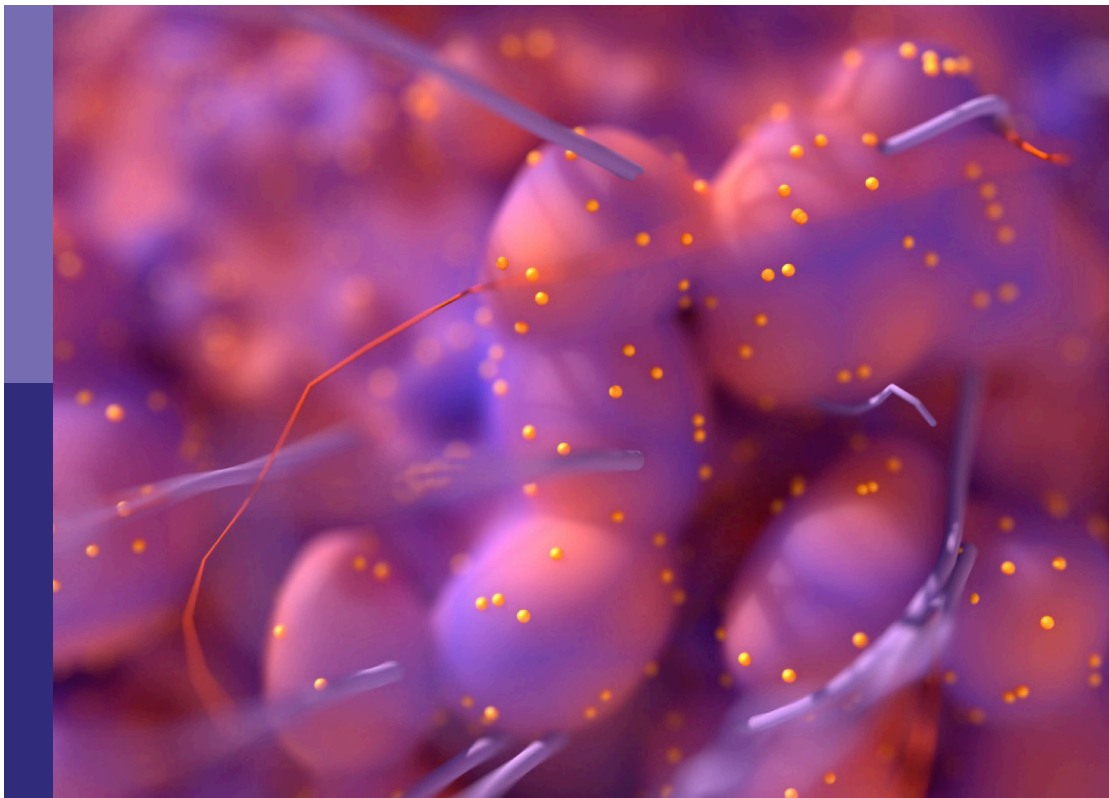
frontiers | Research Topics

Women in skin cancer vol II: 2022

Edited by
Sapna Patel, Selma Ugurel and Pamela Bond Cassidy

Published in
Frontiers in Oncology



ISSN 1664-8714
ISBN 978-2-8325-3306-2
DOI 10.3389/978-2-8325-3306-2

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Women in skin cancer vol II: 2022

Topic editors

Sapna Patel — University of Texas MD Anderson Cancer Center, United States
Selma Ugurel — Essen University Hospital, Germany
Pamela Bond Cassidy — Oregon Health and Science University, United States

Citation

Patel, S., Ugurel, S., Cassidy, P. B., eds. (2023). *Women in skin cancer vol II: 2022*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3306-2

Table of contents

frontiers | Frontiers in Oncology

TYPE Editorial
PUBLISHED 08 August 2023
DOI 10.3389/fonc.2023.1253081

Editorial: Women in skin cancer vol II: 2022

Selma Ugurel[1,2]* and Sapna P. Patel[3]

[1]Department of Dermatology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany, [2]German Cancer Consortium (DKTK), Partner Site Essen/Düsseldorf, Essen, Germany, [3]MD Anderson Cancer Center, The University of Texas, Houston, TX, United States

KEYWORDS

diagnostics, prognostics, personalized treatment approaches, immunotherapy, targeted therapy

Editorial on the Research Topic
Women in skin cancer vol II: 2022

OPEN ACCESS

EDITED AND REVIEWED BY
Vladimir Spiegelman,
Penn State Milton S. Hershey Medical Center, United States

*CORRESPONDENCE
Selma Ugurel
selma.ugurel@uk-essen.de

RECEIVED 04 July 2023
ACCEPTED 24 July 2023
PUBLISHED 08 August 2023

CITATION
Ugurel S and Patel SP (2023) Editorial: Women in skin cancer vol II: 2022.
Front. Oncol. 13:1253081.
doi: 10.3389/fonc.2023.1253081

Introduction

Skin cancer remains a significant public health concern worldwide, with its incidence on the rise in recent years. However, advancements in molecular biology have led to a better understanding of its spectrum of different entities. Moreover, research and technological developments have opened up new horizons in the treatment of these malignancies. This Research Topic explores clinical topics surrounding skin cancer, including diagnostics, prognostics, and new personalized treatment approaches in skin cancer management around the globe.

Diagnostics

Artificial intelligence algorithms have demonstrated remarkable potential in analyzing skin images and assisting in the diagnosis of skin cancer. Machine learning models can analyze large datasets and accurately differentiate between benign and malignant lesions, aiding clinicians in making informed decisions. The article of Kriegsmann et al. describes how the authors localized and categorized skin tumors on whole formalin-fixed paraffin-embedded tissue slides without prior annotation. For this aim, they previously trained a convolutional neuronal network on major non-tumor anatomical tissue structures of the skin as well as the most relevant skin tumor categories. Subsequently, they validated their system on an external test set of tissue slides with very good results: Automated differentiation of BCC, SCC, melanoma, naevi and non-tumor tissue structures was possible, and a high diagnostic accuracy was achieved in the validation (98%) and test

(97%) set. Most importantly, the research team openly provided all images and codes they used for their project to enable other researchers to improve and validate their data.

Prognostics

The field of melanoma prognostics has developed notable advancements in recent years, with the integration of advanced technologies and sophisticated predictive models. Molecular profiling techniques, including gene expression profiling and next-generation sequencing, offer a deeper understanding of the molecular landscape of melanoma, allowing for more precise prognostication, though their use is not fully validated for integration into clinical algorithms at this time. Nevertheless, these approaches enable the identification of gene signatures associated with aggressive disease and may provide insights into potential therapeutic targets. Moreover, machine learning algorithms, by analyzing large datasets and incorporating multiple prognostic factors, facilitate the development of robust prognostic models. The integration of these advanced tools holds immense promise for enhancing prognostication in melanoma and aiding in clinical decision-making. Augustin et al. investigated a large set of 4,790 diabetic patients with cutaneous melanoma for their melanoma recurrence rates, progression free survival (PFS), and overall survival (OS) with and without exposure to metformin, an anti-diabetic drug that has been shown to reduce intratumoral hypoxia, improve T-cell function, and increase the sensitivity to PD-1 blockade in pre-clinical studies. The authors found a reduction in recurrence rate, overall survival, and interestingly also incidence of brain metastasis in patients who received metformin, with the caveat that this is merely an association and not necessarily a correlation finding. These results suggest rationale for ongoing and future clinical trials studying the potential augmentation of checkpoint blockade with metformin in advanced melanoma.

Personalized treatment approaches

Treatment options for skin cancer have evolved beyond traditional methods, with a focus on personalized approaches that consider individual characteristics and genetic factors.

Immunotherapy

One of the most remarkable breakthroughs in skin cancer treatment is the advent of immunotherapy. This revolutionary approach harnesses the power of the immune system to target and destroy cancer cells. Immune checkpoint inhibitors targeting PD1, CTLA4 and other checkpoint molecules, have demonstrated exceptional efficacy in treating advanced melanoma as well as other skin cancer entities. These medications block the proteins that suppress the immune response, allowing immune cells to recognize and attack cancer cells effectively. Immunotherapy has not only shown remarkable response rates but also provided durable remissions and improved overall survival in patients. However, these potent agents also lead to severe side effects, which often limit their further use in affected patients. In this regard, Neuville et al. report a case of capillary leak syndrome associated with a chylothorax in a melanoma patient who has been treated with adjuvant nivolumab (anti-PD1). The knowledge on early recognition and adequate management of these adverse events therefore is of high importance for the clinical care of advanced skin cancer patients. Helbig and Klein give an overview on the treatment of pleomorphic dermal sarcomas with immune checkpoint inhibitors. Their review demonstrates that these rare skin tumors respond well to immunotherapy due to their high tumor mutational burden, which is linked to their suggested UV-induction, as well as their high number of tumor-infiltrating lymphocytes. Lodde et al. report on COVID-19 vaccination which led to unimpaired seroconversion in advanced skin cancer patients under treatment with either immunotherapy or targeted therapy. Notably, an impaired serological response was observed in patients who were immunocompromised due to concomitant diseases or previous chemotherapies, whereas immunosuppressive comedication due to severe adverse events did not impair the serological response to COVID-19 vaccination.

Targeted therapy

Targeted therapies have transformed the management of specific types of skin cancer. In cases where melanomas harbor specific genetic mutations such as BRAF or NRAS, targeted drugs like vemurafenib, cobimetinib, dabrafenib and trametinib can be used. These medications specifically inhibit the abnormal signaling pathways that drive cancer growth, leading to tumor regression and improved patient outcomes. The advent of precision medicine and molecular profiling has revolutionized the selection of appropriate targeted therapies, allowing for personalized treatment strategies tailored to each patient's genetic profile. Shaikh et al. report on a phase-1 clinical trial combining targeted therapy with vemurafenib plus cobimetinib with the PD1 inhibitor pembrolizumab in patients with BRAF-mutant metastatic melanoma. Nine of 30 planned patient were treated and responded well with an objective response rate of 78% (7/9). However, significant adverse events of CTCAE grade 3 to 4 were observed in 8 of 9 patients, leading to an early closure of this study. Salman et al. demonstrate that despite the clear benefits of targeted therapies for BRAF-mutated melanoma in other regions of the world, there is no clear path to prepare Latin Americans for a sustainable personalized medicine approach. Melanoma therefore represents an increasing public health burden with extensive unmet needs in Latin America, a problem which must be solved in the future. Zattarin et al. report a patient with advanced extramammary Paget's disease showing a long lasting benefit from the HER-2 inhibitor trastuzumab, indicating that this approach is worth exploring further in the management of this rare skin cancer entity.

Conclusion

The field of skin cancer research and management is rapidly evolving, with new and hot topics continuously emerging. New methods and techniques for diagnostics and prognostics, as well as personalized treatment approaches are at the forefront of research and development. Through these advancements, we hope to further improve patient outcomes, reduce the burden of this disease, and enhance collaborative efforts in combating skin cancer.

Author contributions

Both authors contributed equally to this work drafting, editing, and approving the final version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

frontiers | Frontiers in Oncology

TYPE Case Report
PUBLISHED 18 August 2022
DOI 10.3389/fonc.2022.925551

OPEN ACCESS

EDITED BY
Selma Ugurel,
University of Duisburg-Essen, Germany

REVIEWED BY
Alexander Kreuter,
University Witten/Herdecke, Germany
Ioana Cosgarea,
Newcastle University, United Kingdom

*CORRESPONDENCE
Emma Zattarin
emma.zattarin@istitutotumori.mi.it

SPECIALTY SECTION
This article was submitted to Skin Cancer, a section of the journal Frontiers in Oncology

RECEIVED 11 May 2022
ACCEPTED 22 July 2022
PUBLISHED 18 August 2022

CITATION
Zattarin E, Nichetti F, Ligorio F, Mazzeo L, Lobefaro R, Fucà G, Peverelli G, Vingiani A, Bianchi GV, Capri G, de Braud F and Vernieri C (2022) Case Report: Prolonged clinical benefit with sequential trastuzumab-containing treatments in a patient with advanced extramammary Paget disease of the groin.
Front. Oncol. 12:925551.
doi: 10.3389/fonc.2022.925551

Case Report: Prolonged clinical benefit with sequential trastuzumab-containing treatments in a patient with advanced extramammary Paget disease of the groin

Emma Zattarin[1]*, Federico Nichetti[1], Francesca Ligorio[1,2], Laura Mazzeo[1], Riccardo Lobefaro[1], Giovanni Fucà[1], Giorgia Peverelli[1], Andrea Vingiani[3,4], Giulia V. Bianchi[1], Giuseppe Capri[1], Filippo de Braud[1,4] and Claudio Vernieri[1,2]

[1]Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, [2]Istituto Fondazione di Oncologia Molecolare Ente del Terzo Settore, The AIRC Institute of Molecular Oncology, Milan, Italy, [3]Department of Pathology and Laboratory Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, [4]Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy

Extramammary Paget disease (EMPD) is a rare form of cutaneous, intraepithelial adenocarcinoma, which typically presents itself as an erythematous plaque originating from apocrine-gland rich regions, such as the vulva, the perianal region, the scrotum, the penis, or the axilla. EMPD patients typically have a good prognosis, with expected 5-year survival of 60%–92%, but it is estimated that about one-third of EMPD patients will develop lymph node or distant metastases. Treatment approaches for EMPD include locoregional therapies such as broad surgical resection, radiotherapy, or topical imiquimod, when the disease is localized, and chemotherapy and biological agents for advanced EMPD. We report the case of a 58-year-old man diagnosed with locally advanced, symptomatic HER2-overexpressing, AR-positive EMPD, who achieved long-term tumor control with a sequence of several trastuzumab-based treatments (more than 30 months with second-line carboplatin plus paclitaxel plus trastuzumab followed by trastuzumab maintenance; 9 months for third-line vinorelbine plus trastuzumab). Even if it is reported that AR expression occurs concomitantly with HER2 overexpression in more than half of the cases of EMPD, to the best of our knowledge, this is the first case report describing androgen receptor blockade therapy in combination with an anti-HER2 agent. Our patient did not benefit from androgen receptor blockade in combination with trastuzumab, thus suggesting that AR expression may simply reflect an intrinsic characteristic of the EMPD cell of origin, rather than tumor dependence upon AR signaling. Given the reported sensibility to anti-HER2 therapy, also new antibody drug conjugates targeting HER2 are worth exploring in the management of advanced EMPD.

KEYWORDS

extramammary Paget disease (EMPD), HER2 overexpression, trastuzumab, prolonged benefit, antiandrogen therapy, rare cancer

Introduction

Extramammary Paget disease (EMPD) is a rare form of cutaneous, slowly growing intraepithelial adenocarcinoma originating from apocrine-gland rich regions, such as the vulva, the perianal region, the scrotum, the penis, or the axilla (1). Similarly to the more common mammary Paget disease (MPD), EMPD typically presents itself as an erythematous plaque that can evolve as erosive, crusty, or eczematous. Clinical features of EMPD resemble inflammatory, non-malignant conditions, thus frequently resulting in misdiagnoses or delayed recognition of the cancerous nature of this disease. EMPD more frequently affects female individuals, with a male-to-female ratio of 1:2.8 in Europe (2), and median age at diagnosis is 65 both in men and in women (3, 4). The overall incidence ranges from 0.1 to 2.4 patients per 1,000,000 person-years (5).

EMPD patients typically have a good prognosis, with expected 5-year survival of 60%–92% (5, 6). However, when EMPD invades the derma, it can spread to loco-regional lymph nodes and/or distant organs (7). It is estimated that about one-third of EMPD patients will develop lymph node involvement or distant metastases (8). In these cases, the expected 5-year survival is <10% (6). As in the case of Paget disease of the breast, human epidermal growth receptor 2 (HER2) overexpression is reported in up to 50% of EMPD patients, and it is associated with a more aggressive clinical behavior, including more frequent dermal invasion and lymph node spread (9). Androgen receptor (AR) is also frequently expressed in EMPD (50%–80% of cases), and similarly to HER2 overexpression, its expression is associated with the presence of invasive disease and more frequent metastatic spread (10–13).

Treatment approaches for localized EMPD include broad surgical resection, radiotherapy, topical imiquimod, and photodynamic therapy; for patients with advanced (locally advanced or metastatic) EMPD, several chemotherapeutic agents, including 5-fluorouracil, taxanes, platinum salts, and vinca alkaloids, have shown moderate antitumor activity in published case series (14–17). More recently, anti-HER2 agents, alone or combined with chemotherapy, have shown promising antitumor activity in patients with HER2-overexpressing EMPD, as summarized in Table 1 (1, 9, 18–28). Androgen blockade therapy also showed some activity in few patients with EMPD expressing AR (27, 28).

Here, we report the case of a 58-year-old man diagnosed with locally advanced, symptomatic HER2-overexpressing, AR-positive EMPD, who experienced prolonged disease control when treated with subsequent lines of trastuzumab plus different cytotoxic agents.

Case description

In August 2015, a 58-year-old man with no significant comorbidities and a 3-year history of a non-healing groin rash, initially misdiagnosed as psoriasis that did not respond to topical antibiotics, antifungal agents, and steroids, was referred to our Institution. At the presentation, lesions were well-demarcated and widely extended over the skin from the umbilical line to both thighs, involving the groins, penis, scrotum, and perianal region. Some of these lesions were erythematous, with some ulcerated and bleeding areas, and others appeared as scaly and eczematous plaques, as shown in Figure 1A. The patient reported severe pain, itching, and dysuria (due to penis involvement). Skin punch biopsy revealed a proliferation of large round neoplastic elements spreading throughout the epidermis with dermal infiltration, coherent with EMPD. Immunoreactivity for cytokeratin 7 (Figure 2A) and EMA and negativity for cytokeratin 20 (Figure 2B) and Melan-A ruled out secondary pagetoid spread from urothelial or colorectal cancer and malignant melanoma (17). HER2 was highly expressed by immunohistochemistry (IHC) [score of 3+ according to CAP guidelines (29)] (Figure 2C), and *in situ* hybridization (ISH) analysis revealed *ERBB2* gene amplification (Figure 2D). Laboratory analyses revealed moderate anemia. A PET/CT scan showed avid fluorine-18-fluorodeoxy-D-glucose (FDG) uptake [maximum standardized uptake value (SUV max), 5] in correspondence with the cutaneous thickening of the suprapubic region, the perineum, and the right thigh, with bilateral inguinal enlarged lymph nodes (SUV max, 3.3), without metastases in visceral organs (Figure 3). Loco-regional approaches were excluded due to the extension of the disease, and the patient was started on systemic therapy. Different patient's treatment lines are summarized in Figure 4. Local disease progression occurred after 4 months of first-line metronomic Capecitabine 1,500 mg/day in December 2015. The patient's quality of life was highly compromised. He experienced a depressive mood, related to the

TABLE 1 Available case series of locally advanced or metastatic HER2-positive EMPD treated with anti-HER2 therapies alone or in combination.

First author	Year of publication	Number of patients	Primary site of EMPD	Inguinal lymph node involvement	Sites of distant metastases	Anti-HER2 therapy	Chemotherapy in combination with anti-HER2 drug	PFS with the anti-HER2-based therapy treatment (months)
Karam (1)	2008	1	vulva	/	/	trastuzumab	/	14
Hanawa (18)	2011	1	vulva	yes	axillary and abdominal lymph nodes	trastuzumab	paclitaxel	12
Wakabayashi (19)	2012	1	vulva	yes	abdominal lymph nodes, lung, liver	trastuzumab	/	20
Gunvén (20)	2012	1	vulva	yes	abdominal lymph nodes, bone	trastuzumab	vinorelbine	36
Barth (21)	2015	1	scrotum	no	cervical lymph nodes, abdominal lymph nodes, bone	trastuzumab	/	>12
Ichiyama (22)	2017	1	vulva	/	/	trastuzumab	paclitaxel	>32
Hsieh (9)	2019	1	vulva	yes	/	trastuzumab	carboplatin + paclitaxel	7
Hsieh (9)*	2019	1	vulva	yes	/	T-DM1	/	6
Lu (23)	2019	2	scrotum	yes	abdominal lymph nodes; bone and liver	trastuzumab	/; paclitaxel	17; 5
Nordmann (24)	2019	1	scrotum	yes	thoracic and abdominal lymph nodes, lung	lapatinib	/	primary resistance
Nordmann (24)	2019	1	scrotum	yes	thoracic and abdominal lymph nodes, lung	trastuzumab	carboplatin	7.5
Bartoletti (25)	2020	4	vulva	yes	abdominal lymph nodes, lung pelvis; abdominal lymph nodes; anus	trastuzumab	paclitaxel	36; 10; 8; 16
Bartoletti (25)*	2020	1	vulva	yes	abdominal lymph nodes, lung	T-DM1	/	>4
Kimura (26)	2020	1	perianal	yes	abdominal lymph nodes	trastuzumab	/	>6

*After progression to first line anti-HER2 therapy. ";" separate the information from different patients.

disabling condition caused by the disease, with important limitations in terms of autonomy, mobilization, and need for hospital access for medication of skin lesions. The patient needed to take antidepressants but refused the psychological support that was offered to him.

