drivers behind the substantial incidence changes observed. Mortality rates for NMSCs are relatively low, with case fatality rates for cSCCs ranging from 2.1%-2.8% (8, 9). Approximately 4.6% of cSCCs recur after excision and 3.7% progress to nodal metastases (9). However, due to their high prevalence, the absolute mortality from NMSCs remains significant. The absolute number of deaths from cSCCs in 2012 in the US was estimated to range from 3932 to 8791 in the white population alone (4). For comparison, from 2012 to 2016, melanoma absolute deaths in the US across all races and ethnicities was a mean of 9,008 per year, while in 2021 this number has decreased to 7,180 (10, 11) With the incidence of NMSCs predicted to rise at a significant rate, effective therapy is an imperative.

IMMUNOGENICITY

NMSCs represent a class of uniquely immunogenic cancers. In melanoma and other malignancies, TMB and expression of

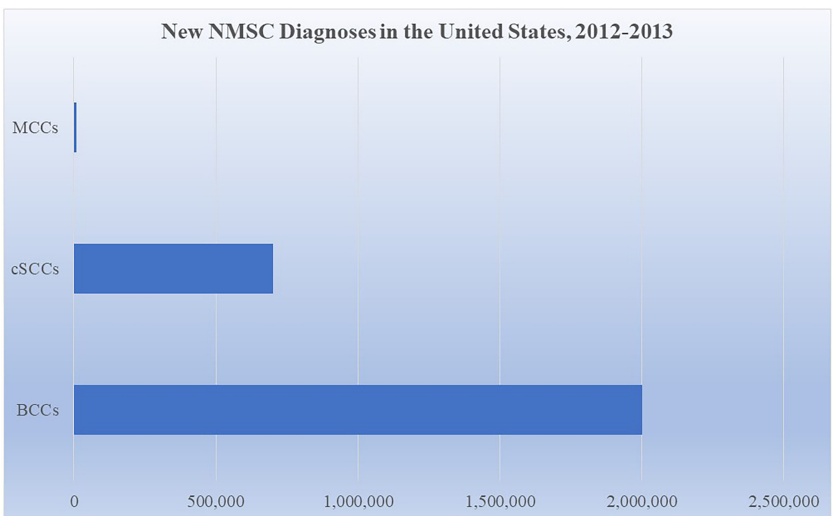

FIGURE 1 | Incidence of new NMSCs in the United States from 2012-2013 (3–5).

PD-L1 have been demonstrated to correlate with response to checkpoint blockade (12). In non-melanoma cutaneous malignancies, important differences exist in some of these immunological characteristics which may impact their responses to immunotherapy.

TMB

In 2017, Chalmers et al. published an analysis of the TMB in 92,439 tissue blocks representing over 100 tumor types (see **Table 1** for comparisons) (12). cSCCs and BCCs were found to have the highest TMB of all cancers surveyed, with 45.2 and 47.3 median mutations/Mb, respectively (12). Merkel cell polyomavirus (MCPyV) associated MCCs exhibit a median TMB of only 1.2 mutations/Mb, while non-virus associated MCCs have a high TMB of 53.9 median mutations/Mb (13). The considerably elevated TMB in non-virus associated NMSCs is believed to reflect the chronic carcinogenic effects of ultraviolet light exposure.

PD-L1 Expression

Absolute PD-L1 expression by tumor cells in BCCs ranges from 22% to 89.9%, while the expression by tumor-infiltrating lymphcytes (TILs) ranges from 82.0% to 94.9% (14, 15). Interestingly, Chang et al., 2017 investigated differences in PD-L1 expression in treated versus treatment-naïve BCCs (15). The cohort included 78 treated BCCs, with treatments comprising radiotherapy (n = 9), systemic chemotherapy (n = 58), and topical chemotherapy (n = 22), and 60 treatment-naïve BCCs. Topical chemotherapy included flourouracil (n=21) and imiquimod (n=1), while systemic agents included hedgehog pathway inhibitors (n=40), platinum agents (n=10), and gefitinib (n=5). Treated BCCs demonstrated greater intensity of PD-L1 expression in both tumors (32% vs 7%, P = .003) and TILs (47% vs 18%, P = .008), suggesting treatment may induce PD-L1 expression. A limitation of this study was that paired samples were not obtained from the same BCC before and after each treatment exposure. Therefore, while PD-L1 expression was associated with the above treatment modalities, the authors were unable to determine the direction of causality. However, as PD-L1 expression correlates with response to immunotherapy in other malignancies, these data imply that previously treated BCCs could possibly be more responsive to checkpoint inhibition.

In cSCCs, absolute PD-L1 expression by tumor cells ranges from 26.5% to 41% with expression by TILs reported to occur in 60% of cases (16, 17). Notably, multiple studies have suggested that high PD-L1 expression and greater intensity of expression correlate with risk of metastatic progression (17, 21). In a 2016 study by Slater and Googe, PD-L1 positivity was recorded in 20% of low grade tumors, 70% of high grade tumors, and 100% of metastases, with expression intensity increasing with grade (17). Of note, the majority of data on cSCCs derives from studies in immunocomptent patients, as compared to the subset of patients who develop cSCCs in the setting of chronic immunosuppression, especially organ transplant recipients. Accordingly, the use of 'cSCC' in this manuscript refers to tumors arising in the immunocompetent unless otherwise specified.

For MPyV-associated MCCs, PD-L1 expression by tumor cells and TILs has been reported at 50% and 56%, respectively (18). For non-virus associated MCCs these values are 0% and 25% (18). PD-L1 expression in MCCs may be a marker of a robust host immune response, with PD-L1 negative MCCs associated with a significantly lower overall survival (18).

Immunogenicity: BCCs *Versus* cSCCs

Higher TMB generally predicts favorable responses to immunotherapy. However, despite BCCs and cSCCs exhibiting similar TMBs, the responses of these tumors to both immune surveillance and immunotherapy diverge significantly. While the incidence of BCC:cSCC is 4:1 in the general population, in immunosuppressed organ transplant recipients, this incidence ratio shifts to favor cSCCs, with an incidence as a high as 1:10 (22). This suggests that SCCs are more frequently recognized by and vulnerable to immune surveillance than BCCs; therefore, in immunosuppressed patients, cSCCs appear more frequently.

The relative immune privilege of BCCs remains a topic of active investigation. However, a variety of characteristics have been noted that may explain it. First, BCCs have reduced capacity for antigen presentation than cSCCs. Most cSCCs display MHC-1, but BCCs have been found to have limited to no MHC-1 expression (22). In addition, BCCs have decreased levels of transporter associated with antigen presentation (TAP-1), which may impair antigen processing prior to presentation (23). However, comparisons of TAP-1 expression between cSCCs and BCCs have not been published. BCCs also exhibit reduced numbers of invasive front, peritumoral, and intratumoral CD8+ cells compared to cSCCs (22). This may be due in part to their aforementioned reduced expression of MHC-1, as it is required for antigen recognition by CD8+ effector T cells. Furthermore, BCCs promote a more favorable local cytokine milieu than cSCCs. Both BCCs and cSCCs express high levels of Il-10, which promotes a Th2 phenotype among surrounding T cells, impairing cell-mediated toxicity (24). Compared to cSCCs, BCCs also exhibit greater expression of Th-2 cytokines IL-4 and IL-5, as well as IL-1beta and IL-6, which

TABLE 1 | Immune Characteristics of NMSCs (12–20).

	TMB (median mutations/Mb)	PD-L1 expression (Tumor)	PD-L1 expression (TILs)
BCC	47.3	22%-89%	82-94%
cSCC (immunocompetent)	45.2	25-41%	60%
MCC (non-virus associated)	53.9	0%	25%
MCC (MPyV-associated)	1.2	50%	56%
Cutaneous melanoma	13.5	30%-35%	50%

have been associated with more aggressive tumor behavior (25). These differences in the molecular immunogenicity of cSCCs and BCCs have implications for their respective clinical responses to immunotheray, as will be discussed below.

IMMUNE CHECKPOINT INHIBITION FOR CUTANEOUS SQUAMOUS CELL CARCINOMA: EXISTING CLINICAL EVIDENCE

Immunotherapy for cSCCs has been trialed throughout the late 20th and early 21st centuries using interferons, interleukins, and imiquimod (26). Results were generally unimpressive, leaving providers searching for new therapies. In contrast to the treatment of cutaneous melanoma, where rapid drug development has led to a considerable array of FDA-approved therapies, the treatment of locally advanced and metastatic cSCCs has only recently seen its first, specific FDA-approved therapies (see **Figure 2** for a comparison of the number of FDA-approved agents approved in cutaneous melanoma and NMSCs from 2005-2021). The advent of checkpoint inhibition with PD-L1/PD-1 inhibitors and its use in cases of advanced cSCC, especially unresectable forms, drew attention for its potential to lead to remarkable results (see **Figure 3**). Historically, it was not until 2016 that a series of case reports lent credence to the potential of PD-L1/PD-1 inhibition to treat locally advanced and metastatic cSCC (26–28). Chang et al. described a report of an unresectable cSCC in a male in his 70s treated with an off-label trial of pembrolizumab, which led to significant tumor reduction and stable disease during the window of observation (27). Assam et al. subsequently reported a dramatic response to off-label pembrolizumab in a 67 year-old male with complete regression of an unresectable cSCC with an *MLH1* mutation (28). Later that year, Falchook et al. published the first case of a patient with metastatic cSCC treated with cemiplimab, then as part of clinical trial NCT02383212 (29).