Based on HER2 overexpression in tumor cells, the patient was candidate to receive anti-HER2-based therapy. Therefore, in January 2016, second-line treatment with carboplatin [area under the curve (AUC) of 5 every 3 weeks] plus paclitaxel (150 mg/mq every 3 weeks) in combination with trastuzumab (provided with an off-label procedure) was initiated. Trastuzumab was administered at a loading dose of 8 mg/kg i.v., followed by 6 mg/kg i.v. maintenance dose every 3 weeks, as for the treatment of HER2-positive breast cancer (30). Echocardiograms were performed prior to treatment start and every 3 months to assess patient's cardiac function. After 4 months, the patient reported a good clinical response, with skin lesions appearing less erythematous and with initial signs of re-epithelization (Figure 1B). This was paralleled by remarkable symptomatic improvement, including reduction in itching, pain, bleeding, crusting, and overall disease extension. Chemotherapy was stopped after six cycles, while triweekly trastuzumab was continued as maintenance treatment for 32 months, with prolonged clinical benefit. During this period, patient symptoms and quality of life improved, and the need for skin lesion medication was reduced. In August 2018, when the patient was receiving trastuzumab maintenance, clinical disease progression occurred (Figure 1C), with worsening of pain and skin lesion bleeding causing anemia, which required blood transfusions. Then, third-line metronomic vinorelbine (oral vinorelbine at the dosage of 50 mg, as given on Monday, Wednesday, and Friday every week) was started, together with i.v. triweekly trastuzumab at 6 mg/kg dosage. The patient experienced clinical benefit from vinorelbine plus trastuzumab

10.3389/fonc.2022.925551

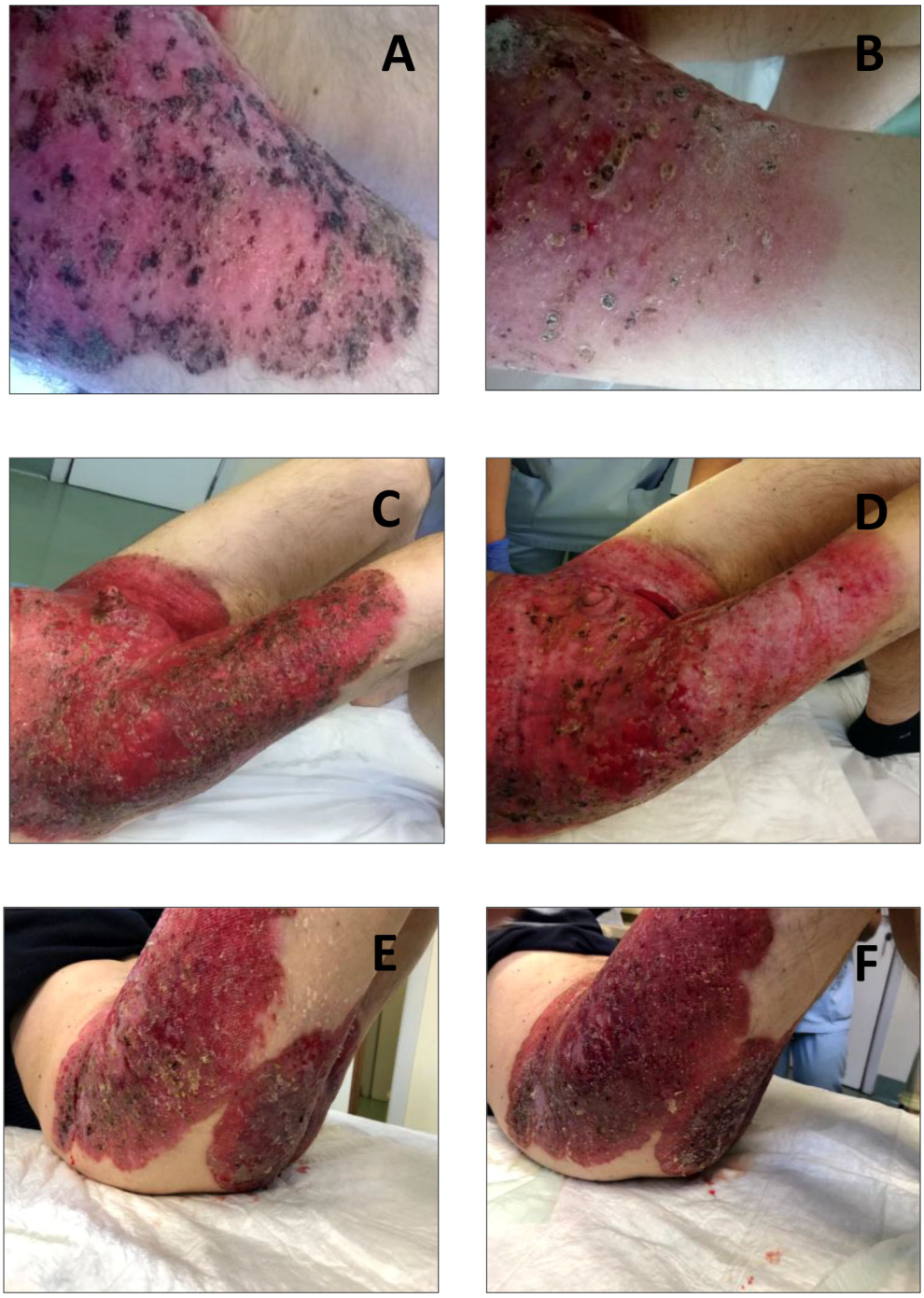


FIGURE 1
Skin scaly and erythematous plaques at different phases of the disease history of our patient. **(A)** Erythematous lesions with some ulcerated and bleeding areas at diagnosis. **(B)** Lesions appearing less erythematous and with initial signs of re-epithelization after 4 months of treatment with carboplatin, paclitaxel plus trastuzumab. **(C)** Clinical disease progression during trastuzumab maintenance therapy. **(D)** Reduction in bleeding and partial epithelialization of skin lesions after starting vinorelbine plus trastuzumab. **(E)** Clinical disease progression during vinorelbine plus trastuzumab. **(F)** Rapid disease progression during anti-androgen therapy plus trastuzumab.

10.3389/fonc.2022.925551

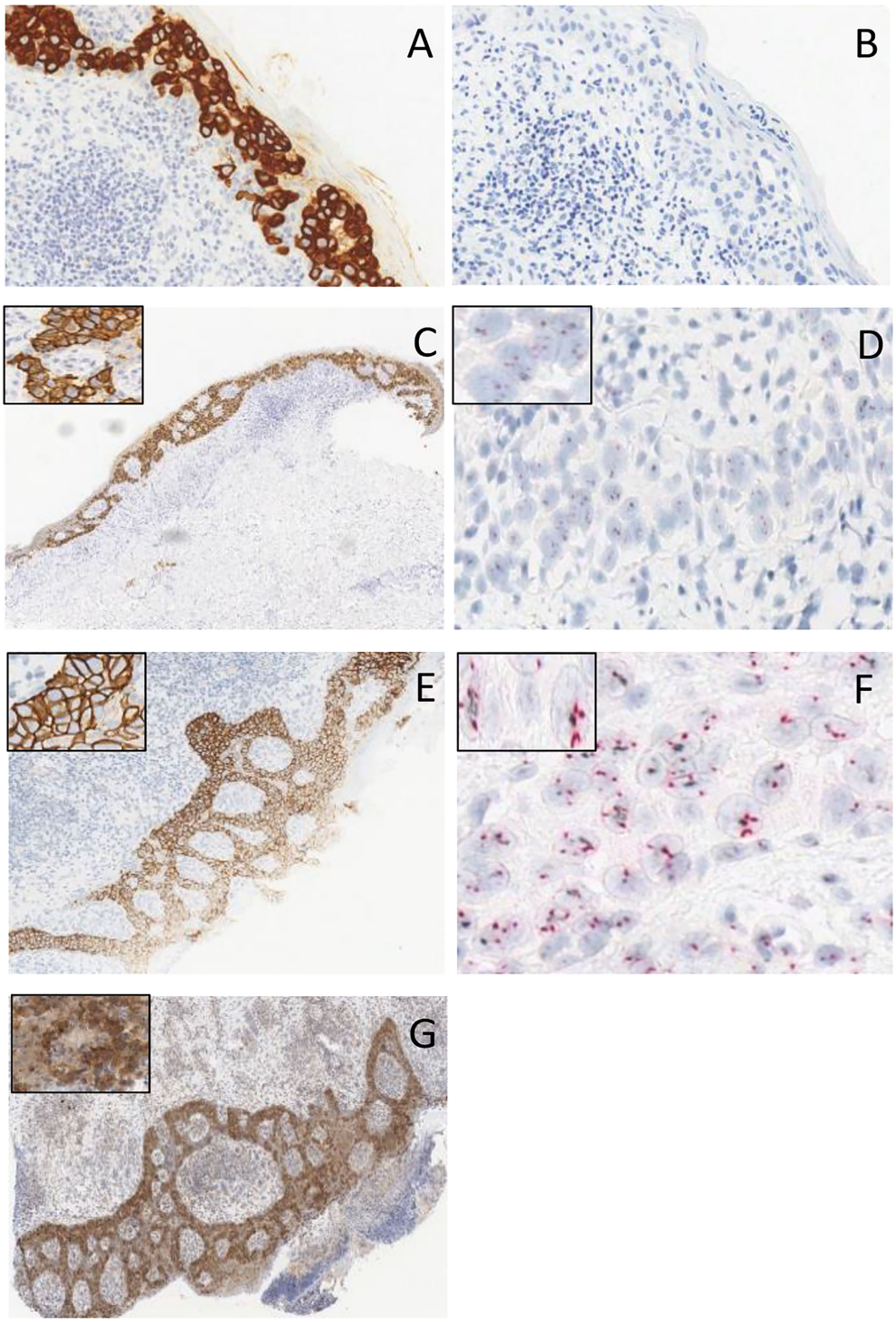


FIGURE 2
(A) Immunoreactivity for cytokeratin 7. **(B)** Negativity for cytokeratin 20. **(C, E)** HER2 strong membranous staining (3+) at immunohistochemical stain magnification, × 10, in two different tumor samples of the patient. **(D, F)** microscopic image in CISH for *HER2* staining in two different tumor samples of the patient. **(G)** Androgen receptor staining at immunohistochemical stain magnification, × 10.

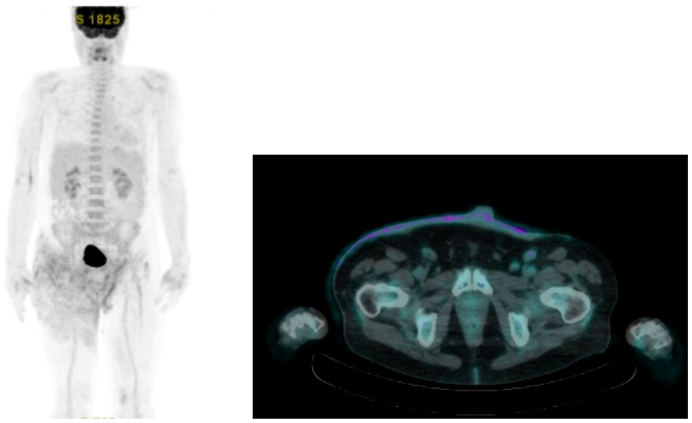


FIGURE 3
FDG PET/CT scan at baseline of patient's clinical history.

combination, with reduction in pain and bleeding and partial re-epithelialization of skin lesions, as shown in Figure 1D (as compared to Figure 1C). Unfortunately, metronomic vinorelbine treatment was poorly tolerated, with the occurrence of febrile neutropenia requiring prolonged hospitalization after 2 months of treatment. In January 2019, after hospital discharge, oral metronomic vinorelbine was resumed at a reduced dose (30 mg on Monday, Wednesday, and Friday every week) in combination with trastuzumab; treatment was continued until May 2019, when clinical disease progression occurred (Figure 1E).

Aiming to re-characterize tumor biology and to find potential therapeutic targets, we performed a new tumor biopsy to combine HER2 IHC/ISH evaluation and AR expression with the analysis of the hotspot regions of 50 cancer-related genes (Cancer Hotspot Panel v2; Thermo Fisher Scientific, Waltham, MA), as assessed by means of targeted next-generation sequencing (NGS) through the Ion Torrent Personal Genome platform "Hot-spot Cancer Panel" (Thermo Fisher Scientific). While confirming the overexpression/amplification of *HER2* (IHC/FISH) (Figures 2E, F), these evaluations also revealed the expression of AR in 90% of tumor cells (Figure 2G). NGS did not reveal mutations in targetable oncogenes. PCR amplification and direct sequencing of microsatellite loci revealed microsatellite stability.

Based on both AR expression and HER2 overexpression, in June 2019, fourth-line treatment with bicalutamide 50 mg p.o. daily, plus leuprorelin 11.25 mg i.m. every 3 months (according to the schedule commonly used in prostate cancer) was started, together with i.v. trastuzumab maintenance; however, the disease rapidly progressed (Figure 1F). In August 2019, endocrine therapy was interrupted and fifth-line treatment with gemcitabine (800 mg day 1, day 8 every 3 weeks) plus i.v. trastuzumab was initiated. The treatment was poorly tolerated,

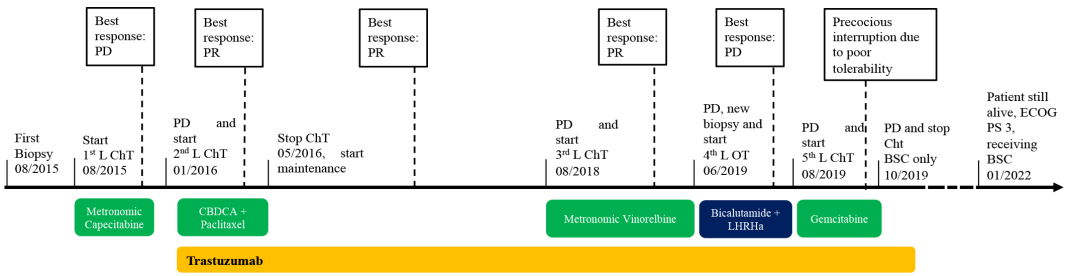


FIGURE 4
Timeline of systemic treatments administrated during patient's disease history. BSC, best supportive care; CBDCA, carboplatin; ChT, chemotherapy; ECOG PS, Eastern Cooperative Group Perfomance Status; LHRHa, LHRH analogue; OT, hormonal therapy; PD, progression disease; PR, partial disease response.

since the patient experienced febrile neutropenia and severe fatigue, which led to permanent treatment interruption after only two cycles. The patient was deemed unfit to tolerate further anticancer treatments, but he continued to receive palliative care.

As for March 2022, the patient is still alive, with a performance status of 3 by ECOG score, symptomatic for severe asthenia, and mobilization difficulties due to loco-regional cutaneous and nodal disease progression, which impair his walking capability and result in severe anemia and frequent blood transfusions. He is receiving daily best supportive care at home.

Statement of patient consent

The patient provided written informed consent for his case to be presented.

Discussion

Inoperable EMPD is typically associated with dismal prognosis, and systemic medical treatment is the only therapeutic choice. In the clinical case that we reported here, a patient with inoperable, loco-regionally advanced EMPD bearing HER2 overexpression (as a consequence of *HER2* gene amplification) achieved long-term tumor control with a sequence of several trastuzumab-based treatments (more than 30 months with second-line carboplatin plus paclitaxel plus trastuzumab followed by trastuzumab maintenance; 9 months for third-line vinorelbine plus trastuzumab).

Owing to its rarity, there is still limited available clinical evidence on the most effective treatment strategies for advanced EMPD, which indeed remains orphan of standard therapies. In the case of limited disease extension, non-surgical treatments, such as radiotherapy, photodynamic therapy, topical imiquimod, or carbon dioxide laser therapy, used alone or in combination, can be considered as valid and effective treatment options (17).

In case reports and case series published to date, chemotherapy has shown moderate antitumor activity (1, 24), and advanced EMPD remains associated with a dismal prognosis (31). With regard to biological therapies, several reports have clearly documented the efficacy of anti-HER2 therapies in HER2-positive EMPD, such as trastuzumab, alone and in combination with paclitaxel, or T-DM1 (Table 1). however, only in a few studies, trastuzumab-based therapies resulted in long-term tumor control, as described in our case. A Japanese phase II single-arm clinical trial enrolled 13 patients with advanced HER2-positive EMPD to receive docetaxel (75 mg/mq every 3 weeks) plus i.v. trastuzumab (UMIN000021311), but results have not been published yet. New antibody drug conjugates (ADCs) targeting HER2, like Trastuzumab-deruxtecan (T-DXd), recently demonstrated meaningful efficacy in HER2-positive advanced breast and gastric cancers and also showed preliminary activity in HER2-positive metastatic colorectal and non-small cell lung cancers (32–34). However, to our knowledge, T-DXd activity in EMPD remains unknown.

The only clinical trial currently enrolling patients with metastatic EMPD is a phase II, single-arm study offering the combination of nivolumab and ipilimumab to advanced rare tumors (NCT02834013). PD-L1 expression is typically low in most EMPDs (35). Nonetheless, a case report described a partial response to ipilimumab 1 mg/kg plus nivolumab 3 mg/kg, which lasted 7 months, in a patient with PD-L1-negative, MSI-stable, low tumor mutational burden metastatic EMPD (36).

The study of Liegl et al. reported that AR expression frequently occurs concomitantly with HER2 overexpression, both in MPD and EMPD (88% of MPD cases and 52% of EMPD cases) (37). Two case reports described a meaningfully decrease of multiple bone and lymph nodes metastases in two patients with AR-overexpressing, advanced EMPD treated with bicalutamide or chlormadinone acetate (anti-androgen drugs), respectively, combined with leuprorelin acetate (luteinizing hormone-releasing agonist) (27, 28). In our patient, combining androgen receptor blockade with trastuzumab did not provide benefit, thus suggesting that AR expression in EMPD cells does not necessarily imply tumor dependence upon AR signaling, nor it predicts response to anti-AR treatments, but it may simply reflect an intrinsic characteristic of the EMPD cell of origin, i.e., glandular cells with apocrine differentiation (38). Due to the high frequency of AR expression in EMPDs (11), future studies should focus on uncovering the determinants of EMPD dependence on the AR pathway to identify patients who could benefit from androgen blockade.

Dissimilarly to other EMPD case reports described in the literature, our patient never developed distant metastases. He presented with cutaneous involvement and bilateral inguinal lymphadenopathies and underwent several subsequent locoregional progression events responsible for a slow but progressive deterioration of clinical conditions. The absence of metastatic spread could be the result of intrinsic molecular characteristics of the tumor and/or of the antitumor and immunomodulatory effects of trastuzumab, which might explain the long-term disease control.

To the best of our knowledge, this is the first case reporting a prolonged clinical benefit and long-term survival after treatment with several trastuzumab-based regimens (i.e., four different treatment combinations, for a total of 45 months of trastuzumab-based therapies), including a trastuzumab–anti-androgen therapy combination, which has never been used in previous published EMPD case reports or case series. In patients with HER2-positive

EMPD, the use of ADCs targeting HER2 should be further explored. In the context of a very rare disease, we suggest that sharing cases of patients achieving prolonged tumor control and long-term survival holds great value and might help clinicians in making decisions when managing these neoplasms.

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.

Author contributions

EZ: conceptualization, manuscript preparing, editing. FN: conceptualization, manuscript preparing, editing. FL: manuscript preparing. LM: draft preparing. RL: manuscript preparing. GF: editing. GP: editing. AV: data collection. GB: conceptualization. GC: editing. FB: conceptualization. CV: conceptualization, manuscript preparing, editing, supervising. All authors contributed to the article and approved the submitted version.

Acknowledgments

We would like to thank AIRC, the Associazione Italiana per la Ricerca sul Cancro, for funding our research (MFAG 22977: PI Claudio Vernieri).

Conflict of interest

FB reports an advisory role at Roche, EMD Serono, NMS Nerviano Medical Science, Sanofi, MSD, Novartis, Incyte, BMS, Menarini; speaker role for BMS, Healthcare Research and Pharmacoepidemiology, Merck Group, ACCMED, Nadirex, MSD, Pfizer, Servier, Sanofi, Roche, AMGEN, Incyte, Dephaforum; Principal Investigator for Novartis, F.Hoffmann-LaRoche Ltd, BMS, Ignyta Operating INC, Merck Sharp and Dohme Spa, Kymab, Pfizer, Tesaro, MSD, MedImmune LCC, Exelixis Inc., LOXO Oncology Incorporated, DAICHI SANKIO Dev. Limited, Basilea Pharmaceutica International AG, Janssen-Cilag International NV, Merck KGAA. CV reports an advisory role for Novartis; travel grants: Lilly, Novartis, Istituto Gentili, Roche, Pfizer; research grants: Roche.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Karam A, Dorigo O. Treatment outcomes in a large cohort of patients with invasive extramammary paget's disease. *Gynecol Oncol* (2012) 125(2):346–51. doi: 10.1016/j.ygyno.2012.01.032

2. van der Zwan JM, Siesling S, Blokx WA, Pierie JP, Capocaccia R. Invasive extramammary paget's disease and the risk for secondary tumours in Europe. *Eur J Surg Oncol* (2012) 38(3):214–21. doi: 10.1016/j.ejso.2011.12.008

3. Wang L, Feng C, Zhou M, Zhou Z, Ding G, Gao P, et al. Tumor wide horizontal invasion predicts local recurrence for scrotal extramammary paget's disease. *Sci Rep* (2017) 7:44933. doi: 10.1038/srep44933

4. Westacott LS, Cominos D, Williams S, Knight B, Waterfield R. Primary cutaneous vulvar extramammary paget's disease involving the endocervix and detected by pap smear. *Pathol 06* (2013) 45(4):426–8. doi: 10.1097/PAT.0b013e328360df63

5. Morris CR, Hurst EA. Extramammary paget disease: A review of the literature-part I: History, epidemiology, pathogenesis, presentation, histopathology, and diagnostic work-up. *Dermatol Surg* (2020) 46(2):151–8. doi: 10.1097/DSS.0000000000002064

6. Weng S, Zhu N, Li D, Chen Y, Tan Y, Chen J, et al. Clinical characteristics, treatment, and prognostic factors of patients with primary extramammary paget's disease (EMPD): A retrospective analysis of 44 patients from a single center and an analysis of data from the surveillance, epidemiology, and end results (SEER) database. *Front Oncol* (2020) 10:1114. doi: 10.3389/fonc.2020.01114

7. Asel M, LeBoeuf NR. Extramammary paget's disease. *Hematol Oncol Clin North Am* (2019) 33(1):73–85. doi: 10.1016/j.hoc.2018.09.003

8. Ohara K, Fujisawa Y, Yoshino K, Kiyohara Y, Kadono T, Murata Y, et al. A proposal for a TNM staging system for extramammary paget disease: Retrospective analysis of 301 patients with invasive primary tumors. *J Dermatol Sci* (2016) 83 (3):234–9. doi: 10.1016/j.jdermsci.2016.06.004

9. Hsieh GL, English DP, Tu P, Folkins A, K KA. Case of metastatic extramammary paget disease of the vulva treated successfully with trastuzumab emtansine. *JCO Precis Oncol* (2019) 2:1–8. doi: 10.1200/PO.17.00204

10. Angelico G, Santoro A, Inzani F, Straccia P, Arciuolo D, Mulè A, et al. Hormonal environment and HER2 status in extra-mammary paget's disease (eMPD): A systematic literature review and meta-analysis with clinical considerations. *Diagn (Basel)* (2020) 10 (12):1040. doi: 10.3390/diagnostics10121040

11. Azmahani A, Nakamura Y, Ozawa Y, McNamara KM, Fujimura T, Haga T, et al. Androgen receptor, androgen-producing enzymes and their transcription factors in extramammary paget disease. *Hum Pathol* (2015) 46(11):1662–9. doi: 10.1016/j.humpath.2015.07.007

12. Fujimoto A, Takata M, Hatta N, Takehara K. Expression of structurally unaltered androgen receptor in extramammary paget's disease. *Lab Invest* (2000) 80(9):1465–71. doi: 10.1038/labinvest.3780153

13. Diaz de Leon E, Carcangiu ML, Prieto VG, McCue PA, Burchette JL, To G, et al. Extramammary paget disease is characterized by the consistent lack of estrogen and progesterone receptors but frequently expresses androgen receptor. *Am J Clin Pathol* (2000) 113(4):572–5. doi: 10.1309/P756-XXCB-TV71-U4XV

14. Tokuda Y, Arakura F, Uhara H. Combination chemotherapy of low-dose 5-fluorouracil and cisplatin for advanced extramammary paget's disease. *Int J Clin Oncol* (2015) 20(1):194–7. doi: 10.1007/s10147-014-0686-2

15. Yoshino K, Fujisawa Y, Kiyohara Y, Kadono T, Murata Y, Uhara H, et al. Usefulness of docetaxel as first-line chemotherapy for metastatic extramammary paget's disease. *J Dermatol Jun* (2016) 43(6):633–7. doi: 10.1111/1346-8138.13200

16. Egashira S, Kajihara I, Kanemaru H, Uemura-Kiyohara M, Yamada-Kanazawa S, Nakahara S, et al. Achieved good response of s-1 and docetaxel combination chemotherapy in two patients with metastatic extramammary paget's disease. *J Dermatol May* (2017) 44(5):e103–4. doi: 10.1111/1346-8138.13693

17. Kibbi N, Owen JL, Worley B, Wang JX, Harikumar V, Downing MB, et al. Evidence-based clinical practice guidelines for extramammary paget disease. *JAMA Oncol 04 01* (2022) 8(4):618–28. doi: 10.1001/jamaoncol.2021.7148

18. Hanawa F, Inozume T, Harada K, Kawamura T, Shibagaki N, Shimada S. A case of metastatic extramammary paget's disease responding to trastuzumab plus paclitaxel combination therapy. *Case Rep Dermatol* (2011) 3(3)223–7. doi: 10.1159/000333002

19. Wakabayashi S, Togawa Y, Yoneyama K, Suehiro K, Kambe N, Matsue H. Dramatic clinical response of relapsed metastatic extramammary paget's disease to trastuzumab monotherapy. *Case Rep Dermatol Med* (2012) 2012:401362. doi: 10.1155/2012/401362

20. Gunvén P, Randén M, Elmberger G, Bozóky B, Floore A. Gene expression profiling guiding diagnosis and therapy of rare mammary-like anogenital gland carcinomas. *Med Oncol Mar* (2012) 29(1):127–32. doi: 10.1007/s12032-010-9769-8

21. Barth P, Dulaimi Al-Saleem E, Edwards KW, Millis SZ, Wong YN, Geynisman DM. Metastatic extramammary paget's disease of scrotum responds completely to single agent trastuzumab in a hemodialysis patient: Case report, molecular profiling and brief review of the literature. *Case Rep Oncol Med* (2015) 2015:895151. doi: 10.1155/2015/895151

22. Ichiyama T, Gomi D, Fukushima T, Kobayashi T, Sekiguchi N, Sakamoto A, et al. Successful and long-term response to trastuzumab plus paclitaxel combination therapy in human epidermal growth factor receptor 2-positive extramammary paget's disease: A case report and review of the literature. *Mol Clin Oncol Nov* (2017) 7(5):763–6. doi: 10.3892/mco.2017.1422

23. Lu X, Zhang P, Zhu Y, Ye D. Human epidermal growth factor receptor 2 amplification as a biomarker for treatment in patients with lymph node-metastatic penoscrotal extramammary paget's disease. *Oncol Lett Mar* (2019) 17(3):2677–86. doi: 10.3892/ol.2019.9930

24. Nordmann TM, Messerli-Odermatt O, Meier L, Micaletto S, Coppetti T, Nägeli M, et al. Sequential somatic mutations upon secondary anti-HER2 treatment resistance in metastatic ERBB2. *Oncotar Nov 19* (2019) 10(62):6647–50. doi: 10.18632/oncotarget.27272

25. Bartoletti M, Mazzeo R, De Scordilli M, Del Fabro A, Vitale MG, Bortot L, et al. Human epidermal growth factor receptor-2 (HER2) is a potential therapeutic target in extramammary paget's disease of the vulva. *Int J Gynecol Cancer 11* (2020) 30(11):1672–7. doi: 10.1136/ijgc-2020-001771

26. Kimura T, Akamatsu Y, Kajihara I, Fukushima S, Ihn H. Case of metastatic extramammary paget's disease treated with trastuzumab-biosimilar monotherapy after s-1 and docetaxel combination chemotherapy. *J Dermatol* (2020) 47(1):e1–2. doi: 10.1111/1346-8138.15096

27. Yoneyama K, Kamada N, Kinoshita K, Kawashima T, Otani M, Endo H, et al. Androgen-deprivation regimen for multiple bone metastases of extramammary paget disease. *Br J Dermatol Oct* (2005) 153(4):853–5. doi: 10.1111/j.1365-2133.2005.06865.x

28. Shirasaki F, Takata M, Ishii T, Fujimoto A, Hatta N, Takehara K. Total androgen blockade therapy for an advanced case of extramammary paget's carcinoma. *Skin Cancer* (2001), 247–50. doi: 10.5227/skincancer.16.247

29. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical Oncology/College of American pathologists clinical practice guideline focused update. *J Clin Oncol* (2018) 36(20):2105–22. doi: 10.1200/JCO.2018.77.8738

30. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* (2001) 344(11):783–92. doi: 10.1056/NEJM200103153441101

31. Fukuda K, Funakoshi T. Metastatic extramammary paget's disease: Pathogenesis and novel therapeutic approach. *Front Oncol* (2018) 8:38. doi: 10.3389/fonc.2018.00038

32. Modi S, Saura C, Yamashita T, Park YH, Kim SB, Tamura K, et al. Trastuzumab deruxtecan in previously treated HER2-positive breast cancer. *N Engl J Med* (2020) 382(7):610–21. doi: 10.1056/NEJMoa1914510

33. Siena S, Di Bartolomeo M, Raghav K, Masuishi T, Loupakis F, Kawakami H, et al. Trastuzumab deruxtecan (DS-8201) in patients with HER2-expressing metastatic colorectal cancer (DESTINY-CRC01): a multicentre, open-label, phase 2 trial. *Lancet Oncol* (2021) 22(6):779–89. doi: 10.1016/S1470-2045(21)00086-3

34. Li BT, Smit EF, Goto Y, Nakagawa K, Udagawa H, Mazières J, et al. Trastuzumab deruxtecan in. *N Engl J Med* (2022) 386(3):241–51. doi: 10.1056/NEJMoa2112431

35. Pourmaleki M, Young JH, Socci ND, Socci ND, Chiang S, Edelweiss M, Li Y, et al. Extramammary paget disease shows differential expression of B7 family members B7-H3, B7-H4, PD-L1, PD-L2 and cancer/testis antigens NY-ESO-1 and MAGE-a. *Oncotarget* (2019) 10(58):6152–67. doi: 10.18632/oncotarget.27247

36. Guercio BJ, Iyer G, Kidwai WZ, Lacouture ME, Ghafoor S, Rossi AM, et al. Treatment of metastatic extramammary paget disease with combination ipilimumab and nivolumab: A case report. *Case Rep Oncol* (2021) 14(1):430–8. doi: 10.1159/000514345

37. Liegl B, Horn LC, Moinfar F. Androgen receptors are frequently expressed in mammary and extramammary paget's disease. *Mod Pathol* (2005) 18(10):1283–8. doi: 10.1038/modpathol.3800437

38. Miyamoto T, Inoue S, Adachi K, Takada R. Differential expression of mucin core proteins and keratins in apocrine carcinoma, extramammary paget's disease and apocrine nevus. *J Cutan Pathol* (2009) 36(5):529–34. doi: 10.1111/j.1600-0560.2008.01087.x

frontiers | Frontiers in Oncology

TYPE Brief Research Report
PUBLISHED 23 August 2022
DOI 10.3389/fonc.2022.879876

OPEN ACCESS

EDITED BY
Vladimir Spiegelman,
Penn State Milton S. Hershey Medical Center, United States

REVIEWED BY
April Rose,
McGill University, Canada
Muhammad Bilal Abid,
Medical College of Wisconsin, United States

*CORRESPONDENCE
Georg C. Lodde
georg.lodde@uk-essen.de

†These authors have contributed equally to this work and share last authorship

SPECIALTY SECTION
This article was submitted to Skin Cancer, a section of the journal Frontiers in Oncology

RECEIVED 20 February 2022
ACCEPTED 02 August 2022
PUBLISHED 23 August 2022

CITATION
Lodde GC, Fiedler M, Dittmer U, Placke JM, Jansen P, Becker JC, Zimmer L, Livingstone E, Schadendorf D, Sondermann W and Ugurel S (2022) COVID-19 vaccination in advanced skin cancer patients receiving systemic anticancer treatment: A prospective singlecenter study investigating seroconversion rates.
Front. Oncol. 12:879876.
doi: 10.3389/fonc.2022.879876

COVID-19 vaccination in advanced skin cancer patients receiving systemic anticancer treatment: A prospective singlecenter study investigating seroconversion rates

Georg C. Lodde[1*], Melanie Fiedler[2], Ulf Dittmer[2], Jan-Malte Placke[1], Philipp Jansen[1], Jürgen C. Becker[1,3,4], Lisa Zimmer[1], Elisabeth Livingstone[1], Dirk Schadendorf[1,4], Wiebke Sondermann[1†] and Selma Ugurel[1,4†]

[1]Department of Dermatology, University Hospital Essen, Essen, Germany, [2]Institute for Virology, University Hospital Essen, Essen, Germany, [3]Translational Skin Cancer Research (TSCR), University of Duisburg/Essen, Essen, Germany, [4]German Consortium for Translational Cancer Research (DKTK), Partner Site Essen/Düsseldorf, Essen/Düsseldorf, Germany

Background: COVID-19 vaccination reduces risk of SARS-CoV-2 infection, COVID-19 severity and death. However, the rate of seroconversion after COVID-19 vaccination in cancer patients requiring systemic anticancer treatment is poorly investigated. The aim of the present study was to determine the rate of seroconversion after COVID-19 vaccination in advanced skin cancer patients under active systemic anticancer treatment.

Methods: This prospective single-center study of a consecutive sample of advanced skin cancer patients was performed from May 2020 until October 2021. Inclusion criteria were systemic treatment for advanced skin cancer, known COVID-19 vaccination status, repetitive anti-SARS-CoV-2-S IgG serum quantification and first and second COVID-19 vaccination. Primary outcome was the rate of anti-SARS-CoV-2-S IgG seroconversion after complete COVID-19 vaccination.

Results: Of 60 patients with advanced skin cancers, 52 patients (86.7%) received immune checkpoint inhibition (ICI), seven (11.7%) targeted agents (TT), one (1.7%) chemotherapy. Median follow-up time was 12.7 months. During study progress ten patients had died from skin cancer prior to vaccination completion, six

patients were lost to follow-up and three patients had refused vaccination. 41 patients completed COVID-19 vaccination with two doses and known serological status. Of those, serum testing revealed n=3 patients (7.3%) as anti-SARS-CoV-2-S IgG positive prior to vaccination, n=32 patients (78.0%) showed a seroconversion, n=6 patients (14.6%) did not achieve a seroconversion. Patients failing serological response were immunocompromised due to concomitant hematological malignancy, previous chemotherapy or autoimmune disease requiring immunosuppressive comedications. Immunosuppressive comedication due to severe adverse events of ICI therapy did not impair seroconversion following COVID-19 vaccination. Of 41 completely vaccinated patients, 35 (85.4%) were under treatment with ICI, five (12.2%) with TT, and one (2.4%) with chemotherapy. 27 patients (65.9%) were treated non adjuvantly. Of these patients, 13 patients had achieved objective response (complete/partial response) as best tumor response (48.2%).

Conclusion and relevance: Rate of anti-SARS-CoV-2-S IgG seroconversion in advanced skin cancer patients under systemic anticancer treatment after complete COVID-19 vaccination is comparable to other cancer entities. An impaired serological response was observed in patients who were immunocompromised due to concomitant diseases or previous chemotherapies. Immunosuppressive comedication due to severe adverse events of ICI did not impair the serological response to COVID-19 vaccination.

KEYWORDS

COVID-19 vaccination, seroconversion, skin cancer, immune checkpoint inhibition, targeted therapy

Introduction

Patients with active cancer disease were reported to be at risk of poor outcomes from severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infection with an increased rate of severe courses and deaths from coronavirus disease 2019 (COVID-19) (1–3). Clinical trials of different COVID-19 vaccines (mRNA-1273, Moderna, Cambridge, USA; BNT162b24, BioNTech-Pfizer, Mainz, Germany; AZD1222, AstraZeneca, Oxford, UK) have demonstrated a reduced risk of SARS-CoV-2 infection and COVID-19 severity and death (4–6). However, these trials did not report on vaccination outcomes of cancer patients. A recent single-center study from Boston, USA reported impaired anti-SARS-CoV-2 antibody responses towards different COVID-19 vaccines in patients with various types of solid and hematologic cancers, describing an association of inferior serological response with the use of chemotherapies and corticosteroids (7). The aim of the present study was to determine the rate of seroconversion after COVID-19 vaccination in advanced skin cancer patients under active systemic anticancer treatment.

Materials and methods

Study design and patient eligibility

This prospective single-center study of a consecutive sample of advanced skin cancer patients was performed from May 2020 until October 2021 at the Department of Dermatology, University Hospital Essen. Inclusion criteria were systemic treatment for advanced skin cancer, known COVID-19 vaccination status, repetitive anti-SARS-CoV-2-S IgG serum quantification and first and second COVID-19 vaccination. Advanced skin cancer patients without systemic treatment, unknown COVID-19 vaccination status and incomplete COVID-19 vaccination were excluded from the study (Figure 1). Primary outcome was the rate of seroconversion in advanced skin cancer patients with systemic anticancer treatment after second dose of COVID-19 vaccination. Data on patient and tumor characteristics, concomitant diseases, immunosuppressive comedications, systemic anticancer therapy, and COVID-19 vaccination status were collected. Systemic anticancer therapies included immune checkpoint

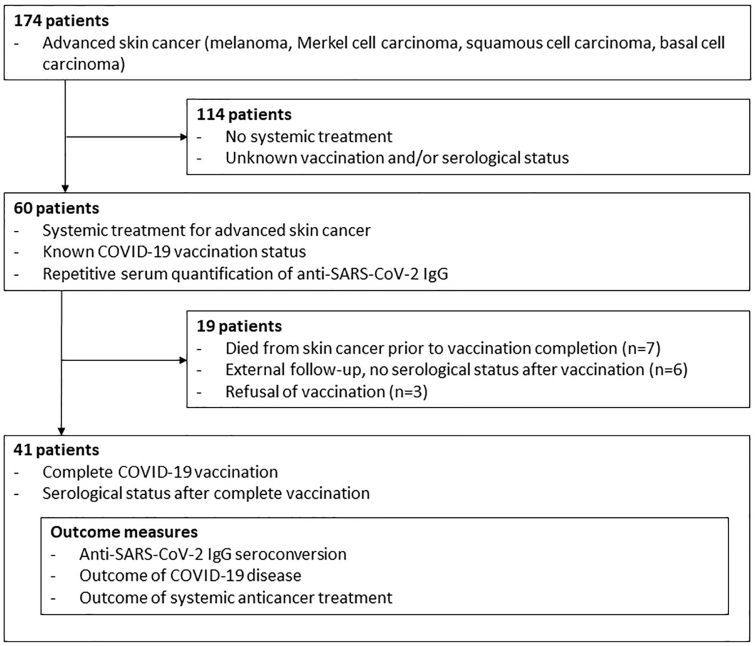


FIGURE 1
Patient Flow.

inhibition (ICI; anti-PD-1/anti-PD-L1/anti-CTLA-4 antibodies), targeted therapy (TT; BRAF+MEK kinase inhibition), and chemotherapy. Concomitant diseases were evaluated using the modified Charlson comorbidity index (CCI) (8). Primary study outcome was the rate of anti-SARS-CoV-2-S IgG seroconversion after second COVID-19 vaccination. Anti-SARS-CoV-2-S antibodies were measured at each dose (ICI) or cycle (TT, chemotherapy) of ongoing anticancer therapy, which corresponds to an at least monthly testing. In cases of treatment completion, serum testing was performed at follow-up visits in 3-months intervals. Secondary study outcome was best tumor response achieved to systemic anticancer treatment within the observation time of the study. It was measured by the institutional interdisciplinary tumor board as physician's assessment according to RECIST (9) within regular clinical practice in staging intervals.