In September of 2018, the FDA approved cemiplimab for metastatic and locally advanced cSCC following results from the aforementioned phase 1, open-label, multi-center, dose-finding trial with expansion cohorts (NCT02383212) as well as its follow-up phase 2 study (NCT02760498) (see **Figure 4** for a summary of FDA approvals of checkpoint inhibitors for NSMCs). In both studies, dosages were standardized at 3 mg per kilogram of body weight every 2 weeks (30). 108 patients, inclusive of locally advanced (n=33) and metastatic (n=75) disease, comprised the evaluable population (31). The ORR for both cohorts was 47%, with complete responders and partial responders representing 4% and 44% of the ORR, respectively (30, 31). Stratified ORR included 41-49% for patients with metastatic disease depending on dosage cohort and 44% for those with locally advanced disease (see **Table 2** for a summary of response kinetics associated with PD-L1 status in key trials) (32, 33). 12-month follow-up data following FDA approval demonstrated median observed time to response of 1.9 months (range: 1.7-9.1) and median progression-free survival of 18.4 months (34). As a comparison, the median overall survival in patients with cSCC treated with traditional chemotherapy alone, including EGFR inhibitors, was 15.3 months (95% CI, 10.4-21.0) overall, with 16.2 months for locally advanced cSCC and 15.3 months for metastatic cSCC (43). The most common treatment-related adverse events observed in patients underoing cemiplimab therapy included diarrhea (28.8%), fatigue (25.4%), and nausea (23.7%) (30). Immune-related adverse events of grade 3 or higher were reported in 13.6% of patients (30).

Following cemiplimab's FDA approval, investigation into the use of immunotherapy in cSCC continued with enthusiasm. In June of 2020, the FDA approved pembrolizumab for patients with recurrent or metastatic cutaneous squamous cell carcinoma. This was based on results from a phase 2 trial (NCT03284424) of 105 patients (35). The ORR was 34.3%, with 3.8% and 30.5% of patients

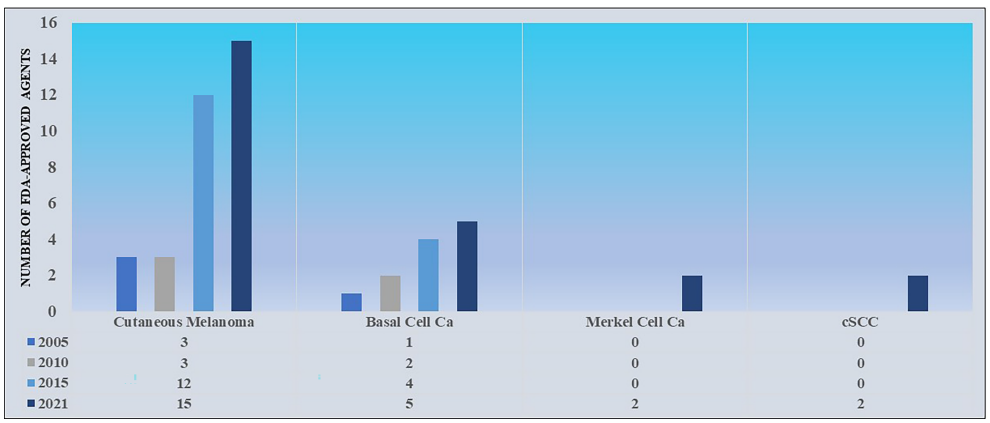

FIGURE 2 | A comparison of the number of FDA-approved agents approved for the treatment of cutaneous melanoma versus NMSCs from 2005-2021. Data sourced from FDA.gov.

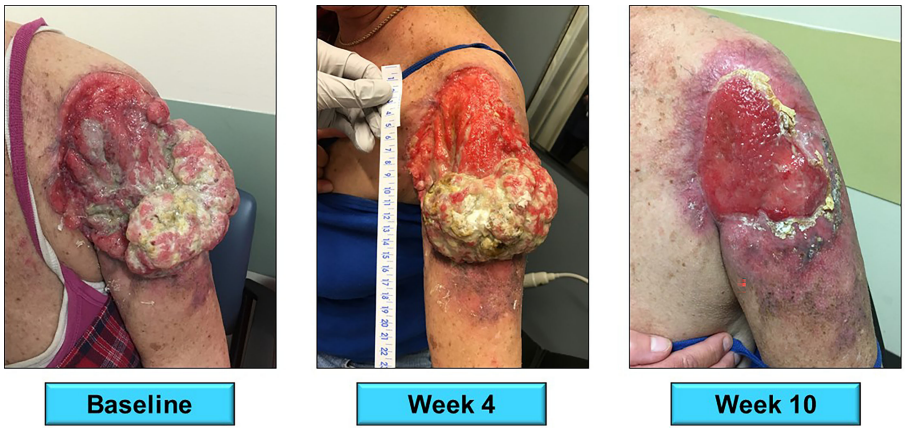

FIGURE 3 | A 59 year-old female presented with locally advanced cSCC of the left upper arm. The tumor had been present for five years per patient history. She received 8 doses of nivolumab 240mg (q2 weeks) from 3/2018 to 8/2018 with complete response. Her response after 10 weeks of therapy is presented above. A subsequent radical resection was negative for residual tumor.

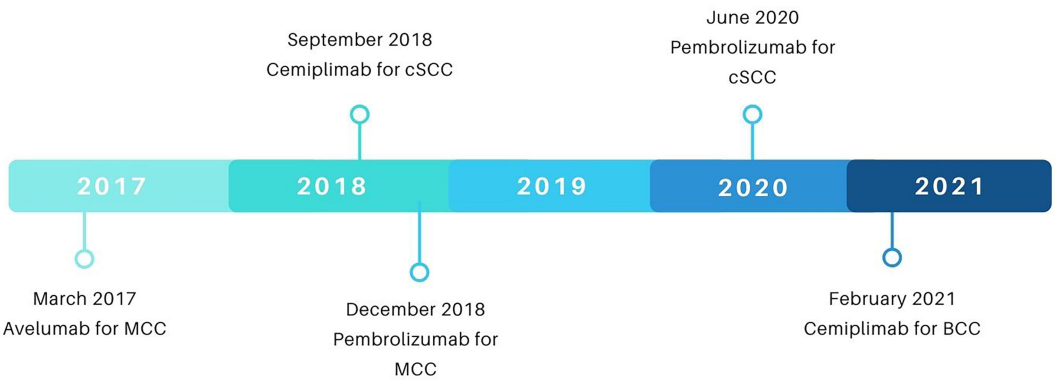

FIGURE 4 | A timeline of FDA approvals of checkpoint inhibitors for NSMCs. Data sourced from FDA.gov.

achieving a complete response or partial response, respectively. Sub-analysis by metastatic versus locally advanced disease was not available at the time of this review. Median duration of response was not reached; however, responses ranged from 2.7 to 13.1 months at the time of review. 79.5% of responders had an ongoing response past 6 months. Median progression free survival was 6.9 months, 12-month progression free survival rate was 32.4%, and 12-month overall survival rate was 60.3%. The most common adverse events were pruritus (14.3%), asthenia (13.3%), and fatigue (12.4%). 5.7% of patients had grade 3 or above treatment-related adverse events. In line with the above data, an additional study of pembrolizumab monotherapy in patients with unresectable cSCC (NCT02883556) demonstrated an ORR of 39% (36).

Cemiplimab remains the mainstay of most clinical regimens, due to more robust data, including higher patient numbers, longer follow-up and numerically better response rates. However, despite these numerical differences, it is unclear if this difference in efficacy between cemiplimab and other PD-1 agents, such as pembrolizumab, is truly significant. Other inhibitors are under active investigation for the treatment of cSCCs, including avelumab, nivolumab, and ipilimumab. Head-to-head comparison studies have not been conducted between these various agents, but would be necessary to definitely evaluate for true differences in efficacy.

IMMUNE CHECKPOINT INHIBITION FOR BASAL CELL CARCINOMA: EXISTING CLINICAL EVIDENCE

The initial evidence for immune checkpoint inhibition activity in BCC came from limited case reports in the mid-to-late 2010s describing responses in locally advanced and metastatic disease. Mohan et al. noted that a patient undergoing treatment with

TABLE 2 | Response and biomarker data in key NMSC immune checkpoint inhibition trials (32–42).

Indication	Trial	Patients	Agent	ORR	Median TTR	Median DOR	Median TMB responders (TMB NR)	ORR for PD-L1-	ORR for PD-L1+	Notes
BCCs										
laBCC	NCT03132636	84	Cemiplimab	31%	Not reported	Not reached	58.2 mut/Mb (23.5)	Not reported	Not reported	Prior HHI failure
mBCC	NCT03132636	28	Cemiplimab	21%	3.2 mo	Not reached	Not reported	Not reported	Not reported	Prior HHI failure
MCCs										
mMCC	NCT02155647	88	Avelumab	33%	Not reported	40.5 mo	Not reported	19%	37%	Prior treatment
mMCC	NCT02155647	116	Avelumab	40%	Not reported	18.2 mo	Not reported	33%	62%	Treatment-naïve
mMCC	NCT02267603	25	Pembrolizumab	56%	2.8 mo	Not reached	Not reported	57%	61%	Treatment-naïve
Advanced MCC	NCT02488759	22	Nivolumab	68%	2.0 mo	Not reached	Not reported	Not reported	Not reported	With or without prior treatment
cSCCs										
laSCC	NCT02760498	78	Cemiplimab	44%	1.9 mo	Not reached	74 mut/Mb (29)	35%	55%	With or without prior treatment
mSCC	NCT02760498	59	Cemiplimab	49%	1.9 mo	Not reached	53.2 mut/Mb (19.4)	Not reported	Not reported	3mg/kg q2w group; with or without prior treatment
mSCC	NCT02760498	56	Cemiplimab	41%	2.1 mo	Not reached	61.4 mut/Mb (13.7)	Not reported	Not reported	350 mg q3w group; with or without prior treatment
laSCC and mSCC	NCT03284424	105	Pembrolizumab	34%	1.5 mo	Not reached	Not reported	20%	33%	With or without prior treatment
laSCC and mSCC	NCT02883556	34	Pembrolizumab	39%	Not reported	Not reached	Not reported	Not reported	Not reported	Treatment-naïve

laBCC, locally advanced basal cell carcinoma; mBCC, metastatic basal cell carcinoma; laSCC, locally advanced squamous cell carcinoma; mSCC, metastatic squamous cell carcinoma; mMCC, metastatic merkel cell carcinoma; OR, Objective response rate; TTR, Time to response; DOR, Duration of response; TMB, Tumor mutational burden; NR, Non-responder; PD-L1-, PD-L1 expression <1%; PD-L1+, PD-L1 expression >=1%; mut/mb, mutations per megabase; mo, month; HHI, Hedgehog inhibitor.