Serum antibody testing

IgG antibodies against SARS-CoV-2 spike protein (anti-SARS-CoV-2-S IgG) in patients' sera were measured by chemiluminescence assays (SARS-CoV-2 S1/S2 IgG or SARS-CoV-2 TrimericS IgG, DiaSorin, Saluggia, Italy) using the LIASION-XL (DiaSorin) following the manufacturer's instructions. The first assay is only semi-quantitative, whereas the second one is quantitative and adjusted to the upcoming WHO standard. Values >15 AU/ml corresponding to 39 BAU/ml, and values >33.8 BAU/ml were considered positive, respectively. Sensitivity/specificity for each assay are 94,4%/98,6% and 96,9%/100%, respectively. For this study only qualitative results were used to document seroconversion.

Data analysis

The study was approved by the ethics committee of the University Duisburg-Essen (21-10141-BO). The period between first serum testing and last patient visit was considered as follow-up time. Descriptive statistics were performed using SPSSv26.0.

Results

Total study cohort

At start of this study, 60 patients with advanced skin cancers received systemic anticancer treatment (Figure 1). Median follow-up time was 12.7 months. Patients were treated for melanoma (n=51, 85.0%), Merkel cell carcinoma (n=6, 10.0%), cutaneous squamous cell carcinoma (n=2, 3.3%), and basal cell carcinoma (n=1, 1.7%); Table 1. Most patients had a modified CCI of 0 (n=44, 73.3%), and an unimpaired performance status (n=53, 88.3%). 52 patients (86.7%) received ICI therapy, seven

TABLE 1 Patient characteristics.

	Total study cohort N (%)	COVID-19 vaccinated N (%)
Total	**60 (100.0)**	**41 (100.0)**
Median age, years (range)	65.0 (41–80)	64.0 (41–80)
Sex		
Female	25 (41.7)	17 (51.5)
Male	35 (58.3)	24 (58.5)
Type of skin cancer		
Melanoma	51 (85.0)	37 (90.2)
Merkel cell carcinoma	6 (10.0)	2 (4.9)
Squamous cell carcinoma	2 (3.3)	1 (2.4)
Basal cell carcinoma	1 (1.7)	1 (2.4)
Charlson comorbidity index[1]		
0	44 (73.3)	31 (75.6)
1-2	13 (21.7)	9 (22.0)
≥3	3 (5.0)	1 (2.4)
Overall performance status (ECOG)		
0	53 (88.3)	38 (92.7)
1-2	6 (10.0)	1 (2.4)
≥3	1 (1.7)	2 (4.9)
LDH (serum)		
Normal	48 (80.0)	35 (85.4)
Increased	12 (20.0)	6 (14.6)
Number of organs involved		
0	19 (31.7)	14 (34.1)
1-3	32 (53.3)	23 (56.1)
>3	9 (15.0)	4 (9.8)
Type of systemic treatment		
Immune checkpoint inhibition	52 (86.7)	35 (85.4)
Monotherapy (PD-1, PD-L1)	38 (63.3)	26 (63.4)
Combination (CTLA-4+PD-1)	14 (23.3)	9 (22.0)
Targeted therapy (BRAF+MEK)	7 (11.7)	5 (12.2)
Chemotherapy	1 (1.7)	1 (2.4)
Treatment setting		
Adjuvant	19 (31.7)	14 (34.1)
Non-adjuvant	41 (68.3)	27 (65.9)
Treatment line		
First-line	35 (58.3)	27 (65.9)
Second-line or higher	25 (41.7)	14 (34.1)
Tumor response to systemic treatment		
Complete response	2 (3.3)	2 (3.3)
Partial response	15 (25.0)	11 (26.8)
Stable disease	6 (10.0)	4 (9.8)
Progressive disease	17 (28.3)	9 (22.0)
No evidence of disease	17 (28.3)	12 (29.3)
Not evaluable	3 (5.0)	3 (7.3)
Survival status		
Alive	50 (83.3)	40 (97.6)
Dead	10 (16.7)	1 (2.4)
Died from skin cancer	10 (16.7)	1 (2.4)
Died from COVID-19	0 (0.0)	0 (0.0)

Characteristics of 60 patients receiving systemic treatment for advanced skin cancer at the time of first serum testing for anti-SARS-CoV-2-S antibodies. The outcome of systemic treatment and the patients' survival status during study progress are also provided. [1] modified, the underlying skin cancer was excluded from comorbidities. LDH, lactate dehydrogenase.

patients (11.7%) received TT, and one patient received chemotherapy. Systemic anticancer treatment was given to 41 patients (68.3%) in a non-adjuvant setting.

At database closure on October 15, 2021, one patient had developed a symptomatic COVID-19 infection. At the time of symptomatic infection, this patient received a systemic treatment with CTLA-4+PD-1 for advanced melanoma. The laboratory data showed normal values for neutrophil granulocytes (5.27/nl, reference 1.7-6.2/nl), lymphocytes (1.42/nl, reference 1.0-3.4/nl) and LDH (234 U/l, reference 120-247 U/l). The patient suffered from mild clinical symptoms including taste disorders, glossodynia and dry coughing. Symptoms were declining spontaneously and the patient was vaccinated four months later when vaccines were available. None of the patients had died from COVID-19.

Patients with serological status after complete COVID-19 vaccination

During study progress ten patients had died from skin cancer prior to vaccination completion, six patients were lost to follow-up and three patients had refused vaccination due to fear of negative interaction with their anticancer treatment or of severe side effects (Figure 1). 41 patients completed COVID-19 vaccination, corresponding to two sequential mRNA and viral vector vaccine applications or a combination of viral vector and mRNA vaccine. From these 41 patients, serological status was known after complete vaccination. 35 (85.4%) were under treatment with ICI, five (12.2%) with TT, and one (2.4%) with chemotherapy (Table 1).

After complete COVID-19 vaccination, 32 patients (78.0%) showed an anti–SARS-CoV-2-S IgG seroconversion (Table 2). Seroconversion was achieved in 26/35 patients treated with ICI, 5/5 patients treated with TT, and 1/1 patient treated with chemotherapy. In three patients (7.3%) anti-SARS-CoV-2-S IgG was detected prior to vaccination, indicating previous SARS-CoV-2 infections which were asymptomatic and previously unknown to the respective patients. At the time of first COVID-19 vaccination, nine patients had active immunosuppressive comedications (Table 2). None of these nine patient were neutropenic at the time of vaccination (reference >1.7/nl). Seven patients received immunosuppressive comedications for treatment of severe adverse events of ICI (corticosteroids, n=6; extracorporeal photopheresis, n=1). All of these seven patients showed anti-SARS-CoV-2-S IgG seroconversion after complete vaccination.

In six of 41 completely vaccinated patients (14.6%) anti-SARS-CoV-2-S IgG antibodies could not be detected in repeated serological testings after vaccination (Table 2; Supplement Table 1). These patients failing seroconversion received ICI (CTLA-4+PD-1, n=2; PD-1, n=4) in time of vaccination. Two patients were treated with immunosuppressive comedications due to concomitant diseases with timing of vaccination.

One patient received IL-12 and IL-23 monoclonal antibody for treatment of chronic inflammatory bowel disease (M. Crohn) for more than 16 months without neutropenia (reference >1.7/nl). Furthermore, the patient had a known Turner syndrome.

The second patient received chimeric monoclonal CD-20 antibody for treatment of mantle cell lymphoma at the time of COVID-19 vaccination. The patient's history revealed first diagnosis of mantle cell lymphoma, stage IV Ann Arbor disease (10), 20 months prior to first COVID-19 vaccination. At time of first diagnosis of mantle cell lymphoma this patient had been treated with a R-CHOP chemotherapy for five months. Autologous stem cell transplantation was performed 15 months prior to COVID-19 vaccination with subsequently CD-20 antibody treatment. This patient had a lymphopenia (0.37/nl, reference 1.0-3.4/nl) with normal values of neutrophil granulocytes (4.14/nl, reference 1.7-6.2/nl) in time of vaccination.

The third patient without seroconversion had a palliative melanoma disease and multiple systemic treatment lines including darcabazine chemotherapy 12 months before COVID-19 vaccination. This patient had a lymphopenia (0.70nl, reference 1.0-3.4/nl) with slightly increased neutrophil granulocytes (6.43/nl, reference 1.7-6.2/nl).

The fourth patient without seroconversion was diagnosed with rectum carcinoma 38 months prior to first COVID-19 vaccination. After total excision of rectum carcinoma the patient had received FOLFOX chemotherapy as adjuvant treatment for five months. In time of vaccination differential blood showed no neutropenia nor lymphopenia.

The fifth patient with failed seroconversion was diagnosed with chronic lymphocytic leukemia 16 years prior to first vaccination. This patient had been treated with chemotherapy (cyclophosphamide, methotrexate, fluorouracil) after first diagnosis of chronic lymphocytic leukemia for six months. At time of vaccination the patient had normal values for neutrophile granulocytes and lymphocytes.

The last patient without seroconversion had a chronic lymphocytic leukemia, Binet stage A (11). This patient presented with leukocytosis (26.7/nl, reference 3.6-9.2) and lymphocytosis (13.9/nl; reference 1-4/nl) at time of vaccination.

Of 41 completely vaccinated patients, 27 patients (65.9%) were treated non adjuvantly. Of these patients, 13 patients had achieved an objective response as best tumor response (48.2%).

Of the 27 patients treated non adjuvantly, 21 patients had a melanoma. Objective response as best tumor response in exclusively non adjuvantly treated melanoma patients was 47.6% (n=10).

Discussion

In our consecutive cohort, 78.0% of skin cancer patients achieved an anti–SARS-CoV-2-S IgG seroconversion after

TABLE 2 COVID-19 vaccination and outcome.

Total	N 41
Vaccination type (first and second vaccination)	
mRNA (2x mRNA-1273, n=1; 2x BNT162b24, n=36)	37
Viral vector (2x AZD1222)	2
Mixed (1x viral vector, 1x mRNA)	2
Immunosuppressive comedication	
None	32
Corticosteroids (methylprednisolon ≥1mg/kg/d, prednisolon ≥100mg/d)	6
Rituximab	1
Extracorporeal photopheresis	1
Ustekinumab	1
Anti-SARS-CoV-2-S IgG (serum)	
Positive prior to vaccination	3
Positive after vaccination (seroconversion)	32
Immune checkpoint inhibition	26
Targeted therapy (BRAF+MEK)	5
Chemotherapy	1
Not positive after vaccination (no seroconversion)	6
Immune checkpoint inhibition	6
MM; concomitant chronic inflammatory bowel disease (M. Crohn); active immunosuppressive therapy (IL-12-/IL-23 monoclonal antibody)	1
MM; concomitant malignancy (mantle cell lymphoma); previous chemotherapy (R-CHOP, R-DHAP) and autologous stem cell transplantation; active immunosuppressive therapy (monoclonal CD-20 antibody)	1
MM; previous chemotherapy (dacarbazine), last treatment 12 months prior to time of vaccination	1
MM; concomitant malignancy (colorectal carcinoma); previous chemotherapy (FOLFOX), last treatment 33 months prior to time of vaccination	1
cSCC; concomitant malignancy (chronic lymphocytic leukemia); previous chemotherapy (cyclophosphamide, methotrexate, fluorouracil), last treatment 15 years prior to time of vaccination	1
MM; concomitant malignancy (chronic lymphocytic leukemia)	1

Characteristics of 41 advanced skin cancer patients with active anticancer therapy who completed COVID-19 vaccination during study progress, corresponding to the time of first vaccine application. Concomitant diseases and comedications of patients failing seroconversion are shown in detail. Abbreviations: MM, melanoma; cSCC, cutaneous squamous cell carcinoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; R-DHAP, rituximab, dexamethasone, cytarabine, cisplatin; FOLFOX, folinic acid, fluorouracil, oxaliplatin.

complete COVID-19 vaccination. Similar response rates have been described previously for patients with different cancer entities without immunosuppressive treatment (8, 12). However, as skin cancers are solid tumors, the rate of seroconversion was lower compared to the study of Thakkar et al. (13).

Only one patient developed symptomatic COVID-19, and no patient died from COVID-19 during study course.

With regard to anticancer treatment, the treatment outcomes observed in our investigated patient cohort are comparable to those reported from similar cohorts before the COVID-19 pandemic. Moreover, the anticancer treatment outcomes of completely vaccinated patients were not inferior to those of incompletely or not vaccinated patients. For non-adjuvant melanoma patients treated with ICI (n=21) we observed objective response rates comparable to previously reported responses in metastatic melanomas (14). Thus, our results strengthen the recent suggestion that advanced skin cancer patients should be offered treatment with ICI or TT in times of the pandemic without delay, as there is currently no evidence that this would increase the risk of severe COVID-19 (15, 16). However, our study is still limited by the low number of patients and the heterogeneity of baseline characteristics.

14.6% of the investigated patients did not develop an anti-SARS-CoV-2-S IgG antibody response despite complete vaccination. All of these patients were under active ICI treatment, yet their failure of seroconversion was likely caused by their underlying immunocompromised status as these

patients either had a concomitant hematological malignancy, a previously obtained chemotherapy, or an immunosuppressive comedication for concomitant autoimmune disease. This observation is in line with recently published data showing lower COVID-19 vaccination efficacy in immunocompromised patients (17). One study reported of seropositive response rates of 76% for patients with active myeloma compared to seropositive response rates of 98% in a healthy cohort study (12). A recently published study described lower rates of seroconversion in patients who underwent immunosuppressive treatments as stem cell transplant or CD-20-antibody treatment (13, 18). Two of six patients with impaired serological response had active immunosuppressive treatment (IL-12-/IL-23 monoclonal antibody, monoclonal CD-20 antibody) in time of vaccination (Table 1). Time since last administration of immunosuppressive therapy might be associated with seroconversion after vaccination (18). However, number of patients is too low for further analyzes of time of vaccination and time of administered immunosuppressive treatment.

In two of six patients with impaired serological response we detected a lymphopenia (<0.37/nl, reference 1.0-3.4/nl). One patient was treated with monoclonal CD-20 antibody for mantle cell lymphoma (mentioned above). The other patient was in palliative metastatic melanoma setting. Lymphopenia has been described previously as risk factor for impaired serological response in hematological malignancies (12). 17.1% (7/41) of the completely vaccinated patients of our study cohort required immunosuppressive comedication, mostly corticosteroids, for the treatment of severe adverse events of ICI therapy. Notably, none of these patients failed to develop an anti-SARS-CoV-2-S IgG seroconversion after COVID-19 vaccination. Management of adverse events of ICI therapy with corticosteroids may not reduce the rate of seroconversion and presents no contraindication for vaccination against COVID-19 (13). This finding begs the question if a delay in COVID-19 vaccination for patients under active treatment with corticosteroids for ICI adverse events is still recommendable (17).

The majority of patients received ICI therapy. ICI therapy may induce autoimmune side effects as pneumonitis (19, 20). Clinical symptoms of autoimmune pneumonitis overlaps with COVID-19 pneumonia. The indistinguishable clinical symptoms of autoimmune induced pneumonitis and COVID-19 pneumonia could lead to undetected breakthrough infections of COVID-19 (21).

Limitations of our study include the relatively small number of patients and the monocentric design. A direct comparison between subgroups of the study cohort is not valid due to the heterogeneity of baseline characteristics with respect to type of skin cancer, tumor stage, treatment setting and type of systemic treatment. Other limitations include the lack of a control group and of cellular data as B cell numbers and T cell response. The field of SARS-CoV-2 diagnostic is highly dynamic. Therefore, we had to use two test generations in this prospective longitudinal study. The first assay is only semi-quantitative, whereas the second one is quantitative and adjusted to the upcoming WHO standard. Serological response to COVID-19 vaccinations in advanced skin cancer patients was analyzed. This study is limited to analyze the efficacy of COVID-19 vaccination in skin cancer patients. Seroconversion has not been correlated with clinical outcomes as hospitalization and mortality rate. However, serological response might be associated with clinical outcomes and could be used to estimate the efficacy of COVID-19 vaccination (22, 23). Larger real-world studies are needed to confirm our preliminary findings.

Conclusion

Rate of seroconversion to COVID-19 vaccination is high in advanced skin cancer patients receiving active systemic anticancer treatment. An impaired serological response to COVID-19 vaccination was observed in patients who were immunocompromised due to concomitant hematological malignancies, previous chemotherapies, or immunosuppressive comedication for underlying autoimmune disease. Lymphopenia might be a risk factor for an impaired serological response to COVID-19 vaccination. Immunosuppressive comedication, in particular by corticosteroids, for treatment of severe side effects of anticancer immunotherapy, did not impair the serological response to COVID-19 vaccination.

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 approved by the institutional ethics committee of the University Duisburg-Essen (21-10141-BO). It was conducted in accordance with the Declaration of Helsinki. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization: SU, WS, and GL.; methodology: SU, MF, WS, and GL; formal analysis: MF, SU, WS, and GL; resources: UD, LZ, DS, EL, WS, SU, and GL; data curation, GL and SU.; writing - original draft preparation: GL and SU; writing and editing: GL, MF, UD, J-MP, PJ, JB, LZ, DS, EL, WS, and SU; visualization: SU, GL, and WS.; supervision: SU; project

administration: GL, SU, and WS; all authors have read and agreed to the final version of the manuscript.

Acknowledgments

We thank all study participants and their families. Part of this work was funded by the Deutsche Forschungsgemeinschaft (DFG, German 582 Research Foundation, RTG 2535, Knowledge- and data-driven personalization of medicine at the 583 point of care). J-MP was supported by the DFG in the framework of the DFG Clinician Scientist Programme UMEA, FU 356/12-1).

Conflict of interest

GL has received travel support from Sun Pharma. MF has given a paid lecture for Dia Sorin. J-MP served as consultant and/or has received honoraria from Bristol-Myers Squibb and Novartis, and received travel support from Bristol-Myers Squibb, Novartis and Therakos. JB declares speaker honoraria from Amgen, MerckSerono, Pfizer, Sanofi; advisory board honoraria from 4SC, Amgen, CureVac, eTheRNA, MerckSerono, Novartis and InProTher; research funding from Alcedis, Boehringer Ingelheim, Bristol-Myers Squibb, IQVIA, and MerckSerono; travel support from 4SC and Incyte. LZ served as consultant and/or has received honoraria from Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, Pierre-Fabre, Sunpharma and Sanofi; Research funding to institution: Novartis; travel support from Merck Sharp & Dohme, Bristol-Myers Squibb, Amgen, Pierre-Fabre, Sanofi, Sunpharma and Novartis, outside the submitted work. EL served as consultant and/or has received honoraria from Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, Medac, Pierre Fabre, Sanofi, Sunpharma and travel support from Amgen, Merck Sharp & Dohme, Bristol-Myers Squibb, Pierre Fabre, Sunpharma and Novartis, outside the submitted work. DS declares relevant financial activities with Roche, Novartis, Bristol-Myers Squibb, Merck Sharp & Dohme, Sanofi, Regeneron, Pfizer, Array, Pierre Fabre, 4SC, Helsinn, Philogen, InFlarX, Merck-Serono, SunPharma, Ultimovacs, and Sandoz. WS reports grants from medi GmbH Bayreuth, grants and personal fees from Novartis and Almirall, personal fees from Abbvie, Amgen, GSK, Lilly, UCB, LEO Pharma, Sanofi, Genzyme, and Janssen outside the submitted work. SU declares research support from Bristol Myers Squibb and Merck Serono; speakers and advisory board honoraria from Bristol Myers Squibb, Merck Sharp & Dohme, Merck Serono, Novartis and Roche, and travel support from Bristol Myers Squibb, Merck Sharp & Dohme, and Pierre Fabre; outside the submitted work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.879876/full#supplementary-material

References

1. Kuderer NM, Choueiri TK, Shah DP, Shyr Y, Rubinstein SM, Rivera DR, et al. Clinical impact of covid-19 on patients with cancer (Ccc19): A cohort study. *Lancet* (2020) 395(10241):1907–18. doi: 10.1016/S0140-6736(20)31187-9

2. Desai A, Sachdeva S, Parekh T, Desai R. Covid-19 and cancer: Lessons from a pooled meta-analysis. *JCO Global Oncol* (2020) 6):557–9. doi: 10.1200/go.20.00097

3. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with covid-19 in the New York City area. *JAMA* (2020) 323 (20):2052–9. doi: 10.1001/jama.2020.6775

4. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mrna-1273 sars-Cov-2 vaccine. *N Engl J Med* (2020) 384(5):403–16. doi: 10.1056/NEJMoa2035389

5. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the Bnt162b2 mrna covid-19 vaccine. *N Engl J Med* (2020) 383(27):2603–15. doi: 10.1056/NEJMoa2034577

6. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the Chadox1 ncov-19 vaccine (Azd1222) against sars-Cov-2: An interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* (2021) 397(10269):99–111. doi: 10.1016/S0140-6736(20)32661-1

7. Naranbhai V, Pernat CA, Gavralidis A, St Denis KJ, Lam EC, Spring LM, et al. Immunogenicity and reactogenicity of sars-Cov-2 vaccines in patients with cancer: The canvax cohort study. *J Clin Oncol* (2022) 40(1):12–23. doi: 10.1200/jco.21.01891

8. Thomas SJ. 1558o - covid-19 vaccine in participants (Ptcpts) with cancer: Subgroup analysis of Efficacy/Safety from a global phase III randomized trial of the Bnt162b2 (Tozinameran) mrna vaccine. *Ann Oncol* (2021) 32(suppl_5):S1129-S1163 101016/annonc/annonc713. doi: 10.1016/j.annonc.2021.08.1551

9. Schwartz LH, Litière S, de Vries E, Ford R, Gwyther S, Mandrekar S, et al. Recist 1.1-update and clarification: From the recist committee. *Eur J Cancer* (2016) 62:132–7. doi: 10.1016/j.ejca.2016.03.081

10. Armitage JO. Staging non-Hodgkin lymphoma. *CA: A Cancer J Clin* (2005) 55(6):368–76. doi: 10.3322/canjclin.55.6.368

11. Binet JL, Auquier A, Dighiero G, Chastang C, Piguet H, Goasguen J, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. *Cancer* (1981) 48(1):198–206. doi: 10.1002/1097-0142(19810701)48:1<198::aid-cncr2820480131>3.0.co;2-v

12. Avivi I, Balaban R, Shragai T, Sheffer G, Morales M, Aharon A, et al. Humoral response rate and predictors of response to Bnt162b2 mrna Covid19 vaccine in patients with multiple myeloma. *Br J Haematology* (2021) 195(2):186–93. doi: 10.1111/bjh.17608

13. Thakkar A, Gonzalez-Lugo JD, Goradia N, Gali R, Shapiro LC, Pradhan K, et al. Seroconversion rates following covid-19 vaccination among patients with cancer. *Cancer Cell* (2021) 39(8):1081–90.e2. doi: 10.1016/j.ccell.2021.06.002

14. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob J-J, Rutkowski P, Lao CD, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* (2019) 381(16):1535–46. doi: 10.1056/NEJMoa1910836

15. Switzer B, Haanen J, Lorigan PC, Puzanov I, Turajlic S. Clinical and immunologic implications of covid-19 in patients with melanoma and renal cell carcinoma receiving immune checkpoint inhibitors. *J ImmunoTherapy Cancer* (2021) 9(7):e002835. doi: 10.1136/jitc-2021-002835

16. Gambichler T, Reuther J, Scheel CH, Becker JC. On the use of immune checkpoint inhibitors in patients with viral infections including covid-19. *J ImmunoTherapy Cancer* (2020) 8(2):e001145. doi: 10.1136/jitc-2020-001145

17. Romano E, Pascolo S, Ott P. Implications of mrna-based sars-Cov-2 vaccination for cancer patients. *J ImmunoTherapy Cancer* (2021) 9(6):e002932. doi: 10.1136/jitc-2021-002932

18. Ollila TA, Lu S, Masel R, Zayac A, Paiva K, Rogers RD, et al. Antibody response to covid-19 vaccination in adults with hematologic malignant disease. *JAMA Oncol* (2021) 7(11):1714–6. doi: 10.1001/jamaoncol.2021.4381

19. Heinzerling L, de Toni E, Schett G, Hundorfean G, Zimmer L. Checkpoint inhibitors. *Dtsch Arztebl Int* (2019) 116(8):119–26. doi: 10.3238/arztebl.2019.0119

20. Eigentler TK, Hassel JC, Berking C, Aberle J, Bachmann O, Grünwald V, et al. Diagnosis, monitoring and management of immune-related adverse drug reactions of anti-Pd-1 antibody therapy. *Cancer Treat Rev* (2016) 45:7–18. doi: 10.1016/j.ctrv.2016.02.003

21. Abid MB. Overlap of immunotherapy-related pneumonitis and covid-19 pneumonia: Diagnostic and vaccine considerations. *J Immunother Cancer* (2021) 9 (4):e002307. doi: 10.1136/jitc-2020-002307

22. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic sars-Cov-2 infection. *Nat Med* (2021) 27(7):1205–11. doi: 10.1038/s41591-021-01377-8

23. Earle KA, Ambrosino DM, Fiore-Gartland A, Goldblatt D, Gilbert PB, Siber GR, et al. Evidence for antibody as a protective correlate for covid-19 vaccines. *Vaccine* (2021) 39(32):4423–8. doi: 10.1016/j.vaccine.2021.05.063

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 17 November 2022
DOI 10.3389/fonc.2022.975342

OPEN ACCESS

EDITED BY
Pamela Bond Cassidy,
Oregon Health and Science University, United States

REVIEWED BY
Georg Christian Lodde,
University Hospital of Essen, Germany
Angela Webb,
Peter MacCallum Cancer Centre, Australia
Arjen Cleven,
Leiden University Medical Center (LUMC), Netherlands

*CORRESPONDENCE
Doris Helbig
doris.helbig@uk-koeln.de
Sebastian Klein
sebastian.klein@uk-essen.de

SPECIALTY SECTION
This article was submitted to Skin Cancer, a section of the journal Frontiers in Oncology

RECEIVED 22 June 2022
ACCEPTED 03 November 2022
PUBLISHED 17 November 2022

CITATION
Helbig D and Klein S (2022) Immune checkpoint inhibitors for unresectable or metastatic pleomorphic dermal sarcomas. *Front. Oncol.* 12:975342. doi: 10.3389/fonc.2022.975342

Immune checkpoint inhibitors for unresectable or metastatic pleomorphic dermal sarcomas

Doris Helbig [1*] and Sebastian Klein [2*]

[1]Department of Dermatology, University Hospital Cologne, Cologne, Germany, [2]Department of Hematology and Stem Cell Transplantation, University Duisburg-Essen, University Hospital Essen, Essen, Germany

Pleomorphic dermal sarcomas (PDS) are rare neoplasms of the skin that occur in UV-exposed sites in the elderly, but represent the most common cutaneous sarcomas. Although the majority of PDS can be surgically removed, local recurrences occur in up to 28%, usually occurring within the first two years after primary excision. Metastases are diagnosed in up to 20% of cases, mainly observed in the skin, lymph nodes and lungs, preferentially affecting patients with underlying hemato-oncologic diseases. Similar to other UV-induced tumors, PDS are inflammatory and immunogenic tumors (with a high number of CD4+/CD8+ tumor-infiltrating lymphocytes (TILs) and checkpoint molecule expression such as PD-L1, LAG-3, TIGIT) with a very high mutational burden. The most common genetic alterations include UV-induced *TP53* loss of function mutations, followed by alterations in the *CDKN2A/B* gene. Rarely, targetable genetic alterations can be detected. Compelling experimental data and clinical reports about PD-1/PD-L1-blocking antibodies in patients with PDS suggest its use as first line treatment in unresectable or metastatic tumor stages. However, individual („off-line") patient management should be discussed in an interdisciplinary tumor board based on molecular genetic testing, mutational burden, PD-L1 expression, and evidence of tumor-infiltrating lymphocytes in addition to comorbities of the individual patient.

KEYWORDS

Immune checkpoint inhibitor, PD-1, PD-L1, pleomorphic dermal sarcoma (PDS), unresectable, metastasized

Introduction

Pleomorphic dermal sarcomas (PDS) are rare neoplasms of the skin with a mesenchymal (fibroblastic) lineage differentiation, arising in UV-exposed locations, typically diagnosed in elderly male individuals (1–3). Although accurate incidence data do not exist, they represent the most common cutaneous sarcomas with increasing incidence due to demographic changes.

Given the similarities in clinic, histology as well as molecular genetics and epigenetics, atypical fibroxanthoma (AFX) and PDS are now considered a spectrum of one entity, but differ in terms of therapy and prognosis (4–10). Histomorphologically, AFX and PDS show similar features. The main difference is that AFX are confined to the dermis whereas PDS involves distinct portions of the subcutis and/or have necrotic tumor portions and/or perineural or lympho-vascular invasion. In AFX, the local recurrence rate after R0 resection is less than 5% (3, 11, 12). While the majority of PDS can be treated by curative excisions, local recurrences occur in up to 28% of patients. Metastases are observed in up to 20%, mainly in the skin, lymph nodes and lungs, preferentially affecting patients with underlying hemato-oncologic diseases (3, 10, 11, 13, 14).

In the last years, it could be shown that PDS are inflammatory and immunogenic tumors with a very high mutational burden. Experimental data and clinical reports indicate that PD-1/PD-L1-blocking antibodies are highly effective in patients with unresectable or metastatic PDS and suggest its use as first line treatment in these advanced tumor stages (2, 15–17).

Genetic alterations

Both, AFX and PDS have a very high mutational burden with UV signature, which is even higher than that of other UV-induced skin tumors such as cutaneous squamous cell carcinomas (cSCC) and malignant melanomas (2, 18–20). Based on very similar gene mutations, gene expressions, copy number variations as well as DNA methylation profiles, AFX and PDS are now accepted to represent a spectrum of the same tumor entity (5, 7–9). In PDS, it has been shown that the tumors exhibit the UV-induced mutation signatures 7a and 7b in almost equal proportions. In other UV-induced tumors such as cSCC, basal cell carcinoma, and melanoma, signature 7a is typically detected, whereas signature 7b is rarely detected. Signature 44, which has been associated with defective DNA mismatch repair (MMR), has been detected in a small number of our investigated PDS (3 of 28); however, is much more common in cSCC (Figure 1) (2).

Gene mutation and gene expression analyses revealed that AFX/PDS have the highest similarity to cSCC. The most frequent genetic alterations are *TP53* loss of function mutations which can be detected in all PDS, followed by genetic alterations in the *CDKN2A/B* gene (*CDKN2A/B* mutations in 68%, deletions in 71%, and both in 46%, while 7% showed even a biallelic loss.) (Figure 1) (2). Other common mutations include *DNHD1*, *GNAS*, *RTN1*, *RTL1*, *ZBTB7A*, *NCKAP5L*, *FAM200A*, *NOTCH1/2*, *FAT1*, and *TERT* promoter mutations (2, 5, 6, 8, 20, 21). In contrast, cSCC, basal cell carcinomas and malignant melanomas show a significantly lower mutation frequency of these frequently mutated genes (2). In a small proportion of PDS, amplifications of *PDGFRA* leading to a PDGFRA expression on protein level and mutations within the kinase domain of *KIT* could be detected.

Immunophenotyping

Immunohistochemical as well as mRNA expression analyses of the immune "microenvironment" have shown that the majority of PDS represent inflammatory and immunogenic tumors with a high number of CD8+ tumor-infiltrating lymphocytes (TILs) and expression of diverse checkpoint molecules such as PD-L1, TIGIT, LAG-3, and CTLA-4 (2, 15, 16).

When we classified a series of PDS tumors into immunologically hot and cold tumors (high versus low amounts of both CD4/CD8+ cells and high versus low PD-L1 expression), we did not detect a significant difference of tumor mutational burden

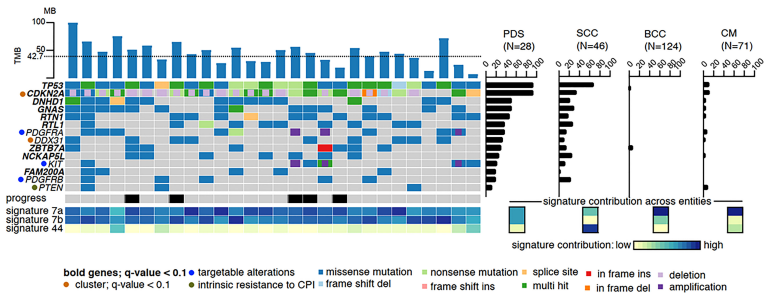


FIGURE 1
High mutational burden and most common mutations of PDS: The dotted line represents the average of variants per megabase. The mutational frequency of pleomorphic dermal sarcoma (PDS) is compared to cutaneous squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and cutaneous melanoma (CM), where the bars indicate their relative prevalence (percentage, right panel). Cross entity comparison of mutational signatures shown below the right panel of mutational frequencies. Clinically, locally or systemically progressed tumors are indicated with a black bar (2).

(TMB). Nevertheless, the TMB is usually high in almost all PDS cases. Differential gene expression analysis between these immunologically hot and cold tumors revealed upregulation of TIGIT in the immunologically hot tumors (2).

In general, elevated levels of immune-related cytokines such as IL1A, IL2, as well as markers that were very recently linked to enhanced response of immunotherapy in malignant melanoma, including CD27, and CD40L have been detected in PDS tumors (22, 23). Moreover, the majority of PDS showed strong MHC-I expression and upregulated HLA class I molecules (*HLA-A, HLA-B, HLA-C* and *HLA-E*, corresponding in humans to the MHC class I) that are involved in tumor neoantigen presentation to tumor-specific CD8+ T lymphocytes leading to tumor cell apoptosis (24–28).

In CD8+high PDS cases (defined as cases with CD8 levels above median), genes such as *CD74*, *LYZ* and *HLA-B* were found to be differentially expressed while the remaining cases revealed enhanced levels of immunosuppressive cytokines including *CXCL14* (29). In addition, the majority of PDS was infiltrated by PD-L1-, PD-1- and LAG-3-expressing immune cells and showed strong MHC-I expression on tumor cells (15).

These results imply that PDS in general, but especially those with a lot of infiltrating CD8+ and/or PD-L1- and LAG-3-expressing TILs as well as MHC-I expression, induce an adequate anti-tumor immune response, which could be enhanced by immune checkpoint inhibitors. Only a small proportion of tumors appear to develop "immune escape" mechanisms, such as downregulation of MHC-I molecules (2, 15, 16).

Treatment of localized stage PDS

Radical excision followed by histopathologic workup is usually performed with curative intent as initial treatment for PDS. An appropriate safety margin should be maintained, as the risk of local recurrence or metastasis can be reduced by wide local excision (3, 12, 13, 30, 31). If this is not possible, a microscopically controlled excision should be performed. Although there are no published data on the radiation sensitivity of AFX/PDS, radiation of the tumor area may be considered if complete tumor excision is not possible. The efficacy of adjuvant radiation with respect to the prognosis of completely excised PDS has not been conclusively established. In an evaluation of a few patients who had received adjuvant postradiation, a positive tendency (fewer local recurrences and/or metastases) of this postradiation could be elicited (3).

Treatment of advanced stage PDS including immune checkpoint inhibition

In case of advanced stage PDS, therapy recommendations should be always discussed and issued in the context of an interdisciplinary tumor board because there is no proven standard therapy. Here, molecular genetic testing, mutational burden, PD-L1 expression, and evidence of tumor-infiltrating lymphocytes (TILs) should be incorporated into individual treatment recommendations.

Since PDS harbor a high mutational burden and mostly exhibit an inflamed, proimmunogenic tumor microenvironment, susceptibility to immune checkpoint inhibition by programmed cell death 1 (PD1)/programmed cell death ligand 1 (PD-L1) inhibitors, (e.g. pembrolizumab, nivolumab) or the anti–CTLA-4 antibody (ipilimumab), or a combination of these agents was presumed in reference to other highly mutated and immunogenic tumors including other skin tumors such as malignant melanoma and cSCC (18, 32, 33). In the meantime, the exceptionally high efficacy of the anti-PD-1 inhibitor pembrolizumab has been described in case reports or small case series (see Table 1) (2, 15–17). Until now, there are no case reports describing the use of other CPI in PDS patients. Nevertheless, larger clinical studies are needed to investigate tumor response to CPI in PDS patients.

For PDS cases with low levels of CD8+ TILs, interventions to increase the infiltration of these inflammatory cells in general need

TABLE 1 Clinical case reports of PDS patients receiving CPI.

Authors	Patient (M/F; age in years)	PDS location	Treatment	TMB	PD-L1 expression	CD8+ TILs	Treatment outcome	Immuno-suppression
Klein S. et al. (2, 16)	1. M;77	Recurrence on the forehead + multiple parietal cutaneous metastases	Pembrolizumab (2 mg/kg/3 weeks)	63.2/MB	Moderate infiltration of PD-L1+ TILs	Moderate	Complete remission after 8 cycles as well as sustained response over 4 years (discontinuation of CPI treatment after 21months)	No

(Continued)

TABLE 1 Continued

Authors	Patient (M/F; age in years)	PDS location	Treatment	TMB	PD-L1 expression	CD8+ TILs	Treatment outcome	Immuno-suppression
	2. M; 88	Inoperable primary (temporal)	Pembrolizumab (200 mg/3 weeks) combined with local RT (70 Gy using 6 MeV electrons in a linear accelerator)	78.0/MB	Limited PD-L1 expression of tumor and TILs	Moderate	Complete remission after 4 months. Patient died shortly thereafter due to CLL progression	CLL
	3. F; 79	Inoperable primary on the cheek with infiltration of the mandibular bone	One single dose of Pembrolizumab, stopped due to autoimmune colitis (grade II)	NA	TPS=80%	NA	Tumor meltdown after single infusion	CLL
Klein O. et al. (17)	M; NA	2 cutaneous metastases on the scalp, parotid gland metastases, 2 lung metastases	Pembrolizumab	NA	NA	Heavy lymphocytic infiltration	Complete remission after 3 months, treatment stop after owing to a flare of pre-existing polymyalgia rheumatica	No

CPI, checkpoint inhibition; M/F, male/female; TILs, Tumor-infiltrating lymphocytes; TMB/MB, Tumor mutational burden/megabases; TPS, Tumor Proportion score; RT, radiotherapy; NA, Not applicable.

to be explored as a future direction for successful treatment with CPI. As shown in other tumor entities, a dual blockade of CTLA-4 and PD-1 or PD-1/PDL-1 and LAG-3 could probably enhance the efficacy of CPI monotherapies, also by rescuing CD8+ T cells more vigorously from exhaustion than single signaling blockade (34, 35).