ipilimumab for metastatic melanoma achieved an incidental regression of locally advanced BCC (44). Ikeda et al. reported a patient with metastatic BCC who achieved near complete remission after treatment with nivolumab (45). Lipson et al. describe a patient with BCC metastatic to the lung who achieved a durable partial response to pembrolizumab (14). Other reports further gave credence to the thesis that formal studies of immune checkpoint inhibition in BCC were warranted (46, 47).

In 2019, the first clinical trial showed immune checkpoint inhibition activity in BCC from a Phase 1/2 investigator-initiated open-label study of pembrolizumab with or without the hedgehog inhibitor vismodegib (NCT02690948) in patients with advanced BCC (48). Of the 9 patients who received pembrolizumab alone, 44% (n=4) achieved partial responses with a median (DOR) of 67.6 weeks. Among the 7 patients receiving pembrolizumab with vismodegib, 29% (n=2) achieved a partial response for a median DOR of 52.8 weeks. Among all patients, the one-year progression free survival (PFS) was 70% and the 1-year overall survival (OS) rate was 94%. The most common immune-related adverse events included dermatitis and fatigue, and one patient experienced grade 3 hyponatremia attributable to pembrolizumab (48).

In February 2021, the FDA approved cemiplimab for patients with locally advanced and metastatic BCC. The approval was based on a phase 2 trial of cemiplimab in patients who had previously failed or were intolerant to hedgehog pathway inhibition (NCT03132636). Among 84 patients with locally advanced disease who were not candidates for curative surgery or radiation therapy (RT), 6% (n=5) of patients achieved a complete response (CR) and 25% (n=21) achieved a partial response, with a median follow-up of 15.1 months (See **Table 2** for a summary of response kinetics associated with PD-L1 status in key trials). Median duration of response (DOR) was not reached, but 85% of responses were ongoing at 12 months. The most common adverse events (AE) in this cohort were fatigue, diarrhea, and pruritis, and 17% of patients discontinued treatment due to AEs (37). Among the 28 evaluable patients in the study with metastatic BCC, 21% (n=6) of patients achieved a PR with a median follow-up duration of 9.5 months. The median DOR was not reached, but the observed duration of responses ranged between 9 and 23 months. The median time to achieve a response was 3.2 months, ranging from 2.1 to 10.5 months. Median progression free survival (PFS) was 8.3 months and median overall survival (OS) was 25.7 months. The most common AEs included fatigue, diarrhea, pruritis, and constipation (38).

IMMUNE CHECKPOINT INHIBITION FOR MERKEL CELL CARCINOMA: EXISTING CLINICAL EVIDENCE

The notion of treating MCC with immune checkpoint inhibitors was first discussed in late 2011 following the approval of

ipilimumab for metastatic melanoma earlier that year (49). Following the then-recent developments linking MCPyV and the immune system to MCC, Bhatia et al. suggested the use of anti-CTLA-4 antibodies such as ipilimumab as potential therapeutic strategies to counteract lymphocytic exhaustion (49). In 2013, several groups reported PD-L1 expression on MCC tumor cells and/or PD-1 expression on TILs in the tumor microenvironment (TME), strengthening the rationale for immunotherapy agents that block the PD-1/PD-L1 axis to be used in MCC treatment (18, 50, 51). In mid-2015, a phase I study of pembrolizumab included one patient with previously-untreated MCC who experienced a DOR of 56+ weeks at time of publication (52). Later that year, Mantripragada and Birnbaum published the first case report of checkpoint inhibitor use in the treatment of MCC which detailed a 42-year-old patient with refractory metastatic MCC who experienced symptomatic relief and shrinkage of heart and pancreatic metastases following four rounds of nivolumab (53).

In 2016, Kaufman et al. published the first results from a clinical trial of immune checkpoint inhibitors in MCC with Part A of the pivotal phase II JAVELIN Merkel 200 trial where they demonstrated objective responses in 32% of 88 refractory metastatic MCC patients treated with avelumab, logging 8 CRs and 20 PRs (54). Notably, 74% of responses persisted beyond one year, greatly improving on the roughly three month DOR seen in first-line chemotherapy at the time (55). FDA approval of avelumab for refractory metastatic MCC followed in March 2017. In 2018, early data from Part B of JAVELIN Merkel 200, which focused on the study of avelumab as a first-line agent in metastatic MCC, indicated a confirmed objective response in 62% of 29 patients with 83% of responders achieving a DOR of 6+ months (56). A later update in 2019 revealed a median duration of response of 18.2 months in 116 patients and median overall survival of 20.3 months, though with a decreased ORR of 39.7% (see **Table 2** for a summary of response kinetics associated with PD-L1 status in key trials) (39). Extended Part A JAVELIN Merkel 200 survival data over a median 65.1 month follow-up period was published in 2021 and revealed median overall survival of 12.6 months and overall survival rates of 30% and 26% at four and five years, respectively (57). Of note, avelumab has been recommended as first-line treatment for metastatic MCC by the NCCN since 2018 (58).

In mid-2016, Nghiem et al. published results from the KEYNOTE-017 trial, which investigated pembrolizumab in 25 advanced MCC patients without prior systemic therapy (40). Sixteen percent (n=4) of patients experienced a CR and 40% (n=10) achieved a PR for an overall objective response rate of 56%. Of note, response was observed in patients with both MCPyV+ and MCPyV- tumors and response to pembrolizumab was not found to be correlated with PD-L1 expression. The FDA granted approval to pembrolizumab in late 2018 for recurrent locally advanced or metastatic MCC ahead of the release of updated data from Nghiem et al's KEYNOTE-017 trial, which featured an overall response rate of 56% and increased the strength for pembrolizumab as a first-line agent in advanced MCC (59).

The first significant data exploring the role of nivolumab in treating advanced MCC was presented in 2017 by Topalian et al. as part of the CheckMate358 trial. Of 22 evaluable patients, 14% (n=3) had CR and 55% (n=12) had PR for a 68% objective response rate (71% in treatment-naïve individuals and 63% in those with 1-2 prior systemic therapies) (41). Most recently in 2020, data from CheckMate358 examining nivolumab as neoadjuvant therapy before surgical resection revealed pathological CR in 17 of 36 individuals (47.2%) who underwent surgery and tumor reduction of ≥30% in 18 of 33 individuals (54.5%) of people who could be radiographically evaluated (60).

Avelumab, pembrolizumab, and nivolumab all demonstrate significant promise in the treatment of MCC; nonetheless, adverse events reported in the trials of these therapies align with previously reported adverse effects in checkpoint inhibitors. The most common adverse effects among the main MCC trials were fatigue, infusion-related reactions, diarrhea, nausea, and lab abnormalities (e.g. elevated liver enzymes) (39, 54, 57, 60). Of note, avelumab is associated with a high rate of infusion reactions, with 25% of patients receiving avelumab experiencing an infusion reaction versus less than 10% of patients receiving other immune checkpoint inhibitors (61).

HETEROGENEITY OF RESPONSES

NMSCs differ in their responses to checkpoint inhibition, a fact which likely reflects the subtle differences in their immunological characteristics, as described in the section on Immunogenicity above. These distinctions are important for both future drug development as well as the establishment of clear clinical expectations during treatment.

cSCC and BCC

The greater immunogenicity of SCCs compared to BCCs is reflected in their respective responses to immunotherapy, both in terms of overall response rate and median time to response. In patients with metastatic BCC, cemiplimab produced an overall response rate (ORR) of 21% by investigator assessment, while, in patients with metastatic cSCC, the overall response rate was 47% (30, 38). Among patients with metastatic BCC who responded to cemiplimab, the median time to achieve a response was 3.2 months, ranging from 2.1 to 10.5 months, versus 2.3 months, ranging from 1.7 to 7.3 months, for those with metastatic cSCC who responded to cemiplimab (30, 38). cSCCs appear to respond more vigorously and more quickly to immunotherapy than BCCs.

MCPyV-Associated MCC and Non-MCPyV-Associated MCC

While non-Merkel cell polyomavirus (MCPyV)-associated MCCs display high tumor mutational burden at a median 53.9 mutations/Mb, MCPyV-associated MCCs do not. Rather, they are associated with a cohort of low-TMB MCCs with a median TMB of 1.2 mutations/Mb (see **Table 1**) (13). Despite these considerable difference in tumor neoantigen expression, response rates to checkpoint inhibition were 50% in TMB-high/UV-driven MCCs and 41% in TMB-low/MCPyV-positive tumors, a non-significant difference (p=0.63) (13). The similarity in responses between these

tumor types suggests that the viral antigens in MCPyV-associated MCCs increase the immunogenicity of the respective tumor to a level equivalent to MCCs with a high mutational burden related to UV exposure, leaving them both susceptible to immunotherapy.