In case of contraindications for a CPI treatment and if oncogenic alterations are detected, targeted therapies should be discussed, although there is no experience with targeted therapies in PDS to date (2, 7, 8). In relation to this, rarely detected *PDGFRA* or *KIT* amplifications/mutations could be of interest as several drugs have proven to induce long-term remissions in PDGFR-expressing cancers, such as gastrointestinal stromal tumors, dermatofibrosarcoma protuberans, or myeloid malignancies (36–40). Furthermore, it has been shown that tumors with a loss of *CDKN2A/B* may benefit from CDK4/6 inhibitors, such as palbociclib, abemaciclib or ribociclib, all approved for the treatment of metastasized breast cancer (41–44).

In patients with advanced stage PDS treated with CPI, further investigation of predictors is still needed. However, all existing studies suggest a high efficacy of immune checkpoint blockade in inoperable or metastatic PDS patients.

Author contributions

All authors contributed to the article and approved the submitted version.

Funding

We acknowledge support by the Open Access Publication Fund of the University of Duisburg-Essen.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer GL declared a shared affiliation with the author SK to the handling editor at the time of review.

Publisher's note

References

1. Anderson HL, Joseph AK. A pilot feasibility study of a rare skin tumor database. *Dermatol Surg* (2007) 33(6):693–6. doi 10.1111/j.1524-4725.2007.33145.x

2. Klein S, Quaas A, Noh KW, Cartolano M, Abedpour N, Mauch C, et al. Integrative analysis of pleomorphic dermal sarcomas reveals fibroblastic differentiation and susceptibility to immunotherapy. *Clin Cancer Res* (2020) 26 (21):5638–45. doi: 10.1158/1078-0432.CCR-20-1899

3. Persa OD, Loquai C, Wobser M, Baltaci M, Dengler S, Kreuter A, et al. Extended surgical safety margins and ulceration are associated with an improved prognosis in pleomorphic dermal sarcomas. *J Eur Acad Dermatol Venereol* (2019) 33(8):1577–80. doi: 10.1111/jdv.15493

4. Withers AH, Brougham ND, Barber RM, Tan ST. Atypical fibroxanthoma and malignant fibrous histiocytoma. *J Plast Reconstr Aesthet Surg* (2011) 64(11): e273–8. doi: 10.1016/j.bjps.2011.05.004

5. Griewank KG, Wiesner T, Murali R, Pischler C, Muller H, Koelsche C, et al. Atypical fibroxanthoma and pleomorphic dermal sarcoma harbor frequent NOTCH1/2 and FAT1 mutations and similar DNA copy number alteration profiles. *Mod Pathol* (2018) 31(3):418–28. doi: 10.1038/modpathol.2017.146

6. Griewank KG, Schilling B, Murali R, Bielefeld N, Schwamborn M, Sucker A, et al. TERT promoter mutations are frequent in atypical fibroxanthomas and pleomorphic dermal sarcomas. *Mod Pathol* (2014) 27(4):502–8. doi: 10.1038/modpathol.2013.168

7. Helbig D, Quaas A, Mauch C, Merkelbach-Bruse S, Buttner R, Emberger M, et al. Copy number variations in atypical fibroxanthomas and pleomorphic dermal sarcomas. *Oncotarget* (2017) 8(65):109457–67. doi: 10.18632/oncotarget.22691

8. Helbig D, Ihle MA, Putz K, Tantcheva-Poor I, Mauch C, Buttner R, et al. Oncogene and therapeutic target analyses in atypical fibroxanthomas and pleomorphic dermal sarcomas. *Oncotarget* (2016) 7(16):21763–74. doi: 10.18632/oncotarget.7845

9. Koelsche C, Stichel D, Griewank KG, Schrimpf D, Reuss DE, Bewerunge-Hudler M, et al. Genome-wide methylation profiling and copy number analysis in atypical fibroxanthomas and pleomorphic dermal sarcomas indicate a similar molecular phenotype. *Clin Sarcoma Res* (2019) 9:2. doi: 10.1186/s13569-019-0113-6

10. Helbig D, Ziemer M, Dippel E, Erdmann M, Hillen U, Leiter U, et al. S1-guideline atypical fibroxanthoma (AFX) and pleomorphic dermal sarcoma (PDS). *J Dtsch Dermatol Ges* (2022) 20(2):235–43. doi: 10.1111/ddg.14700

11. Helbig D. Hemato-oncological diseases as risk factor for recurrence or metastasis of pleomorphic dermal sarcoma. *Front Oncol* (2022) 12:873771. doi: 10.3389/fonc.2022.873771

12. Tolkachjov SN, Kelley BF, Alahdab F, Erwin PJ, Brewer JD. Atypical fibroxanthoma: Systematic review and meta-analysis of treatment with mohs micrographic surgery or excision. *J Am Acad Dermatol* (2018) 79(5):929–34.e6. doi: 10.1016/j.jaad.2018.06.048

13. Miller K, Goodlad JR, Brenn T. Pleomorphic dermal sarcoma: Adverse histologic features predict aggressive behavior and allow distinction from atypical fibroxanthoma. *Am J Surg Pathol* (2012) 36(9):1317–26. doi: 10.1097/PAS.0b013e31825359e1

14. Tardio JC, Pinedo F, Aramburu JA, Suarez-Massa D, Pampin A, Requena L, et al. Pleomorphic dermal sarcoma: A more aggressive neoplasm than previously estimated. *J Cutan Pathol* (2016) 43(2):101–12. doi: 10.1111/cup.12603

15. Klein S, Mauch C, Wagener-Ryczek S, Schoemmel M, Buettner R, Quaas A, et al. Immune-phenotyping of pleomorphic dermal sarcomas suggests this entity as a potential candidate for immunotherapy. *Cancer Immunol Immunother* (2019) 68 (6):973–82. doi: 10.1007/s00262-019-02339-3

16. Klein S, Persa OD, Mauch C, Noh KW, Pappesch R, Wagener-Ryczek S, et al. First report on two cases of pleomorphic dermal sarcoma successfully treated with immune checkpoint inhibitors. *Oncoimmunology* (2019) 8(12):e1665977. doi: 10.1080/2162402X.2019.1665977

17. Klein O, McTigue C, Wong ZW, Syme DB, Hunter-Smith DJ. Complete response of metastatic pleomorphic dermal sarcoma to anti-PD-1 therapy. *Br J Dermatol* (2020) 183(6):e189. doi: 10.1111/bjd.19309

18. Migden MR, Khushalani NI, Chang ALS, Lewis KD, Schmults CD, Hernandez-Aya L, et al. Cemiplimab in locally advanced cutaneous squamous cell carcinoma: Results from an open-label, phase 2, single-arm trial. *Lancet Oncol* (2020) 21(2):294–305. doi: 10.1016/S1470-2045(19)30728-4

19. Rischin D, Migden MR, Lim AM, Schmults CD, Khushalani NI, Hughes BGM, et al. Phase 2 study of cemiplimab in patients with metastatic cutaneous squamous cell carcinoma: Primary analysis of fixed-dosing, long-term outcome of weight-based dosing. *J Immunother Cancer* (2020) 8(1):1–8. doi: 10.1136/jitc-2020-000775

20. Lai K, Harwood CA, Purdie KJ, Proby CM, Leigh IM, Ravi N, et al. Genomic analysis of atypical fibroxanthoma. *PLoS One* (2017) 12(11):e0188272. doi: 10.1371/journal.pone.0188272

21. Sakamoto A, Oda Y, Itakura E, Oshiro Y, Tamiya S, Honda Y, et al. H-, K-, and n-ras gene mutation in atypical fibroxanthoma and malignant fibrous histiocytoma. *Hum Pathol* (2001) 32(11):1225–31. doi: 10.1053/hupa.2001.28953

22. Auslander N, Zhang G, Lee JS, Frederick DT, Miao B, Moll T, et al. Robust prediction of response to immune checkpoint blockade therapy in metastatic melanoma. *Nat Med* (2018) 24(10):1545–9. doi: 10.1038/s41591-018-0157-9

23. Wasiuk A, Testa J, Weidlick J, Sisson C, Vitale L, Widger J, et al. CD27-mediated regulatory T cell depletion and effector T cell costimulation both contribute to antitumor efficacy. *J Immunol* (2017) 199(12):4110–23. doi: 10.4049/jimmunol.1700606

24. Chowell D, Krishna S, Becker PD, Cocita C, Shu J, Tan X, et al. TCR contact residue hydrophobicity is a hallmark of immunogenic CD8+ T cell epitopes. *Proc Natl Acad Sci U.S.A.* (2015) 112(14):E1754–62. doi: 10.1073/pnas.1500973112

25. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* (2015) 348(6230):124–8. doi 10.1126/science.aaa1348

26. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* (2014) 371(23):2189–99. doi: 10.1056/NEJMoa1406498

27. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* (2015) 350(6257):207–11. doi: 10.1126/science.aad0095

28. Qian J, Luo F, Yang J, Liu J, Liu R, Wang L, et al. TLR2 promotes glioma immune evasion by downregulating MHC class II molecules in microglia. *Cancer Immunol Res* (2018) 6(10):1220–33. doi: 10.1158/2326-6066.CIR-18-0020

29. Starnes T, Rasila KK, Robertson MJ, Brahmi Z, Dahl R, Christopherson K, et al. The chemokine CXCL14 (BRAK) stimulates activated NK cell migration: Implications for the downregulation of CXCL14 in malignancy. *Exp Hematol* (2006) 34(8):1101–5. doi: 10.1016/j.exphem.2006.05.015

30. Lonie S, Yau B, Henderson M, Gyorki D, Angel C, Webb A. Management of pleomorphic dermal sarcoma. *ANZ J Surg* (2020) 90(11):2322–4. doi: 10.1111/ans.15909

31. Soleymani T, Tyler Hollmig S. Conception and management of a poorly understood spectrum of dermatologic neoplasms: Atypical fibroxanthoma, pleomorphic dermal sarcoma, and undifferentiated pleomorphic sarcoma. *Curr Treat Options Oncol* (2017) 18(8):50. doi: 10.1007/s11864-017-0489-6

32. Krieger T, Pearson I, Bell J, Doherty J, Robbins P. Targeted literature review on use of tumor mutational burden status and programmed cell death ligand 1 expression to predict outcomes of checkpoint inhibitor treatment. *Diagn Pathol* (2020) 15(1):6. doi: 10.1186/s13000-020-0927-9

33. Maubec E, Boubaya M, Petrow P, Beylot-Barry M, Basset-Seguin N, Deschamps L, et al. Phase II study of pembrolizumab as first-line, single-drug therapy for patients with unresectable cutaneous squamous cell carcinomas. *J Clin Oncol* (2020) 38(26):3051–61. doi: 10.1200/JCO.19.03357

34. Nghiem PT, Bhatia S, Lipson EJ, Kudchadkar RR, Miller NJ, Annamalai L, et al. PD-1 blockade with pembrolizumab in advanced merkel-cell carcinoma. *N Engl J Med* (2016) 374(26):2542–52. doi: 10.1056/NEJMoa1603702

35. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non-Small-Cell lung cancer. *N Engl J Med* (2015) 373(2):123–35. doi: 10.1056/NEJMoa1504627

36. Nishida T, Doi T, Naito Y. Tyrosine kinase inhibitors in the treatment of unresectable or metastatic gastrointestinal stromal tumors. *Expert Opin Pharmacother* (2014) 15(14):1979–89. doi: 10.1517/14656566.2014.937707

37. Metzgeroth G, Walz C, Erben P, Popp H, Schmitt-Graeff A, Haferlach C, et al. Safety and efficacy of imatinib in chronic eosinophilic leukaemia and hypereosinophilic syndrome: A phase-II study. *Br J Haematol* (2008) 143 (5):707–15. doi: 10.1111/j.1365-2141.2008.07294.x

38. Rutkowski P, Klimczak A, Lugowska I, Jagielska B, Wagrodzki M, Debiec-Rychter M, et al. Long-term results of treatment of advanced dermatofibrosarcoma protuberans (DFSP) with imatinib mesylate - the impact of fibrosarcomatous transformation. *Eur J Surg Oncol* (2017) 43(6):1134–41. doi: 10.1016/j.ejso.2017.03.011

39. Ugurel S, Mentzel T, Utikal J, Helmbold P, Mohr P, Pfohler C, et al. Neoadjuvant imatinib in advanced primary or locally recurrent dermatofibrosarcoma protuberans: a multicenter phase II DeCOG trial with long-term follow-up. *Clin Cancer Res* (2014) 20(2):499–510. doi: 10.1158/1078-0432.CCR-13-1411

40. Fu Y, Kang H, Zhao H, Hu J, Zhang H, Li X, et al. Sunitinib for patients with locally advanced or distantly metastatic dermatofibrosarcoma protuberans but resistant to imatinib. *Int J Clin Exp Med* (2015) 8(5):8288–94.

41. Eilers G, Czaplinski JT, Mayeda M, Bahri N, Tao D, Zhu M, et al. CDKN2A/p16 loss implicates CDK4 as a therapeutic target in imatinib-resistant dermatofibrosarcoma protuberans. *Mol Cancer Ther* (2015) 14(6):1346–53. doi: 10.1158/1535-7163.MCT-14-0793

42. Shah A, Bloomquist E, Tang S, Fu W, Bi Y, Liu Q, et al. FDA Approval: Ribociclib for the treatment of postmenopausal women with hormone receptor-positive, HER2-negative advanced or metastatic breast cancer. *Clin Cancer Res* (2018) 24(13):2999–3004. doi: 10.1158/1078-0432.CCR-17-2369

43. Sledge GWJr., Toi M, Neven P, Sohn J, Inoue K, Pivot X, et al. MONARCH 2: Abemaciclib in combination with fulvestrant in women with HR+/HER2-advanced breast cancer who had progressed while receiving endocrine therapy. *J Clin Oncol* (2017) 35(25):2875–84. doi: 10.1200/JCO.2017.73.7585

44. Turner NC, Ro J, Andre F, Loi S, Verma S, Iwata H, et al. Palbociclib in hormone-Receptor-Positive advanced breast cancer. *N Engl J Med* (2015) 373 (3):209–19. doi: 10.1056/NEJMoa1505270

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 22 November 2022
DOI 10.3389/fonc.2022.1022967

OPEN ACCESS

EDITED BY
Hongzan Sun,
Shengjing Hospital of China Medical University, China

REVIEWED BY
XS Zhao,
Southern Medical University, China
Wei Wei,
Xi'an Polytechnic University, China

*CORRESPONDENCE
Mark Kriegsmann
mark.kriegsmann@med.uni-heidelberg.de

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work and share last authorship

SPECIALTY SECTION
This article was submitted to Skin Cancer, a section of the journal Frontiers in Oncology

RECEIVED 19 August 2022
ACCEPTED 24 October 2022
PUBLISHED 22 November 2022

CITATION
Kriegsmann K, Lobers F, Zgorzelski C, Kriegsmann J, Janßen C, Meliß RR, Muley T, Sack U, Steinbuss G and Kriegsmann M (2022) Deep learning for the detection of anatomical tissue structures and neoplasms of the skin on scanned histopathological tissue sections.
Front. Oncol. 12:1022967.
doi: 10.3389/fonc.2022.1022967

Deep learning for the detection of anatomical tissue structures and neoplasms of the skin on scanned histopathological tissue sections

Katharina Kriegsmann[1†], Frithjof Lobers[2†], Christiane Zgorzelski[3], Jörg Kriegsmann[4,5], Charlotte Janßen[6], Rolf Rüdinger Meliß[7], Thomas Muley[8], Ulrich Sack[2], Georg Steinbuss[1‡] and Mark Kriegsmann[3*‡]

[1]Department of Hematology, Oncology and Rheumatology, Heidelberg University, Heidelberg, Germany, [2]Department of Clinical Immunology, Medical Faculty, University of Leipzig, Leipzig, Germany, [3]Institute of Pathology, Heidelberg University, Heidelberg, Germany, [4]MVZ Histology, Cytology and Molecular Diagnostics Trier, Trier, Germany, [5]Proteopath Trier, Trier, Germany, [6]Center for Industrial Mathematics (ZeTeM), University of Bremen, Bremen, Germany, [7]Institute for Dermatopathology, Hannover, Germany, [8]Translational Lung Research Centre (TLRC) Heidelberg, Member of the German Centre for Lung Research (DZL), Heidelberg, Germany

Basal cell carcinoma (BCC), squamous cell carcinoma (SqCC) and melanoma are among the most common cancer types. Correct diagnosis based on histological evaluation after biopsy or excision is paramount for adequate therapy stratification. Deep learning on histological slides has been suggested to complement and improve routine diagnostics, but publicly available curated and annotated data and usable models trained to distinguish common skin tumors are rare and often lack heterogeneous non-tumor categories. A total of 16 classes from 386 cases were manually annotated on scanned histological slides, 129,364 100 x 100 µm (~395 x 395 px) image tiles were extracted and split into a training, validation and test set. An EfficientV2 neuronal network was trained and optimized to classify image categories. Cross entropy loss, balanced accuracy and Matthews correlation coefficient were used for model evaluation. Image and patient data were assessed with confusion matrices. Application of the model to an external set of whole slides facilitated localization of melanoma and non-tumor tissue. Automated differentiation of BCC, SqCC, melanoma, naevi and non-tumor tissue structures was possible, and a high diagnostic accuracy was achieved in the validation (98%) and test (97%) set. In summary, we provide a curated dataset including the most common neoplasms of the skin and various anatomical compartments to enable researchers to train, validate and

improve deep learning models. Automated classification of skin tumors by deep learning techniques is possible with high accuracy, facilitates tumor localization and has the potential to support and improve routine diagnostics.

KEYWORDS

deep learning, pathology, artificial intelligence, dermatopathology, digital pathology, deep learning - artificial neural network

1 Introduction

Skin cancer is the most common cancer type in the United States (1). Patient management and prognosis is variable and depends on the entity, molecular changes, as well as on the clinical stage at the time of diagnosis (2). Skin cancer is a highly heterogeneous group composed of non-melanotic and melanotic neoplasms (3). Among the non-melanotic neoplasms, basal cell carcinoma (BCC) and squamous cell carcinomas (SqCC) are the most common (4) and usually well treatable. Despite major advances in treatment, most deaths from skin cancer are still due to melanoma (5). Thus, the correct diagnosis is paramount for treatment selection and prognosis.

Currently, the diagnosis of different cutaneous tumor types is based on physical examination, dermatoscopy and ultimately histological evaluation of an excision specimen. While reliable diagnosis can be made in a substantial number of tissue specimen on a regular standard stain alone, a significant subset of neoplastic skin lesions requires additional immunohistology and molecular studies for definite classification. In particular, the differentiation between BCC and SqCC, as well as between naevi and melanoma may be challenging. As the incidence of skin cancer is increasing, while the number of pathologists and dermato-pathologists is decreasing in many countries, the introduction of new methods to support skin tumor diagnostics is desirable (6).

The use of deep learning methods applied to clinical images, dermatoscopy images or scanned histopathological slides holds great promise to support cancer diagnostics in general (7, 8), and skin cancer diagnostics in particular (5, 9). In the past, the feasibility and potential to classify different diseases on scanned histological slides has been demonstrated for automated localization and diagnosis of melanoma (9, 10), the differentiation between naevi and melanoma (11), and the differentiation between basaloid, squamous, melanocytic and other skin tumors (12).

Major problems identified in previous studies for routine diagnostic application of such algorithms are: (i) no consideration of non-tumor skin categories or inclusion of only one non-tumor skin class, (ii) the need for manual annotation of the tissue region of interest prior to automated tumor classification, and (iii) non-availability of raw data images for validation purposes. In the current study, we therefore annotated major non-tumor anatomical tissue structures of the skin and major skin tumor categories and subsequently trained a convolutional neuronal network using an up-to-date workflow. We localized and categorized skin tumors on whole slides without prior annotation, validated our data on an external test set and provide all images and code to enable other researchers to improve and validate their data.

2 Methods

2.1 Patient data

Whole slides from patients with BCC (n = 93), SqCC (n = 100), naevi (n = 98) and melanoma (n = 87) were extracted from the archive of the Institute of Pathology, Heidelberg University, the MVZ for Histology, Cytology and Molecular Diagnostics Trier and the Institute for Dermatopathology Hannover. Diagnoses were made according to the World Health Organization (WHO) Classification of Skin Tumours (13). All slides with representative tumor regions were scanned using an automated slide scanner (Aperio AT2, Leica Biosystems, Nussloch, Germany) with 400 x magnification, as previously described (14). Image data were anonymized and are provided along with this manuscript (Link: https://heidata.uni-heidelberg.de/privateurl.xhtml?token=366931ac-50a2-43f9-880f-88d63e07d493). Moreover, an independent external dataset of melanoma whole slides was downloaded from the website of the Cancer Imaging Archive (CPTAC-CM) (15). After quality review 62 cases were included as an external test set, while 41 of these cases were melanoma and 21 were tumor-free skin. The analysis was approved by the local ethics committee of Heidelberg University.

2.2 Image data

Scanned histopathological slides were imported into QuPath (16) (v.0.1.2, University of Edinburgh, Edinburgh, UK) and

annotated (F.L. and M.K.) for the following 16 categories: chondral tissue, dermis, elastosis, epidermis, hair follicle, skeletal muscle, necrosis, nerves, sebaceous glands, subcutis, eccrine glands (sweat glands), vessels, BCC, SqCC, naevi and melanoma. Image patches 100 x 100 µm (~395 x 395 px) in size were generated in QuPath, extracted on the local hard drive and subsequently reviewed. Blurry images were deleted. Representative image patches are displayed in Figure 1.

2.3 Splitting of datasets

Images from patients were separated into a training, validation and test set. All image patches from one patient were used in only one of the respective sets. Since there are only three cases with elastosis, we assigned the case with most elastosis patches to the training, the case with the second most patches to the test and the remaining case to the validation set. All other cases were assigned randomly to one of the sets. The sets were not changed during the analyses. The splits by image patches and patients are displayed in Table 1.

2.4 Hard- and software

For training we used a p3.2xlarge instance from Amazon Web Services with a single V100 GPU while for inference we used a Lenovo P1 Gen 2 laptop. Further we used the Scientific Data Storage (SDS) service from Heidelberg University. Training and inference were performed using a singularity container image based on the TensorFlow Docker container image. For random augmentation we used the respective function in the

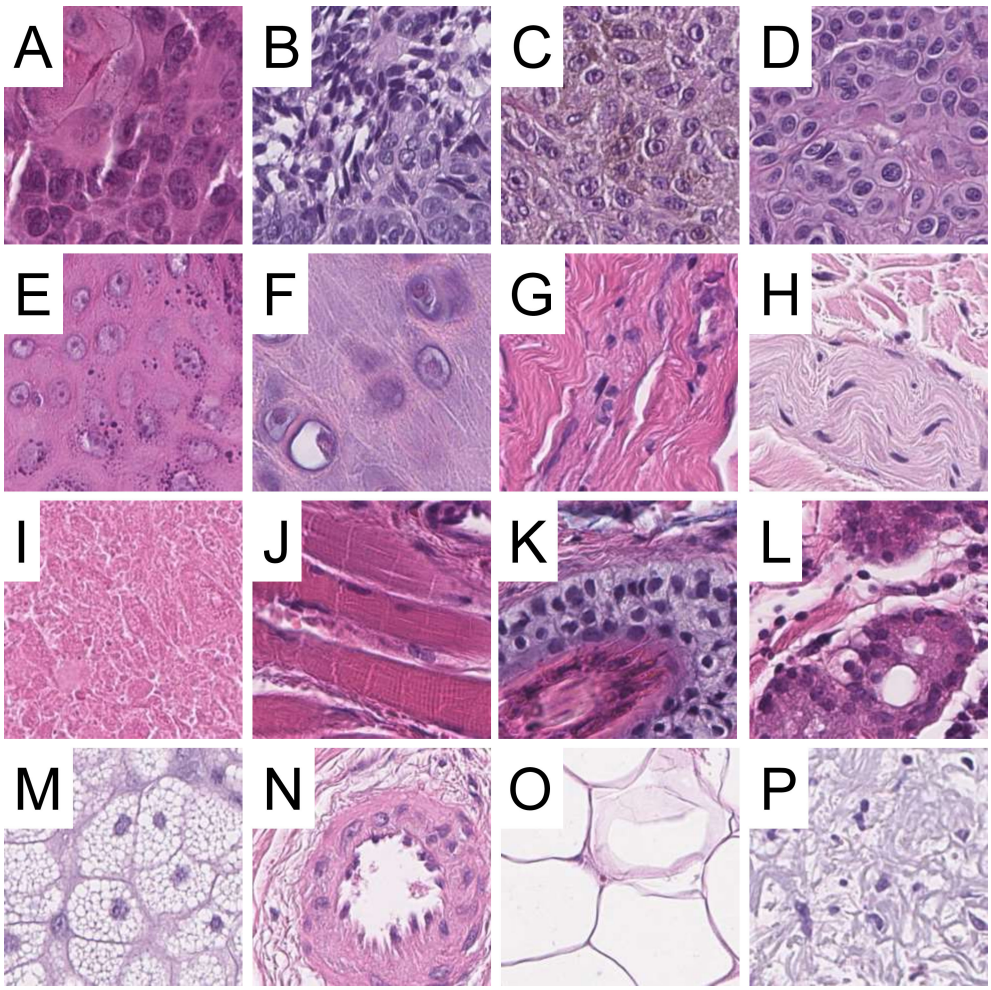


FIGURE 1
Examples of image patches included in the dataset. Squamous cell carcinoma **(A)**, basal cell carcinoma **(B)**, melanoma **(C)**, naevi **(D)**, epidermis **(E)**, chrondral tissue **(F)**, dermis **(G)**, nerves **(H)**, necrosis **(I)**, skeletal muscle **(J)**, hair follicles **(K)**, sweat glands/eccrine glands **(L)**, sebaceous glands **(M)**, vessels **(N)**, subcutis **(O)** and elastosis **(P)**. All images are 100 x 100 µm (395 x 395 px) in size, scale bar = 20 µm.

10.3389/fonc.2022.1022967

TABLE 1 Number of image patches and patients in the training, validation and test set.

Class	Training, n (%)		Validation, n (%)		Test, n (%)	
	by patches	by patient	by patches	by patient	by patches	by patient
Chondral tissue	4442 (62)	9 (50)	743 (10)	2 (11)	1992 (28)	7 (39)
Dermis	15878 (70)	134 (69)	1857 (8)	20 (10)	4875 (22)	39 (20)
Elastosis	136 (65)	1 (33)	6 (3)	1 (33)	66 (32)	1 (33)
Epidermis	10419 (74)	130 (70)	1086 (8)	19 (10)	2613 (19)	36 (19)
Hair follicle	1437 (71)	104 (71)	250 (12)	15 (10)	325 (16)	27 (18)
Skeletal muscle	6159 (80)	47 (73)	904 (12)	7 (11)	669 (9)	10 (16)
Necrosis	1641 (54)	24 (67)	468 (15)	5 (14)	924 (30)	7 (19)
Nerves	1201 (64)	93 (68)	219 (12)	13 (10)	464 (25)	30 (22)
Sebaceous glands	7268 (67)	94 (69)	1074 (10)	13 (9)	2565 (24)	30 (22)
Subcutis	7370 (61)	64 (65)	1245 (10)	9 (9)	3438 (29)	26 (26)
Sweat glands	2533 (71)	94 (71)	220 (6)	11 (8)	818 (23)	27 (20)
Vessels	1068 (65)	109 (71)	136 (8)	14 (9)	439 (27)	31 (20)
BCC	6919 (78)	71 (76)	1063 (12)	12 (13)	941 (11)	10 (11)
SqCC	6793 (61)	61 (61)	919 (8)	10 (10)	3470 (31)	29 (29)
Naevi	7923 (75)	72 (73)	944 (9)	8 (8)	1762 (17)	18 (18)
Melanoma	7784 (67)	59 (68)	1220 (10)	9 (10)	2678 (23)	19 (22)

BCC, basal cell carcinoma; SqCC, squamous cell carcinoma.

image python module. The code is available at Link: https://heidata.uni-heidelberg.de/privateurl.xhtml?token=366931ac-50a2-43f9-880f-88d63e07d493.

2.5 Training and validation of models

Each model is based on the EfficientNetV2 architecture (17), was trained for a total of 30 epochs and with a learning rate of either 0.01, 0.001 or 0.001. We used a batch size of 64 for the smaller S models and 32 for the medium sized M models (cf. Larger Model). We used the AMSGrad optimizer (a variant of the Adam optimizer (18) with $\beta1 = 0.9$, $\beta2 = 0.999$ and $\epsilon\hat{} = 1.0*10^{-7}$). During training the data was sampled such that there was no class imbalance. We used random augmentation (19) with n=2 to reduce overfitting (M is different for the models, see below). Each model configuration (a set of model hyperparameters, e.g. the learning rate) was trained three times to account for the randomness involved model training (e.g. the random weights initialization). We first trained a few models to find a good learning rate, then tested if a learning rate schedule, progressive training (17) or a larger model improved prediction quality.

Hyperparameters for the initial models were as follows: image input size of 300 x 300 px, a dropout of 0.3 and M=15 for random augmentation. Using the learning rate scheduler, the learning rate was linearly increased from 0 to its base value (e.g. 0.01) for the first five epochs as in the original EfficientNetV2 publication (so called warm-up) (17) and subsequently exponentially decayed with a rate of 0.97. In progressive training the training set was split in different stages. In each stage, i.e. after a certain number of epochs, size of input images and regularization (such as dropout) were increased. The aim of progressive training was to improve training speed by using smaller image sizes on early epochs. Hyperparameters for the first 15 epochs were: image size of 128 x 128 px, top dropout 0.1 and M = 5 for random augmentation. Hyperparameters for the last 15 epochs were: image size of 300 x 300 px, top dropout 0.3 and M = 15 for random augmentation. For the larger model, we used the EfficientNetV2 M instead of the EfficientNetV2 S. We had to decrease the batch size to 32 such that the model and data fitted into the GPU memory. For all EfficientNetV2 M models, progressive learning was applied with the following hyperparameters: for the first 15 epochs: image size of 128 x 128 px, top dropout 0.1 and M = 5 for random augmentation; for the last 15 epochs: image size of 380 x 380 px, top dropout 0.4 and M = 20 for random augmentation. All models were subsequently compared and the model with the best performance was selected.

For each training run we recorded cross entropy loss, balanced accuracy (BAC) and Matthews correlation coefficient (MCC) for training as well as validation data (20). While we recorded and display all three metrics, we used MCC to select the best model. The validation set results were evaluated using confusion matrices. To visualize the proximity of the different classes, the last convolutional layer after the last pooling operation of the validation data was subjected to dimension reduction using uniform manifold approximation and projection (UMAP) computed *via* the Python package umap-learn.

2.6 Evaluation of the test set and on the external set

The best performing model (cf. Section 2.5) was applied to the test set. As all data were scanned with one scanner system and all cases were derived from institutions in Germany, we additionally applied our algorithm on an external set of melanomas where preprocessing of the tissue and most scanning conditions were unknown and staining properties were different. As the external test data had a high burden of artefacts, all slides were manually reviewed and only cases that passed a quality control were used for further analysis. However, remaining slides still had a relatively high burden of artefacts such as blurry areas, tissue tears, variation in tissue thickness, dust particles, high amount of necrosis and overall low tissue and staining quality. In addition, the magnification of the external slides was 200x which was different compared to the magnification of our data which was 400x. We decided to still test our algorithm on this set to evaluate suboptimal input data.

3 Results

3.1 Model training and optimization

In total 129,364 image tiles from 386 cases were used for training. Within all broader categories of models, a learning rate of 0.001 seemed to perform best regarding MCC on the validation data. The initial models (Supplementary Figure 1), the models trained with a learning rate scheduler (Supplementary Figure 2), models trained using progressive learning (Supplementary Figure 3) and larger models (Supplementary Figure 4) are depicted in the supplementary materials.

We compared the best performing models to choose the final model (Figure 2). The best performing model based on either loss, BAC or MCC are shown in Table 2. We decided for the model performing best in terms of MCC which was trained using the following configuration: EfficientNetV2 S, batch size of 64, progressive learning and learning rate of 0.001.

3.2 Evaluation on the validation data

A confusion matrix shows generally high concordance between actual and predicted classes on the validation set based on image patches (Figure 3A). Within the tumor category, naevus was mostly misclassified as melanoma (2.75%) and vice versa (4.18%). Moreover, SqCC was most commonly misclassified as BCC (8.81%) and vice versa (1.51%). Among the non-tumor categories, the classes with misclassifications of > 5% were elastosis (33.33%), vessels (9.56%) and nerves (5.94%), that were misclassified as dermis. The non-tumor category that had highest misclassification rates was epidermis which was misclassified as SqCC (5.43%) and vice versa (3.70%, Figures 3B, C).

To render a final diagnosis on a whole slide in the routine diagnostic scenario, the image patch-based result may not be very informative. Thus, we evaluated the proportion of the tumor image tiles that were correct on the case level (Figure 4). Overall, only two out of 81 (2.5%) tumor patients had very low proportions of image tiles that voted for the correct diagnosis. Interestingly, in both cases the correct diagnosis was squamous cell carcinoma. When examining both cases in detail (Supplementary Figure 5), one case would have finally been misclassified based on a majority vote for the final tumor class. In the other case, misclassifications in the non-tumor category would have luckily led to the correct diagnosis based on a

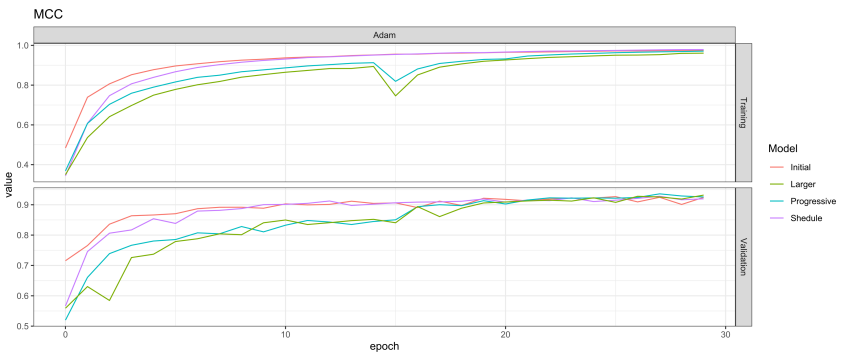


FIGURE 2
Matthews correlation coefficient (MCC) for the best models. MCC increases with the initial model, the model with learning rate scheduler, the model with progressive training and the larger model across the training process in the training as well as in the validation set. Models which used progressive learning show a drop at epoch 15 which corresponds to the changing hyperparameter settings at this point of time. MCC, Matthews correlation coefficient.

TABLE 2 The three best models after training based on loss, balanced accuracy and Matthews correlation coefficient.

Metric	Model ID	Epoch	Training			Validation		
			loss	BAC	MCC	loss	BAC	MCC
Loss	19	27	0.08955	0.96953	0.96750	0.18650	0.91650	0.93590
BAC	20	28	0.08871	0.96920	0.96760	0.18998	0.94276	0.93178
MCC	19	27	0.08955	0.96953	0.96750	0.18650	0.91650	0.93590

BAC, balanced accuracy; ID, Identifier; MCC, Matthews correlation coefficient.

majority vote for the final diagnosis, which would result in a final diagnostic accuracy of 98.7% (80/81).

3.3 Evaluation on the test data

For test data a confusion matrix showed a high degree of concordance between actual and predicted classes based on image patches (Supplementary Figure 6). The test data generally exhibited the same misclassifications as described on the validation data. Likewise, on the case level (Supplementary Figure 7) the diagnostic accuracy regarding the tumor classes was high with 74 out of 76 (97.4%) correct classifications. The respective confusion matrices of the two misclassified cases are provided in Supplementary Figure 8. When non-tumor and tumor classes were taken together, 73 out of 76 (96.0%) of cases were correctly classified. Among the three cases with a wrong classification result, one case was a SqCC misclassified as BCC and two cases were melanoma misclassified as SqCC.

3.4 Visualization of the resemblance of the images

To visualize the resemblance of the images, the output of last convolutional layer after the final pooling operation was subjected to dimension reduction. A UMAP diagram confirms that classes that are very different morphologically such as skeletal muscle, sebaceous glands and chondral tissue, are separated clearly from other image categories. On the other hand, melanocytic lesions such as naevi and melanoma show proximity and also some overlap. UMAP diagram of validation data (Figure 5) and test data (Supplementary Figure 9) are displayed.

3.5 Evaluation on the external test set

A total of 62 external slides passed the initial quality control. Quality issues were noted even in the remaining cases that passed the quality control. Examples of quality issues of the external test set are provided in Supplementary Figure 10. Of the 41 melanoma cases, 32 were predicted as melanoma (78%), 7 cases were predicted as BCC (17%), one case (2%) was predicted as SqCC and naevus, respectively. Non-tumor skin cases had generally a proportion of tiles predicted as tumor <5% of all image tiles and were rather randomly distributed throughout the whole slide.

4 Discussion

In the past, deep learning techniques have been shown to support diagnosis and prognosis prediction in many neoplastic and non-neoplastic diseases, such as but not limited to prostate cancer (21), lung cancer (22), breast cancer (23), pancreatic cancer (8), colon cancer (24), skin cancer (25, 26), cancer of unknown primary (27), or scoring of fibrosis or fat in non-neoplastic liver disease (28, 29).

In skin diseases, the technique has been mainly used in neoplastic diseases so far. While most reports focus on either melanocytic (9–11, 30) or non-melanocytic (26) lesions, data on both melanocytic and non-melanocytic lesions, non-tumor skin lesions or anatomical tissue structures are scarce (12, 26). Although there are honorable exceptions (31), most of the published studies on deep learning on histopathological slides do not make their annotated data, the full dataset of image patches and/or their code available. Our study narrows this gap by providing image data of 16 different classes including normal anatomical tissue structures, reactive solar elastosis and the most common neoplastic skin lesions. This complements the dataset from Thomas et al., who provided a publicly available comprehensive segmented dataset including 12 different classes for non-melanoma skin cancer and anatomical tissue structures. We hope our data will enable researchers to validate our and their results and to develop new methods for the application of deep learning to support pathologists and ultimately improve patient care. Of note, we believe it is necessary to avoid a common non-tumor skin category and to separate morphologically distinct classes during training, as the different anatomical tissue structures are morphologically highly heterogeneous, which has also been highlighted by a UMAP diagram in the current study. The introduction of non-tumor skin categories will also enable automated classification of whole slides, without prior annotation of the tumor area, which is important to achieve a workflow that is faster than the current

10.3389/fonc.2022.1022967

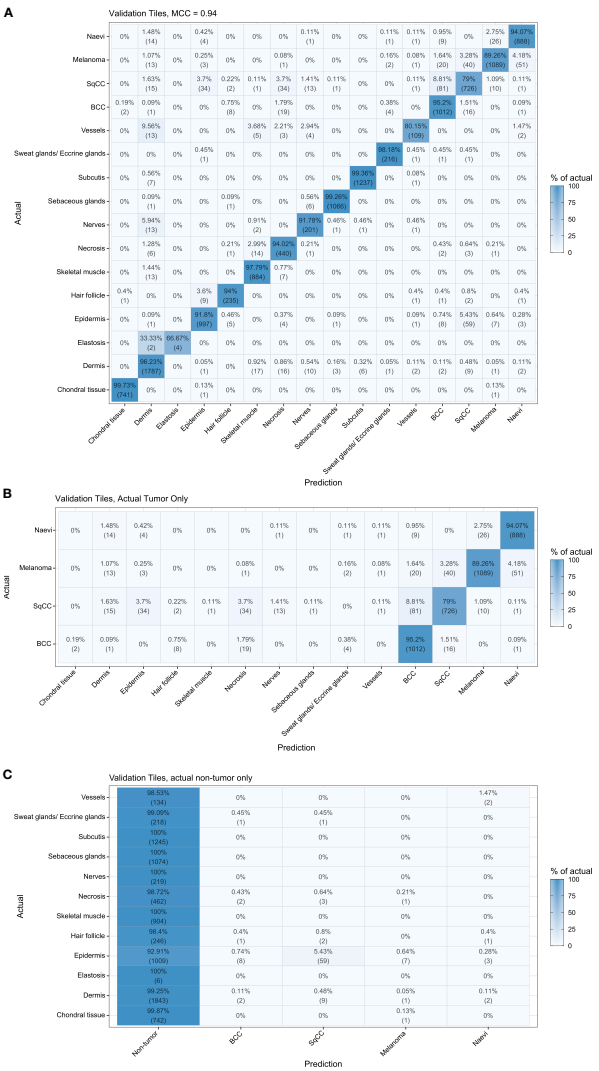


FIGURE 3
Confusion matrix of the validation set. High concordance between actual and predicted classes can be observed. The algorithm shows a higher rate of misclassification of elastosis and vessels with dermis, which can be explained since elastotic changes and vessels are commonly observed in the dermis **(A)**. In tumor categories **(B)** a higher rate of misclassifications was observed for squamous cell carcinomas that were predicted as basal cell carcinoma. The misclassification of non-tumor categories as tumor was rare but observed with epidermis, misclassified as squamous cell carcinoma **(C)**. BCC, basal cell carcinoma; SqCC, squamous cell carcinoma.

analog setting, although we are aware of recent developments that may overcome this issue (32). Additionally, the identification of anatomical tissue structures may be suitable for automatic tissue orientation with subsequent automatic distance measurements of the tumor margin (26).