FUTURE DIRECTIONS

Checkpoint inbibition in NMSCs is an area of active, ongoing investigation. **Tables 3–5** present a summary of current and future trials for cSCCs, BCCs, and MCCs.

Neoadjuvant Therapy

Neoadjuvant therapy for cutaneous melanoma is currently being investigated, with recent data suggesting promising results. In a meta-analysis of six clinical trials, 33% of patients achieved a pathologic complete response (pCR) with neoadjuvant immunotherapy (43% combination and 20% monotherapy) (62). In patients with pCR, near pCR or partial pathologic response with immunotherapy, the two-year relapse free survival was 96% (62). The efficacy of neoadjuvant immunotherapy in cutaneous melanoma has inspired similar trials in NMSCs.

TABLE 3 | Active and upcoming trials in immune checkpoint inbibition for cSCC.

Identifier	Treatment Setting/Trial Phase	Immune Checkpoint Inhibitor(s) Involved	Other Involved Agent (s) including RT	Recruitment Status
NCT02760498	Unresectable Locally Advanced cSCC or Metastatic cSCC/Phase I	Cemiplimab	None	Recruiting
NCT02955290	Stage III-IV cSCC of the Head and Neck/Phase I-II	Nivolumab, Pembrolizumab	CIMAvax (EGF vaccine)	Recruiting
NCT02964559	Locally Advanced cSSC or Metastatic cSCC/Phase II	Pembrolizumab	None	Active, not recruiting
NCT03082534	Unresectable Locally Advanced cSCC/Phase II	Pembrolizumab	None	Recruiting
NCT03284424	Locally Advanced cSCC, Metastatic cSCC, or Recurrent cSCC/Phase II	Pembrolizumab	None	Active, not recruiting
NCT03565783	Stage III-IV cSCC of the Head and Neck/Phase II	Cemiplimab	None	Recruiting
NCT03666325	Unresectable Locally Advanced cSCC or Metastatic cSCC/Phase II	Pembrolizumab	Cetuximab	Not yet recruiting
NCT03737721	Unresectable cSCC/Phase II	Avelumab	RT	Recruiting
NCT03833167	High-Risk Locally Advanced cSCC/Phase III	Pembrolizumab	None	Recruiting
NCT03834233	Locally Advanced cSCC or Metastatic cSCC/Phase II	Nivolumab	None	Active, not recruiting
NCT03889912	Recurrent and Resectable cSCC/Phase I	Cemiplimab	None	Active, not recruiting
NCT03944941	Nonresectable Locally Advanced cSCC or Metastatic cSCC/Phase II	Avelumab	Cetuximab	Recruiting
NCT03969004	High risk cSCC/Phase III	Cemiplimab	None	Active, not recruiting
NCT04050436	Locally Advanced cSCC or Metastatic cSCC/Phase II	Cemiplimab	Cetuximab, RP1 (oncolytic virus)	Recruiting
NCT04154943	Stage II-IV (M0) cSCC/Phase II	Cemiplimab	None	Recruiting
NCT04204837	Stage III-IV cSCC/Phase II	Nivolumab	None	Active, not recruiting
NCT04242173	Unresectable Locally Recurrent cSCC or Metastatic cSCC/Phase II	Cempilimab	None	Recruiting
NCT04315701	Resectable High Risk Localized cSCC or Resectable Locally Recurrent cSCC or Resectable Regionally Advanced cSCC/Phase II	Cempilimab	None	Recruiting
NCT04339062	Locally Advanced cSCC or Metastatic cSCC in people with either prior allogeneic HSCT or renal transplant/Phase I	Cempilimab	None	Recruiting
NCT04428671	Resectable High Risk cSCC/Phase I	Cemiplimab	None	Recruiting
NCT04611321	Unresectable Locally Advanced cSCC or Metastatic cSCC/Phase I-II	IBI318 (anti-PD-1/anti-PD-L1)	None	Recruiting
NCT04620200	Resectable Stage III-IVa cSCC (Stage I-II cSCC if Extensive/Mutilating Surgery is Required)/Phase II	Nivolumab, Ipilimumab	None	Recruiting
NCT04632433	High Risk Resectable Stage III cSCC/Phase II	Cemiplimab	None	Not yet recruiting
NCT04710498	Resectable cSCC/Phase II	Atezolizumab	None	Not yet recruiting
NCT04808999	Resectable High Risk cSCC or Resectable Locooregional cSCC/Phase II	Pembrolizumab	None	Not yet recruiting
NCT03901573	*Locoregionally Advanced cSCC/MCC Needing Systemic Treatment or Metastatic cSCC/MCC/Phase Ib-II	Atezolizumab	NT-17 (IL-7 agonist)	Recruiting
NCT03816332	*Stage III-IV MCC, Unresectable MCC, Unresectable BCC, Metastatic BCC, Metastatic cSCC/Phase I	Nivolumab, Ipilimumab	Tacrolimus	Recruiting
NCT02978625	Advanced BCC/MCC/cSCC or Non-Refractory BCC/MCC/cSCC/Phase II	Nivolumab	TVEC	Recruiting

**Melanoma(s) are included in these trials.*

TABLE 4 | Active and upcoming trials in immune checkpoint inhibition for BCC.

Identifier	Treatment Setting/Trial Phase	Immune Checkpoint Inhibitor(s) Involved	Other Involved Agent(s) including RT	Recruitment Status
NCT03132636	Locally Advanced BCC or Metastatic BCC/Phase II	Cemiplimab	None	Active, not recruiting
NCT03521830	Locally Advanced BCC or Metastatic BCC/Phase II	Nivolumab, Ipilimumab, Relatlimab (anti-LAG-3)	None	Recruiting
NCT04323202	Locoregionally Advanced and Resectable BCC/Phase II	Pembrolizumab	None	Recruiting
NCT04679480	Locally Advanced BCC, Metastatic BCC, or Presence of >5 BCCs/Phase II	Cemiplimab	Sonidegib (small molecule Hedgehog pathway inhibitor)	Recruiting
NCT03816332	*Stage III-IV MCC, Unresectable MCC, Unresectable BCC, Metastatic BCC, Metastatic cSCC/Phase I	Nivolumab, Ipilimumab	Tacrolimus	Recruiting
NCT02978625	Advanced BCC/MCC/cSCC or Non-Refractory BCC/MCC/cSCC/Phase II	Nivolumab	TVEC	Recruiting

**Melanoma(s) are included in these trials.*

TABLE 5 | Active and upcoming trials in immune checkpoint inhibition for MCC.

Identifier	Treatment Setting/Trial Phase	Immune Checkpoint Inhibitor(s) Involved	Other Involved Agent(s) including RT	Recruitment Status
NCT02196961	Completely Resected MCC/Phase II	Nivolumab	None	Active, not recruiting
NCT02584829	Stage IV MCC/Phase I-II	Avelumab	IFN-beta, MCPyV-specific CD8+ cells, RT	Active, not recruiting
NCT03071406	Stage IV MCC/Phase II	Nivolumab, Ipilimumab	RT	Recruiting
NCT03271372	Stage III MCC/Phase III	Avelumab	None	Recruiting
NCT03304639	Stage III-IV MCC/Phase II/Phase II	Pembrolizumab	RT	Active, not recruiting
NCT03599713	Advanced/Stage IV MCC	Retifanlimab (anti-PD1)	None	Recruiting
NCT03712605	Completely Resected Stage I-III MCC/Phase III	Pembrolizumab	RT	Recruiting
NCT03747484	Nonresectable MCC or Stage IV MCC/Phase I-II	Avelumab, Pembrolizumab	FH-MCVA2TCR (Autologous MCPyV-specific T-cells)	Recruiting
NCT03783078	Locoregionally Advanced MCC or Stage IV MCC/Phase III	Pembrolizumab	None	Active, not recruiting
NCT03798639	Stage III MCC/Phase I	Nivolumab, Ipilimumab	RT	Active, not recruiting
NCT03853317	Stage IV MCC/Phase II	Avelumab	N-803 (IL-15 superagonist), haNK (CD16-targeted NK cells)	Recruiting
NCT03988647	Stage IV MCC/Phase II	Pembrolizumab	RT	Recruiting
NCT04261855	Stage IV MCC/Phase Ib-II	Avelumab	RT	Recruiting
NCT04291885	Stage I-III MCC/Phase II	Avelumab	None	Recruiting
NCT04393753	Stage III-IV MCC/Phase II	Avelumab	Domatinostat (HDAC inhibitor)	Recruiting
NCT04792073	Refractory Stage III-IV MCC/Phase II	Avelumab	RT	Recruiting
NCT03901573	*Locoregionally Advanced cSCC/MCC Needing Systemic Treatment or Metastatic cSCC/MCC/Phase Ib-II	Atezolizumab	NT-17 (IL-7 agonist)	Recruiting
NCT03816332	*Stage III-IV MCC, Unresectable MCC, Unresectable BCC, Metastatic BCC, Metastatic cSCC/Phase I	Nivolumab, Ipilimumab	Tacrolimus	Recruiting
NCT02978625	Advanced BCC/MCC/cSCC or Non-Refractory BCC/MCC/cSCC/Phase II	Nivolumab	TVEC	Recruiting

**Melanoma(s) are included in these trials.*

Numerous phase 1 and 2 trials are investigating neoadjuvant checkpoint inhibition for the treatment of recurrent or metastatic BCC and cSCC. Based on promising response rates from a recent case series, a phase 1 trial was initiated in mid-2020 to evaluate the response and recurrence rates of BCCs to neoadjuvant pembrolizumab with an additional year of adjuvant treatment after resection if required (NCT04323202). Neoadjuvant administration of checkpoint inhibitors is also an active area of clinical research for cSCCs with trials investigating neoadjuvant cemiplimab (NCT03889912, NCT04428671, NCT04632433), nivolumab (NCT04620200), atezolizumab (NCT04710498), and pembrolizumab (NCT04808999) to begin recruiting soon.

Adjuvant Therapy

Adjuvant therapy utilizing checkpoint inhibition has demonstrated considerable efficacy in cutaneous melanoma, with studies suggesting the use of checkpoint inhibitors following resection in Stage III and IV can reduce the risk of disease relapse by 40–50% (63, 64).Due to encouraging results

from initial studies of adjuvant therapy, current trials are investigating head-to-head comparisons of checkpoint inhibitors, combination therapy, and the use of adjuvant therapy in earlier stages of disease (2). The success of adjuvant therapy in cutaneous melanoma has led to its investigation in the treatment of NMSCs as well.

The use of checkpoint inbibition as adjuvant therapy for advanced NMSCs is a current focus of numerous upcoming and ongoing studies. Notable trials include the use of adjuvant pembrolizumab after resection in BCCs (NCT04323202), adjuvant nivolumab following complete MCC resection (NCT02196961), pembrolizumab following surgery and radiotherapy for cSCCs (NCT03833167), and cemibilimab following both surgery and radiotherapy (NCT03969004) as well as surgery alone (NCT04428671) for cSCCs.

IMPROVING THE EFFICACY OF CHECKPOINT INHIBITION

Hedgehog Inhibition (BCC)

While the aforementioned investigator-initiated open-label study of pembrolizumab with or without hedgehog inhibition in advanced BCC did not find a difference in response between the single agent arm and the dual treatment arm, this approach is still undergoing clinical investigation given strong pre-clinical evidence that implicates hedgehog signaling in promoting an immunosuppressive tumor microenvironment (65). HHI in BCC increases chemokines involved in T cell recruitment and influx of T cells, suggesting a potential for synergy between HHI and checkpoint inhibition in advanced BCC patients (66). To this end, a phase 2 trial is investigating cemiplimab in combination with pulsed sonidegib for patients with advanced BCC (NCT04679480).

Cetuximab *(cSCC)*

Cetuximab is an EGFR-inhibitor approved for multiple indications associated with squamous cell carcinoma of the head and neck, including concomitant administration with platinum-based agents and radiotherapy as well as monotherapy in cases unresponse to platinum-based therapy. Recent studies have suggested the potential of cetuximab to treat unresectable cSCC, and numerous trials are now investigating the efficacy of combination therapy with cetuximab and various checkpoint inhibitors, including pembrolizumab (NCT03082534, NCT03666325) and avelumab (NCT03944941). An abstract at the 2021 ASCO meeting suggested ceteuximab may have a role in the treatment of patients immediately after progression on immunotherapy. In a small cohort study, patients who were initiated on cetuximab immediately following immunotherapy failure experienced an ORR of 54%, with 1 complete and 6 partial responses (67).

HDACis (MCC)

Domatinostat is a selective class I histone deacetylase inhibitor, which functions to upregulate the expression of cancer germline antigens and MHC class I/II molecules, among other modifications in the tumor microenvironment, boosting the innate immune response (22). Domatinostat is currently being tested alongside avelumab in a trial for patients with MCC refractory to previous immune checkpoint therapy (NCT04393753).

Radiation (MCC, BCC, cSCC)

The use of radiation in conjunction with checkpoint inhibitor therapy remains an area of active investigation. In addition to its role in directly killing tumor cells, radiotherapy has shown further potential benefit in cancer care through auxiliary means that include modulation of the tumor microenvironment, increased tumor-associated antigen expression, increased cytokine release, and stimulation and proliferation of immune cells such as CD8+ cytotoxic T-cells (68). The abscopal effect, which describes the regression of a tumor or tumors distant from the site of local radiotherapy, is believed to reflect the immune-sensitizing effect of radiotherapy and has been observed in cases of cSCC and MCC (69–71). Greater understanding of these effects has underscored the hypothesis of a synergy between radiotherapy and immunotherapies in cancer. This idea has resulted in several ongoing trials in MCC, BCC, and cSCC aimed at determining the efficacy of radiotherapy in conjunction with various checkpoint inhibitors.

Dual Checkpoint Blockade (MCC, BCC, cSCC)

Given the success of dual immune checkpoint inhibition in various solid tumors, a phase 2 clinical trial in locally advanced and metastatic BCC patients is investigating the use of nivolumab in combination with ipilimumab or relatlimab, an investigational monoclonal antibody that blocks the immune checkpoint receptor LAG-3 (NCT03521830). Similarly, a phase 2 trial is underway examining the response rates of advanced cSCC to IBI318, an anti-PD-1/PD-L1 bispecific antibody (NCT04611321). Though avelumab has become the de-facto neoadjuvant therapy in metastatic MCC, cases of MCC refractory to initial anti-PD-L1 monotherapy have been documented. In the specific case of avelumab-refractory MCC, case reports have suggested a nivolumab + ipilimumab regimen may overcome this resistance with documented durability of response (72–74). This regimen is currently being assessed with and without stereotactic radiation therapy for treatment of avelumab-resistant metastatic MCC (NCT03071406).

Direct Comparisons

Currently, there are no current or future studies assessing head-to-head efficacy of different immune checkpoint inhibitors across NMSCs. While certain checkpoint inhibitors, such as cempibilmab for cSCC and avelumab for MCC, are used more often in the clinical setting, it remains unknown if there are significant inter-class differences.

Other Immunotherapies

Other novel immunomodulatory agents are being investigated as concomitant therapies to boost the efficacy of immune checkpoint inhibition in the treatment of NMSCs. Oncolytic viruses are an active area of research. A phase 2 study of talimogene

laherparepvec, an oncolytic herpesvirus, in combination with nivolumab for the treatment of cSCCs, BCCs, and MCCs (NCT02978625) is ongoing. In addition, cemiplimab in combination with RP1, an oncolytic herpesvirus that encodes a fusogenic GALV-GP R-protein and GM-CSF, is being studied for the treatment of advanced cSCC (NCT04050436). An additional trial of tumor antigen vaccination with recombinant Human EGF-rP64K/Montanide ISA 51 in addition to nivolumab or pembrolizumab is in progress (NCT02955290). The administration of exogenous cytokines is also under investigation, with a study of NT-17, an IL-7 agonist, in combination with atezolizumab for the treamtent of advanced MCC and cSCC (NCT03901573). Several trials of MCC therapy involve the administration of recombinant immune cells. One current trial examines a treatment of avelumab combined with CD-16 targeted NK cells (haNK) and a novel IL-15 superagonist (N-803) in patients with MCC refractory to a first-line checkpoint inhibitor (NCT03853317). An additional trial for patients with unresectable or metastatic MCC involves the co-administration of a checkpoint inhibitor with autologous T-cells that have been genetically engineered to recognize and target MCPyV (NCT03747484).

Future Biomarkers

Further advancement in the field of immunotherapy will depend on the expanded study of biomarkers that can serve as predictors of response and resistance to checkpoint inhibition. While TMB is known to correlate with response to PD-1 blockade, it alone does not fully predict outcomes, as some non-responders have high TMB. Therefore, identifying other factors that can influence the efficacy of immune checkpoint inhibition will enable tailored treatment. Such factors that require further investigation include known biomarkers, such as PD-L1 expression and infiltrating T cells, as well as genomic studies. In one recent example, non-amplification short variant mutations in PD-L1, were identified in 1.6% of cSCCs, potentially heralding resistance to checkpoint inhibition (75).

Use in Solid Organ Transplantation

A current critical question in the field of immunoncoology is the appropriate use of checkpoint inhibition in the setting of solid organ transplantation. Transplant recipients carry a greatly increased risk of developing cancer, especially NMSCs (22, 76–78). For example, in kidney transplant patients, cSCCs represent 70% of all malignancies post-transplant and are estimated to affect over 50% of kidney transplant recipients (78). Checkpoint inhibitors have been used safely to address advanced disease in transplant patients (76–78). However, the risk of rejection stands between 25-50% according to recent reports (76–78). Therefore, novel ways to maintain the efficacy of checkpoint inhibition and minimize the risk of rejection are required.

CONCLUSION

NMSCs represent a significant global health burden that is set to grow ever larger with time, as medical advances permit both a rising average life expectancy and, associatively, an increased risk for NMSC development. Breakthroughs in immunotherapy first touted in the treatment of melanoma have now shown promising data in the treatment of advanced NMSCs, where previously few to no effective therapies were available. The immunogenicity of NMSCs makes them an attractive target for immunotherapy, and, accordingly, clinical trials in this space are being initiated at a rapid pace. Immune checkpoint inhibition has begun to demonstrate clinical efficacy in treating NMSCs of all subtypes. Future studies will further define the array of checkpoint inhibitors that offer maximal efficacy as well as the crucial concomitant therapies necessary to optimize their therapeutic potential.

AUTHOR CONTRIBUTIONS

Conceptualization, CS, AD, JG, AK, LG, and RC. Methodology, CS, AD, JG, and AK. Writing—original draft preparation, CS, AD, JG, AK, YS, and AC. Writing—review and editing, CS, AD, JG, AK, YS, AC, LG, and RC. Tables and figures, CS, AD, JG, and AK. Supervision, YS, AC, LG, and RC. Project administration, CS. All authors contributed to the article and approved the submitted version.

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REVIEW
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Immunotherapy for Cutaneous Squamous Cell Carcinoma: Results and Perspectives

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Although initial surgical excision cures 95% of patients, a minority of cutaneous squamous cell carcinomas (cSCCs) are judged to be unresectable, either locally advanced or with unresectable regional lymph nodes or distant metastases. These patients are offered systemic treatments. Response rate to chemotherapy is relatively low and not durable, as well as the results obtained with epidermal growth factor inhibitors (EGFRi). Like other cutaneous tumors, cSCCs have high immunogenicity, driven by the high mutational burden, the ultraviolet signature, and the overexpressed tumor antigens. Two checkpoint inhibitors, cemiplimab and pembrolizumab, achieved high response rate and survival with fewer toxicities than other available systemic agents. These promising results prompted to investigate new combination strategies of systemic therapy and surgery or radiotherapy. Subgroup analysis showed promising role of immunotherapy to facilitate surgery in locally advanced cSCC and, in a small group of patients, long-term survivals without resection. However, some cSCCs treated with immunotherapy develop either early or late resistance, so new drugs and new combinations are in a clinical study to overcome the mechanism underpinning these resistances. The present review focuses on the progress with immunotherapy to date and on new therapeutic strategies for cSCC.

Keywords: cutaneous squamous cell carcinoma, immunocheck point inhibitors, neoadjuvan, adjuvant, transplantation, future perspective

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INTRODUCTION

Cutaneous squamous cell carcinoma (cSCC) is the second most common non-melanoma skin cancer and accounts for 20% of all deaths from skin cancer (1, 2). The estimated incidence of new cSCC cases in the UK is between 15 and 35 per 100,000 people and is increasing (3). The vast majority of patients have a limited disease, so can be successfully managed with a variety of simple procedures, such as cryotherapy and curettage, topical treatments (fluorouracil, imiquimod), or simple surgical excision. When the lesions are more advanced, Mohs micrographic surgery, more extensive surgical resection, or radiotherapy or their combinations are generally sufficient to control the locoregional disease. Only 5% of cSCCs are unresectable, locally advanced, or with non-resectable regional lymph nodes or distant metastases. This quote of patients represents the indication for systemic treatments.

Only limited data are available on the role of systemic chemotherapy in the treatment of advanced cSCC. Cisplatin-based combinations appear to be the most active regimens (4, 5) and have been adapted from those used for SCC occurring at other non-cutaneous sites. Based on non-randomized trials, systemic chemotherapy is able to achieve partial response (PR) in about 34–44% of the cases, with median progression-free survival (PFS) and overall survival (OS) of about 5 and 11 months, respectively (5, 6).

The epidermal growth factor receptor (EGFR) is highly expressed in many epithelial tumors. Although its tumoral expression is inversely correlated with clinical outcome (7), the degree of overexpression does not appear to correlate with the efficacy of EGFR inhibitors (8). In prospective studies on EGFR inhibition with antibodies or small molecules in patients with advanced cSCC, an objective response was reported in 10–31% of patients, and the median time of OS was 11–13 months (8–12). A phase 2 study on cetuximab reported an objective response of 28% and a mean OS of 8.1 months (10). Therefore, advanced cSCC is a life-threatening condition for patients treated with cytotoxic chemotherapy or EGFR inhibitors and is associated with substantial morbidity, quality of life impact, and health care burden. Patients over 65 years of age are more likely than younger patients to require dose reductions in the first cycle of chemotherapy, emphasizing the need for new therapeutic approaches in a predominantly elderly population (13, 14).

WHY ARE CUTANEOUS SQUAMOUS CELL CARCINOMA SO IMMUNOGENIC?

Long-term sun exposure leading to DNA damage is postulated to account for the high mutational burden, approximately 45 mutations per megabase (15–18). Furthermore, tumor suppressor genes are most frequently altered, with the UV signature being a key mutational difference (15). Because UV have a relevant role in an early phase of cutaneous cancers pathogenesis, several molecular studies demonstrated that cSCC has a high mutational burden, which likely results in higher levels of tumor neoantigens that may be targets for the immune system. Additionally, the strong link between immunosuppression and the risk of cutaneous squamous cell carcinoma (19) indicates that natural immunosurveillance has a strong role in controlling this tumor type. There are several posited mechanisms for immunosurveillance escape, in cSCC the more relevant being the promotion of an immune-tolerant microenvironment (20). This happens by manipulation of cytokines (increased secretion of IL-6, IL-10, and TGF-beta; consumption of IL-2) that encourages infiltration of Treg cells, myeloid-derived suppressor cells (MDSCs), and other cell types that negatively modulate immune response. These cells can then actively suppress proliferation of CD4+ and CD8+ T lymphocytes that would otherwise recognize tumor antigens (20, 21). cSCC also upregulates the expression of immune checkpoint molecules such as PD ligand 1 (PD-L1) that promote peripheral T cell exhaustion (22).

On the basis of these preclinical data, immune checkpoint inhibitors were tested in advanced cSCC with good results.

CEMIPLIMAB AND PEMBROLIZUMAB FOR LOCOREGIONALLY ADVANCED OR METASTATIC DISEASE

Cemiplimab and pembrolizumab are fully human monoclonal antibodies belonging to the class of immunoglobulin G4 (IgG4) class, which binds to the PD-1 and blocks its interaction with its ligands PD-L1 and PD-L2. The involvement of PD-1 with its PD-L1 and PD-L2 ligands, which are expressed by antigen-presenting cells and may be expressed by tumor cells and/or other cells in the tumor microenvironment, results in inhibition of the T-cell function, such as proliferation, cytokine secretion, and cytotoxic activity.

Cemiplimab efficacy was investigated in cSCC in two expansion cohorts of phase I multicohort study (n=26 patients) (23, 24), and then in a phase II EMPOWER-CSCC 1 study (n=193 patients) (25–29); both trials had an open-label, multicenter design. The phase I clinical trial demonstrated the safety and the activity of cemiplimab in cSCC. The response rate, as assessed by independent central review, was 50% [95% confidence interval (CI), 30 to 70] with a duration of response that exceeded 6 months in 7 of the 13 responding patients (23). These data have been confirmed across the three parallel treatment groups of phase II clinical trial [i.e., 3 mg/kg once every 2 weeks in Groups 1 (mcSCC) and 2 (lacSCC); 350 mg once every 3 weeks in group 3 (mcSCC)]. Prior systemic treatment for cancer had been received by 33.7% of patients, while 90.2% of patients had previously had surgery for their cancer and 67.9% had received prior radiotherapy (30). An objective response was observed in 49.2, 43.6, and 41.1% of patients, in groups 1, 2, and 3, respectively; with a median time to response of 1.9 months in groups 1 and 2 and 2.1 months in group 3 (25, 26). The response seen with cemiplimab in patients with advanced cSCC appeared to be durable; at the interim analyses, the median duration of response (DOR) and median survival was not reached, after a median follow-up of 16.5, 8.1, and 9.3 months for groups 1, 3, and 2, respectively (25, 26). In mcSCC cohort patients with a DOR ≥12 months was 22 of 29 responders (26) and in lacSCC patients 12 of 34 responders (25). Median OS and PFS were yet to be reached by any treatment group.

Across cSCC, pembrolizumab was investigated in two phase II trials, the KEYNOTE 629 (n= 105 patients) and CARSKIN trial (n=57 patients in expansion cohort); both trials were open-label, single-arm, multicenter design. In KEYNOTE 629, majority of the patients had received one or more prior systemic therapies (87%) or RT (74%) (31). In the entire study population, the objective response rate was 34%, with complete and partial response rates reported in 4 and 31%, respectively. Among the cohort of 36 patients with confirmed disease response, approximately two-thirds (69%) experienced durable responses longer than 6 months. At a median follow-up of approximately 10 months, median PFS was 7 months, and 1-year OS was 60% (31).

In the investigated initiated CARSKIN trial, where only treatment-naïve patients were enrolled, the objective response rate in the entire study population was 42%, with a complete and

partial response rate of 7 and 35%, respectively (32). In the expansion cohort, the objective response rate was higher among those with programmed cell death ligand 1 (PD-L1)-positive disease (55%) *versus* PD-L1-negative disease (17%) (P=0.02) (32). In the primary cohort, after a median follow-up of 22.4 months, any of 16 responders experienced a subsequent disease progression. In this cohort, the median PFS and OS were 7 and 25 months, respectively (32) (See **Table 1**).

Based on these results, even if the lack of major evidence of phase 3 trials and in the absence of a direct comparison between chemotherapy and immunotherapy, FDA and EMA approved cemiplimab and pembrolizumab for the treatment of advanced and metastatic cSCC. NCCN and ESMO guidelines recommend them as first-line therapy.

SAFETY AND ADVERSE EVENTS OF IMMUNOTHERAPY IN ADVANCED AND METASTATIC cSCC

Because of the advanced age and comorbidities in patients with cSCC, safety is one of the most important challenges. Across the above-presented trials, most treatment-related adverse events (TRAEs) were G1 to 2, and only 13–19% were G ≥3. Less than 10% of patients discontinued the treatment because of toxicities, with a low number of treatment-related deaths (25–27, 31, 32). Investigators distinguished "treatment-related adverse events (TRAE)" from "immune-related adverse events (irAE)," the second one linked to the probable immune-pathogenesis. Most immune-mediated AEs were G1 or 2 and non-serious. In KN629, the most frequently reported ones were hypothyroidism (8.8%), pneumonitis (3.8%), hyperthyroidism (3.1%), and severe skin reactions (3.1%), while there were no grade 4 to 5 irAEs (31) (See **Table 2**).

We may therefore conclude that in advanced and metastatic cSCC, treatment with both cemiplimab and pembrolizumab is safe, with a spectrum of toxicities similar to what observed in other solid tumors. However, because comorbidities in patients with cSCC may be high, any added toxicities impact on patient's frailty, therefore suggesting the importance of an early recognition and treatment of immune-mediated AEs.

IMMUNOTHERAPY IN ALLOGENIC ORGAN TRANSPLANTATION

It is well-known that the state of immune tolerance induced by broad immunosuppression to prevent allograft rejection leads to an increased risk of the development of cSCC.

TABLE 1 | Overview of results from Keynote 629, Carskin and EMPOWER trial.

	EMPOWER – CSCC 1 (25–27)			KEYNOTE 629 (31)	CARSKIN (32)
	Group1*	Group2*	Group3*		
Drug		Cemiplimab		Pembrolizumab	Pembrolizumab
Dose	3mg/Kg q2W mCSCC	3mg/Kg q2W laCSCC	350 mg q3W	200 mg q3W	200 mg q3W
Number of pts enrolled	59	78	56	105	57
Prior systemic treatment (%pts)	56	15	36	87	Treatment naive
Median follow-up (months)	16.5	8.1	9.3	11.4	22.4
ORR % (95% CI)	49 (36–63)	44 (32–55)	41 (28–55)	34 (25–44)	42 (29–56)
DCR % (95% CI)	71 (58–82)	79 (69–88)	64 (50–77)	52 (42–62)	60 (46–72)
mDOR (months)	NR	NR	NR	NR	NR
Kaplan–Meier 12-month estimate of DOR, % (95% CI)	89 (69–96.3)	88 (66–95)	NE	66 (NR)	93 (82–100)

** Group 1, 3 mg/kg once every 2 weeks mCSCC; Group 2, 3 mg/kg once every 2 weeks laCSCC; Group 3, 350 mg once every 3 weeks.*
CSCC, cutaneous squamous cell carcinoma; laCSCC, local advanced CSCC; mCSCC, metastatic CSCC; ORR, overall response rate; DCR, disease control rate; mDOR, median duration of response; NR, not reached; NE, not explain.

TABLE 2 | Overview of adverse events from Keynote 629, Carskin and EMPOWER trial.

	EMPOWER – CSCC 1 (25–27)			KEYNOTE 629 (31)	CARSKIN (32)
	Group1*	Group2*	Group3*		
Drug		Cemiplimab		Pembrolizumab	Pembrolizumab
Dose	3 mg/kg q2W mCSCC	3 mg/kg q2W laCSCC	350 mg q3W	200 mg q3W	200 mg q3W
Any TRAE	58%	78%	64%	67%	71%
TRAE grade ≥3	19%	10%	13%	6%	7%
Treatment-related death	0	1 pt (aspiration pneumonia)	0	1 pt (cranial nerve neuropathy)	1 pt
TRAE-led discontinuation	8%	8%	10%	12%	na
Most frequent TRAE					
1st	Fatigue (27%)	Fatigue (28%)	Rash (13%)	Pruritus (14%)	Fatigue (18%)
2nd	Arthralgia (8%)	Pruritus (22%)	Fatigue (11%)	Asthenia (13%)	Diarrhea (13%)
3rd	Diarrhea (8%)	Diarrhea (17%)	Hypothyroidism (11%)	Fatigue (12%)	Hypothyroidism (13%)

**Group 1, 3 mg/kg once every 2 weeks mCSCC; Group 2, 3 mg/kg once every 2 weeks laCSCC; Group 3, 350 mg once every 3 weeks.*
CSCC, cutaneous squamous cell carcinoma; laCSCC, local advanced CSCC; mCSCC metastatic CSCC; TRAE, treatment related adverse events.

Both CTLA-4 and PD-1/PD-L1 play a key role in immunotolerance required for allograft survival (33, 34). In a preclinical study, the injection of anti-CTLA-4 immunoglobulin in the perioperative period led to the acute rejection of liver allograft but did not have any effect on graft survival when it was injected after the establishment of peripheral tolerance (33). On the contrary, the early infusion of anti-PD-1 antibodies prevented the induction of peripheral tolerance, and infusion at a later stage led to the complete loss of allograft (33–35). Although this has not been proved in humans, several reports in literature are warning about the high rates (quite 40%) of allograft rejection in patients with cancer who were treated with an ICI leading to organ failure in 71% of the patients who experienced rejection (36). The majority of graft rejections happened after 1–2 doses of ICIs, although no one has demonstrated that the loss of immunotolerance secondary to ICI is dose- and time-independent.

Accordingly, prospective studies using ICIs in organ-transplanted patients with cancer are needed. The only prospective study reported to date is a small phase I clinical trial (37) testing the safety of nivolumab in four renal transplant recipients with multiple myeloma, head and neck SCC, renal cell carcinoma, and bladder cancer. The patients were required to have no human leukocyte antigen donor-specific antibodies (DSAs). Patients received one, two, three, and nine doses of nivolumab, respectively. None of the patients had a graft rejection, and only one patient (who received nine doses) experienced a partial response. Another phase I trial (38) is open and accruing patients with renal transplants diagnosed with unresectable or metastatic cutaneous carcinoma (melanoma-cSCC-basal cell carcinoma or Merkel cell carcinoma) to receive prednisone, tacrolimus, and nivolumab with the addition of ipilimumab upon the progression of the disease. The primary endpoint of the study is response rate. As of today, because of the high risk of allograft loss and the poor data of clinical benefit, the use of ICI should be discussed with patients clearly before the initiation of treatment, and these patients should be closely monitored for signs of rejection.

FUTURE STRATEGIES: ADJUVANT AND NEOADJUVANT IMMUNOTHERAPY IN cSCC

Clinical trials in melanoma showed that in the advanced metastatic treatment setting, patients with lower tumor burdens were more likely to experience long-term survival after anti–PD-1 therapy (39, 40). This suggests that postoperative (adjuvant) anti–PD-1 therapy directed against residual micrometastatic disease might prolong RFS and OS. The same impact in survival could be foreseen by anticipating immunotherapy before surgery (neoadjuvant), where using PD-L1 blockade with primary tumor in place could leverage higher levels of endogenous tumor antigen to enhance T-cell priming.

In fact, anti–PD-(L)1 rejuvenates tumor-specific cytotoxic T cells that already reside in the tumor microenvironment (TME), causing their activation, proliferation, and trafficking to micrometastatic deposits. Moreover, having tumor-draining lymph nodes (TDLN) in place could increase the antigen presentation by dendritic cell to T cells. Recently, Yost, Chang, and colleagues showed that after anti–PD-1 therapy of cutaneous squamous cell carcinomas, T cell clonal expansion was due to new clones "appearing" in the TME rather than expansion of clones already in the tumor before initiation of anti–PD-1 therapy; these findings suggest that other clones not present initially in the tumor traffic in it upon anti–PD-1 treatment (41). The melanoma trial by Blank et al. comparing neoadjuvant *versus* adjuvant regimens of anti–PD-1 plus anti–CTLA-4 found a greater expansion of tumor-resident T-cell clones in the peripheral blood of patients enrolled on the neoadjuvant arm (42). These clones persisted in the periphery for weeks after tumor resection (41).

Based on this strong biological rationale and on data from melanoma studies, several ongoing trials are investigating the role of checkpoint inhibitors in neoadjuvant settings for patients with cSCC. They differ from each other at first for inclusion criteria, because they enroll only patients with high-risk tumor, but the risk of recurrence is established by different factors. To date, the most used reference to identify high-risk patients is the American Joint Committee on Cancer staging 8th edition (AJCC-8) (43). High-risk cSCCs are therefore defined according to tumor diameter, lymph node size, the number of positive lymph nodes and their location(s) (ipsilateral, contralateral, bilateral), and extranodal extension. However, the AJCC-8 is relevant only for head-and-neck cSCCs, which might limit its usefulness. Other risk factors are considered by the Brigham and Women's Hospital (BWH)-staging system (43), when having all of these characteristics: tumor diameter ≥2 cm, tumor invasion beyond subcutaneous fat, perineural invasion ≥0.1 mm and poorly differentiated cSCC, or bone invasion.

After all, the correct selection of patient population in the neoadjuvant setting is an important challenge. Special considerations are centered on risk-benefit expectations in patient populations among which a proportion would be cured by surgery alone and on the other hand the risk of severe and prolonged immune-related adverse effects.

Another point of difference between trials in these settings is the primary endpoint used as surrogate of survival. Disease-free survival is the historical surrogate of overall survival in the adjuvant setting, although differences exist across neoadjuvant immunotherapy clinical studies. This is because the role of response rate as a surrogate of survival in this setting is not well established. Pathologic response criteria for neoadjuvant cancer therapy were first developed in the context of chemotherapy as a parameter portending clinical outcomes. Pathologic complete response (pCR), the most stringent criterion, is defined as the absence of any viable tumor in the definitive surgical resection specimen. To date, pCR and major pathologic response (MPR) defined as describing a treatment effect resulting in ≤10% residual viable tumor are the most commonly used metrics for assessing response to neoadjuvant immunotherapy, although differences exist across clinical studies.

Similar to the precedent established with non-immunologic neoadjuvant therapies, the degree of pathologic response may help assign patients to postsurgical observation or intervention, and ongoing studies will answer this question.

If these new therapeutic strategies would translate into a clinical benefit, new questions will arise regarding the type of surgery and the role of postoperative radiotherapy. At first on the surgical treatment, if an aggressive or cosmetically disfiguring surgery would have been recommended in the absence of neoadjuvant therapy, would the same surgical approach still be required for tumors exhibiting a major response to neoadjuvant treatment? Limited surgical interventions could be used in patients whose on-treatment tumor biopsies show a complete or major pathologic response, for example, as provided in melanoma in an extension cohort of NCT02977052.

FUTURE STRATEGIES: IMMUNOTHERAPY CONCOMITANT TO RADIATION

Another innovative strategy in cSCC therapy is the combination of immunotherapy with radiotherapy. The discovery that radiation-induced damage to tumor tissues and normal tissues in the radiation field can trigger the activation of the immune system *via* well-known damage-signaling cascades, immunogenic cell death, or both has led to a paradigm change in the use of radiotherapy. Preclinical and clinical investigations revealed a complex interplay between radiotherapy, irradiated cells and tissues, and the immune system (44–46); for example, exposure to radiotherapy was shown to upregulate major histocompatibility complex I (MHC I) expression in tumor cells, modulate immunosuppressive barriers in the tumor microenvironment, activate restrictive tumor vessels, trigger the recruitment of immune effector cells to the local tumor, and even elicit systemic tumor-specific immune responses (47, 48). The efficacy of synergy between radiotherapy and checkpoint inhibitors in squamous cell carcinoma of the skin is being studied in the UNSCARRed trial: a single-arm, interventional study combining avelumab with radical radiotherapy, accrual is ongoing.

NEW IMMUNOTHERAPY DRUGS AND COMBINATIONS

Increased understanding of the underlying immunologic mechanisms is leading to the identification of several additional potential targets to unleash the immune system and control malignancy. These approaches include new checkpoint inhibitors, agonist of costimulatory receptors, manipulation of T cells, oncolytic viruses, cytokines, and vaccines. In addition to implement the response to immunotherapy and increase survival, new pharmacological combinations are under investigation.

New Checkpoint Inhibitors and Combinations

Data on anti-CTLA4 (e.g., Ipilimumab or tremelimumab) for cSCC are limited; their association with anti-PD1 is still in study in the neoadjuvant setting (NCT04620200) and in allograft patients (NCT03816332) (38).

TIGIT is another inhibitory receptor co-expressed with PD-1 on tumor antigen-specific CD8+ T cells and CD8+ tumor-infiltrating lymphocytes (TILs). It is highly expressed by Tregs in peripheral blood mononuclear cells of healthy donors and patients with cancer and further upregulated in the TME (49). Promising data on anti-TIGIT monoclonal antibody Tiragolumab efficacy in NSCLC had been presented at ASCO virtual meeting 2020 (50). As far as we know, there are no trials ongoing with this drug in cSCC. CD47 is another promising target. It is upregulated in essentially every cancer type to inhibit innate immune cells from phagocytosing the tumor cells. A humanized anti-CD47 monoclonal antibody demonstrated promising results in non-Hodgkin lymphoma (51), and the evaluation of the activity in cSCC is ongoing (NCT04502888).

Another therapeutic strategy, formulated to increase the response rate of immunotherapy and to overcome mechanisms of resistance to progression, is the addition of an anti-EGFR agent. The hypothesis of I-TACKLE (NCT03666325) trial is that the adjunct of an anti EGFR agent as cetuximab could reverse the primary and secondary resistance to pembrolizumab, with a synergistic effect able to counteract pathway redundancy (i.e., the presence of several concurrent pathways which need to be addressed together) and boosting T-cell priming. Enrollment is underway, and the results of a first analysis are expected soon. Another trial with avelumab concurrently with cetuximab is currently ongoing (NCT03944941).

Checkpoint inhibitors and modulators of DNA damage response (DDR) is another pharmacological association being studied in several neoplasms. PARPi-mediated catastrophic DNA damage is a favorable factor for ICI therapy, and the relationship between tumor mutation burden and efficacy of ICI has been confirmed in previous studies (52). Apart from tumor mutation burden, DDR-mediated immune responses collaborate with ICI, which remodel tumor immune microenvironment and boost the cancer immunity cycle (53). In this way, PARPi-mediated acute inflammation remodels tumor immune microenvironment and drives a systemic Th1-skewing immune response. In cSCC, a trial is ongoing with pembrolizumab plus abexinostat (NCT03590054), a pan-histone deacetylase inhibitor and inhibitor of RAD51, which is involved in repairing DNA double-strand breaks.

Agonists of Costimulatory Receptors

Multiple costimulatory receptors are involved in the immune response to tumors and hence are potential targets for cancer immunotherapy. Inducible T-cell costimulator (ICOS), CD40, CD28 agonists are only some examples of costimulatory receptors, studied in preclinical animal models and some of them also in early phases of clinical development (54). CD40 has been identified as an interesting immunotherapy target in

human cancers by virtue of its ability to stimulate helper T-cell immune response and macrophage differentiation (55). CD40 ligand (CD40L) gene therapy has been shown to increase tumor-infiltrating T cells *in vivo* and demonstrated an oncolytic effect. SL-172154 is an engineered monoclonal antibody that consists of Sirpα linked to CD40L, providing checkpoint blockade (CD47 axis) and potent costimulation (CD40 axis). By blocking this signal through Sirpα binding, the surface of the tumor cells is coated with the drug, allowing the CD40L side to bind to CD40 on APCs, which will lead to enhanced antigen presentation to CD8+ and CD4+ T lymphocytes and tumor cell phagocytosis. The trial with SL-172154 in cSCC is ongoing (NCT04502888).

Cytokines

Initial approaches to immunotherapy harnessed the numerous downstream effects of cytokines. IL2 and INFa demonstrate mild efficacy in melanoma and renal cell carcinoma. Other interleukin analogs or interleukin receptor agonists have been studied in the preclinical setting, with poor results. Today promising results are expected by the combination between atezolizumab and NT-I7 (recombinant human IL-7-hybrid Fc), which acts through IL-7 receptor (IL-7R), which is expressed on naïve and memory CD4+ and CD8+ T cells. Thus, IL-7 promotes proliferation, maintenance, and functionality of these key T-cell subsets mediating immune responses. The trial with this association in cSCC is ongoing (NCT03901573).

Oncolytic Viruses

Oncolytic viruses mediate antitumor effects in several ways. Viruses can be engineered to efficiently infect cancer cells preferentially over normal cells, to promote the presentation of tumor-associated antigens, to activate signals that promote a less immune-tolerant tumor microenvironment, and to serve as transduction vehicles for the expression of immune-modulatory cytokines (56). Injection of oncolytic viruses may synergize with checkpoint inhibitors by increasing CD8+ T cell infiltration and IFN gamma signaling as well as upregulating PD-L1 in the microenvironment (57).

RP1 is an oncolytic HSV that encodes a fusogenic GALV-GP R- protein and granulocyte-macrophage colony-stimulating factor (GM-CSF). RP1 demonstrated tolerable safety and tumor regression alone and with nivolumab in patients with several tumor types, including cSCC (57). Another promising oncolytic virus is Talimogene laherparepvec (TVEC). It is again an HSV-1, modified to lose the neurovirulence and include the capacity to express GM-CSF. This allows for preferential replication within tumor cells resulting in cell lysis. Additionally, the release of virally derived GM-CSF along with antigens derived from ruptured tumor cells can induce a systemic tumor-specific immune response which may lead to regression of distant uninjected lesions (58).

Vaccines

Many attempts have been made to harness the adaptive immune recognition of a cancer-related antigen to effect antitumor responses. There are many methods of vaccination, and the choice of antigen, schedules of administration, and adjuvants can influence an adaptive immune response. Antigen choices range from simple peptides, which are easy to administer but affect a narrow antigen spectrum and are often restricted by specific HLA class 1, to whole-cell preparations that offer a broader range of antigens but are more costly and time-consuming to prepare (59). Given the increasing understanding of the importance of immune recognition of not only multiple tumor antigens but specific ones for each patient, current studies to develop therapeutic vaccines are beginning to explore the use of individualized pooled antigens. Several efforts on these strategies are being made in cSCC.

Other drugs with alternative mechanisms of action, external to the immune synapse, are under investigation. Among the molecular ones suitable to stimulate antitumor immune effects, a toll-like receptor 9 (TLR9) agonist, CMP-001, activates plasmacytoid dendritic cells (pDCs) and triggers interferon alpha (IFNα) release. These lead to a cascade of antitumor immune effects. Ongoing trials are testing CMP-001 in combination with checkpoint inhibitors in several solid tumors, also in cSCC (NCT03684785).

CONCLUSIONS

The treatment of locally advanced metastatic cSCC remains a challenge. Chemotherapy may achieve response rata in about one-third of the patients, as targeted agents against EGFR (4, 5, 10); however, the main limitations of these approaches are the relatively short duration of response and the adverse events, often not compatible with the frailty of the patients. In contrast, immune checkpoint inhibitors showed a high response rate, a long duration of response, and a better toxicity profile (25–27, 31, 32). This has broadened the therapeutic armamentarium to be offered to cSCC patients and the possibility to treat also elderly patients with comorbidities otherwise not amenable to systemic treatments. However, new questions arise from this new approach. First of all, the search for predictive factors of response to treatment remains an unresolved challenge, with no molecular or clinical factor able to identify patients with a greater possibility of obtaining a benefit from immunotherapy. Moreover, it is not clear up to now how long to continue the treatment in patients who achieve a complete response with immune checkpoint inhibitors. The role of immunotherapy as adjuvant or neoadjuvant treatment is being investigated in ongoing clinical trials, thus representing a new frontier for multidisciplinary approaches. Behind this, if immunotherapy has proven to be an effective strategy, new drug combinations with novel mechanisms of actions are being investigated to improve the results till now considered and to overcome the resistance mechanisms.

AUTHOR CONTRIBUTIONS

PB contributed to conception of the study. AA wrote the first draft of the manuscript. PB and AA contributed to manuscript revision, read, and approved the submitted version.

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