Technically, we have used the EfficientNetV2, which has achieved high top-1 and top-5 accuracies on the ImageNet reference dataset and is a modern and efficient alternative to larger and more computationally expensive architectures available (17). In the past other algorithms, specifically for segmentation or specific organ systems have been published (33, 34). The architecture used in this study has been successfully applied to histological images previously (35). We have successfully applied techniques like image augmentation of progressive learning that have been suggested to find a well performing model in the current study (17). To train a reliable deep learning model, a large number of images is usually necessary to account for technical and biological variation. In this regard, a higher number of patient samples is commonly preferred over a large number of images. The number of patients included for training, validation and testing in the current investigation is within the reported range of previous studies

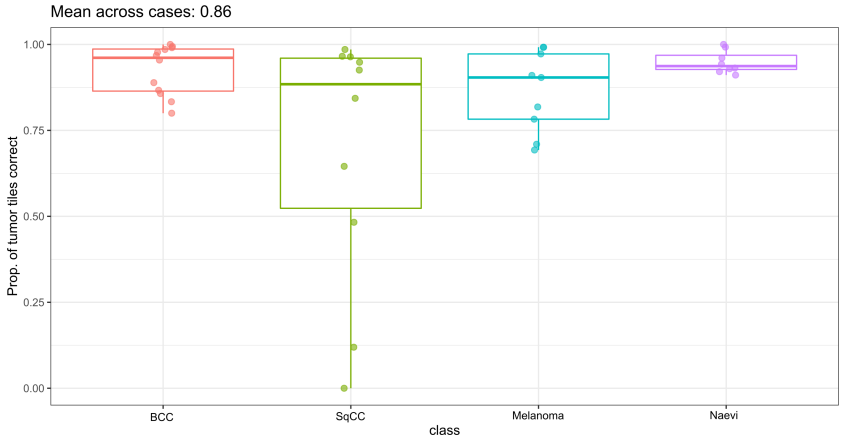


FIGURE 4
Proportion of tumor image tiles that was correctly classified on patient level in the validation set. Most tumors were correctly classified on patient level. Two patients with squamous cell carcinomas were misclassified. BCC, basal cell carcinoma; SqCC, squamous cell carcinoma.

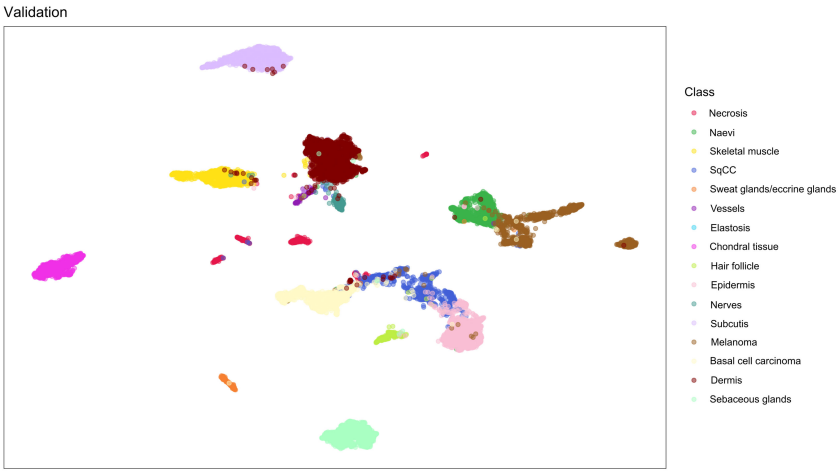


FIGURE 5
Dimension reduction using uniform manifold approximation and projection based on the last convolutional layer after the last pooling operation of the validation data. Close proximity of image classes that resemble each other morphologically such as melanocytic tumors can be observed. On the other hand, image categories that are morphologically very different such as skeletal muscle, sebaceous glands or chrondral tissue show distinct clusters. BCC, basal cell carcinoma; SqCC, squamous cell carcinoma.

(9, 11, 26). Currently, there is no consensus on the minimum number of cases that should be included in a deep learning study. Algorithms that require manual annotation comprise often a much lower number of patients as compared to approaches that do not need manual annotation. The previously reported studies on deep learning on histopathological slides have included between dozens to >1000 cases per entity (32, 36). The largest manually annotated publicly available dataset on skin cancer subtypes comprises 290 whole slides. Our study includes a total of 16 classes from 386 manually annotated cases which is within the reported range. Thus, we think that our approach is valid although there is no firmly established standard to calculate sample sizes for deep learning studies. Our training and validation workflow included not only a training and validation set, but also an internal and an external test set. This strategy is regarded as good scientific practice (37). Although we tested only for melanoma and normal tissue in

the external test set, we still believe that the approach is legit, as melanoma is by far the disease with the worst prognosis and because the external dataset exhibited severe artifacts and was therefore suitable to test for plausibility of our model.

Moreover, we show the application of our model that was trained on small image patches, to whole slides, which allows rapid identification of anatomical tissue structures and neoplastic lesions. This may result in a faster and more focused review of tissue sections in the routine diagnostic setting, as regions of interest are highlighted. Moreover, areas with high tumor cell content are automatically highlighted for potential dissection and subsequent molecular analyses.

The performance of our model on the image level is within the range that has been reported by others (9–11). Our model had weaknesses in the distinction of BCC and SqCC, naevi and melanoma, epidermis and SqCC and elastosis, vessels and nerves with dermis. All these misclassifications can be explained: First, BCC may exhibit squamous differentiation and SqCC may look basaloid. Second, the distinction of naevi and melanoma may be challenging and the criteria for correct classification include the assessment on low magnification power. As we provided only high magnification power images for training and cytology not always resolve the differential, some misclassified images were expected. Third, SqCC is derived from the epidermal compartment and both classes are therefore composed of the same cells. Especially in highly differentiated SqCC the morphological difference to epidermis may be minimal to absent on high-power. Fourth, elastosis, vessels and nerves are all located within the collagenous dermal skin compartment. Thus, the classification of an image containing e.g. vessels as dermis, is not necessarily a misclassification.

Although, our algorithm showed a decreased performance of 78% for melanoma and 84% in all whole slides on the external test set considering a majority vote, we still believe that the performance is reasonably good and support the use of our classifier for research, given the rather poor overall quality of this external cohort.

The limitations of our study include the number of patients and the number of different entities included to train the model. The full morphological spectrum of BCC, SqCC, naevi and melanoma cannot fully be displayed with the number of patients included in this study. Likewise, the number of cutaneous neoplasms is by far larger as the four most common tumor types included in this study and entities not trained, cannot be identified by the classifier. Based on the above-mentioned limitations the application of such a deep learning model can only be a diagnostic supplement and should always be conducted under supervision of an expert pathologist or dermatopathologist, to avoid potentially harmful consequences for patients.

In summary, we show that the automated identification and classification of common skin tumors is possible by deep learning on scanned histological tissue sections and may contribute to an efficient workflow in routine diagnostics.

Data availability statement

The datasets for this study can be found here: https://doi.org/10.11588/data/7QCR8S and here: https://wiki.cancerimagingarchive.net/pages/viewpage.action?pageId=33948224. The code to conduct the analysis can be found here: https://doi.org/10.11588/data/7QCR8S.

Ethics statement

The study was approved by the Ethics committee of Heidelberg University, #315/20. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conception and design of the study, MK and KK. Data acquisition, FL. Data curation, MK, CZ, FL, JK, and RRM. Data analysis, KK, FL, CZ, GS, and MK. Visualization, KK, FL, GS, and MK. Financial support, KK, GS, JK, US, MK, and TM. Supervision, KK, JK, US, and MK. Manuscript draft, FL and MK. Review for important intellectual content, KK, FL, JK, CZ, CJ, RM, US, TM, GS, and MK. All authors contributed to the article and approved the submitted version

Acknowledgments

The authors gratefully acknowledge the data storage service SDS@hd supported by the Ministry of Science, Research and the Arts Baden-Württemberg (MWK) and the German Research Foundation (DFG) through grant INST 35/1314-1 FUGG and INST 35/1503-1 FUGG. Data used in this publication (external test set) were generated by the National Cancer Institute Clinical Proteomic Tumor Analysis Consortium (CPTAC).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1022967/full#supplementary-material

References

1. Guy GPJr., Machlin SR, Ekwueme DU, Yabroff KR. Prevalence and costs of skin cancer treatment in the U.S. 2002-2006 and 2007-2011. *Am J Prev Med* (2015) 48:183–7. doi: 10.1016/j.amepre.2014.08.036

2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistic. *CA Cancer J Clin* (2022) 72:7–33. doi: 10.3322/caac.21708

3. Yeh I. Update on classification of melanocytic tumors and the role of immunohistochemistry and molecular techniques. *Semin Diagn Pathol* (2022) 39:248–56. doi: 10.1053/j.semdp.2022.02.001

4. Stern RS. Prevalence of a history of skin cancer in 2007: Results of an incidence-based model. *Arch Dermatol* (2010) 146:279–82. doi: 10.1001/archdermatol.2010.4

5. Tawbi HA, Schadendorf D, Lipson EJ, Ascierto PA, Matamala L, Castillo Gutierrez E, et al. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. *N Engl J Med* (2022) 386:24–34. doi: 10.1056/NEJMoa2109970

6. Markl B, Fuzesi L, Huss R, Bauer S, Schaller T. Number of pathologists in Germany: comparison with European countries, USA, and Canada. *Virchows Arch* (2021) 478:335–41. doi: 10.1007/s00428-020-02894-6

7. Kriegsmann M, Haag C, Weis CA, Steinbuss G, Warth A, Zgorzelski C, et al. Deep learning for the classification of small-cell and non-Small-Cell lung cancer. *Cancers (Basel)* (2020) 12. doi: 10.3390/cancers12061604

8. Kriegsmann M, Kriegsmann K, Steinbuss G, Zgorzelski C, Kraft A, Gaida MM. Deep learning in pancreatic tissue: Identification of anatomical structures, pancreatic intraepithelial neoplasia, and ductal adenocarcinoma. *Int J Mol Sci* (2021) 22. doi: 10.3390/ijms22105385

9. Li T, Xie P, Liu J, Chen M, Zhao S, Kang W, et al. Automated diagnosis and localization of melanoma from skin histopathology slides using deep learning: A multicenter study. *J Healthc Eng* (2021) 2021:5972962. doi: 10.1155/2021/5972962

10. De Logu F, Ugolini F, Maio V, Simi S, Cossu A, Massi D, et al. Recognition of cutaneous melanoma on digitized histopathological slides *via* artificial intelligence algorithm. *Front Oncol* (2020) 10 1559. doi: 10.3389/fonc.2020.01559

11. Ba W, Wang R, Yin G, Song Z, Zou J, Zhong C, et al. Diagnostic assessment of deep learning for melanocytic lesions using whole-slide pathological images. *Transl Oncol* (2021) 14:101161. doi: 10.1016/j.tranon.2021.101161

12. Ianni JD, Soans RE, Sankarapandian S, Chamarthi RV, Ayyagari D, Olsen TG, et al. Tailored for real-world: A whole slide image classification system validated on uncurated multi-site data emulating the prospective pathology workload. *Sci Rep* (2020) 10 3217. doi: 10.1038/s41598-020-59985-2

13. Elder DEM, Scolyer RA, Willemze R. *WHO classification of skin tumours*. Lyon, France: IARC (2018).

14. Steinbuss G, Kriegsmann K, Kriegsmann M. Identification of gastritis subtypes by convolutional neuronal networks on histological images of antrum and corpus biopsies. *Int J Mol Sci* (2020) 21. doi: 10.3390/ijms21186652

15. Clark K, Vendt B, Smith K, Freymann J, Kirby J, Koppel P, et al. The cancer imaging archive (TCIA): Maintaining and operating a public information repository. *J Digit Imaging* (2013) 26:1045–57. doi: 10.1007/s10278-013-9622-7

16. Bankhead P, Loughrey MB, Fernandez JA, Dombrowski Y, Mcart DG, Dunne PD, et al. QuPath: Open source software for digital pathology image analysis. *Sci Rep* (2017) 7:16878. doi: 10.1038/s41598-017-17204-5

17. Tan M, Le QV. *EfficientNet: Rethinking model scaling for convolutional neural networks* (2019). Available at: https://ui.adsabs.harvard.edu/abs/2019arXiv190511946T (Accessed May 01, 2019).

18. Kingma DP, Ba J. *Adam: A method for stochastic optimization* (2014). Available at: https://ui.adsabs.harvard.edu/abs/2014arXiv1412.6980K (Accessed December 01, 2014).

19. Cubuk ED, Zoph B, Shlens J, Le QV. *RandAugment: Practical automated data augmentation with a reduced search space* (2019). Available at: https://ui.adsabs.harvard.edu/abs/2019arXiv190913719C (Accessed September 01, 2019).

20. Grandini M, Bagli E, Visani G. *Metrics for multi-class classification: an overview* (2020). Available at: https://ui.adsabs.harvard.edu/abs/2020arXiv200805756G (Accessed August 01, 2020).

21. Bulten W, Pinckaers H, Van Boven H, Vink R, De Bel T, Van Ginneken B, et al. Automated deep-learning system for Gleason grading of prostate cancer using biopsies: a diagnostic study. *Lancet Oncol* (2020) 21:233–41. doi: 10.1016/S1470-2045(19)30739-9

22. Coudray N, Ocampo PS, Sakellaropoulos T, Narula N, Snuderl M, Fenyo D, et al. Classification and mutation prediction from non-small cell lung cancer histopathology images using deep learning. *Nat Med* (2018) 24:1559–67. doi: 10.1038/s41591-018-0177-5

23. Ehteshami Bejnordi B, Veta M, Johannes Van Diest P, Van Ginneken B, Karssemeijer N, Litjens G, et al. Diagnostic assessment of deep learning algorithms for detection of lymph node metastases in women with breast cancer. *JAMA* (2017) 318:2199–210. doi: 10.1001/jama.2017.14585

24. Echle A, Ghaffari Laleh N, Quirke P, Grabsch HI, Muti HS, Saldanha OL, et al. Artificial intelligence for detection of microsatellite instability in colorectal cancer-a multicentric analysis of a pre-screening tool for clinical application. *ESMO Open* (2022) 7:100400. doi: 10.1016/j.esmoop.2022.100400

25. Mahbod A, Schaefer G, Wang C, Dorffner G, Ecker R, Ellinger I. Transfer learning using a multi-scale and multi-network ensemble for skin lesion classification. *Comput Methods Programs BioMed* (2020) 193:105475. doi: 10.1016/j.cmpb.2020.105475

26. Thomas SM, Lefevre JG, Baxter G, Hamilton NA. Non-melanoma skin cancer segmentation for histopathology dataset. *Data Brief* (2021) 39:107587. doi: 10.1016/j.dib.2021.107587

27. Lu MY, Chen TY, Williamson DFK, Zhao M, Shady M, Lipkova J, et al. AI-Based pathology predicts origins for cancers of unknown primary. *Nature* (2021) 594:106–10. doi: 10.1038/s41586-021-03512-4

28. Yu Y, Wang J, Ng CW, Ma Y, Mo S, Fong ELS, et al. Deep learning enables automated scoring of liver fibrosis stages. *Sci Rep* (2018) 8:16016. doi: 10.1038/s41598-018-34300-2

29. Taylor-Weiner A, Pokkalla H, Han L, Jia C, Huss R, Chung C, et al. A machine learning approach enables quantitative measurement of liver histology and disease monitoring in NASH. *Hepatology* (2021) 74:133–47. doi: 10.1002/hep.31750

30. Liu Y, Jain A, Eng C, Way DH, Lee K, Bui P, et al. A deep learning system for differential diagnosis of skin diseases. *Nat Med* (2020) 26:900–8. doi: 10.1038/s41591-020-0842-3

31. Matek C, Krappe S, Munzenmayer C, Haferlach T, Marr C. Highly accurate differentiation of bone marrow cell morphologies using deep neural networks on a large image data set. *Blood* (2021) 138:1917–27. doi: 10.1182/blood.2020010568

32. Chen CL, Chen CC, Yu WH, Chen SH, Chang YC, Hsu TI, et al. An annotation-free whole-slide training approach to pathological classification of lung cancer types using deep learning. *Nat Commun* (2021) 12:1193. doi: 10.1038/s41467-021-21467-y

33. Lemke SB, Weidemann T, Cost AL, Grashoff C, Schnorrer F. A small proportion of talin molecules transmit forces at developing muscle attachments *in vivo*. *PLoS Biol* (2019) 17:e3000057. doi: 10.1371/journal.pbio.3000057

34. Chen H, Li C, Wang G, Li X, Mamunur Rahaman M, Sun H, et al. GasHis-transformer: A multi-scale visual transformer approach for gastric histopathological image detection. *Pattern Recognition* (2022) 130:108827. doi: 10.1016/j.patcog.2022.108827

35. Liu D, Wang W, Wu X, Yang J. *"EfficientNetv2 model for breast cancer histopathological image classification"*. (2022).

36. Achi HE, Belousova T, Chen L, Wahed A, Wang I, Hu Z, et al. Automated diagnosis of lymphoma with digital pathology images using deep learning. *Ann Clin Lab Sci* (2019) 49:153–60.

37. Reinke A, Tizabi MD, Eisenmann M, Maier-Hein L. Common pitfalls and recommendations for grand challenges in medical artificial intelligence. *Eur Urol Focus* (2021) 7:710–2. doi: 10.1016/j.euf.2021.05.008

frontiers | Frontiers in Oncology

TYPE Brief Research Report
PUBLISHED 23 November 2022
DOI 10.3389/fonc.2022.1022496

OPEN ACCESS

EDITED BY
Giuseppe Palmieri,
University of Sassari, Italy

REVIEWED BY
Douglas Johnson,
Vanderbilt University Medical Center, United States
Gabriella Liszkay,
National Institute of Oncology (NIO), Hungary

*CORRESPONDENCE
Saba S. Shaikh
saba.shaikh@fccc.edu

SPECIALTY SECTION
This article was submitted to Skin Cancer, a section of the journal Frontiers in Oncology

RECEIVED 18 August 2022
ACCEPTED 19 October 2022
PUBLISHED 23 November 2022

CITATION
Shaikh SS, Zang Y, Hanmer J, Wang H, Lin Y, Davar D, Zarour HM, Kirkwood JM and Najjar YG (2022) Phase I trial of pembrolizumab plus vemurafenib and cobimetinib in patients with metastatic melanoma. *Front. Oncol.* 12:1022496. doi: 10.3389/fonc.2022.1022496

Phase I trial of pembrolizumab plus vemurafenib and cobimetinib in patients with metastatic melanoma

Saba S. Shaikh[1,2]*, Yan Zang[2], Janel Hanmer[3], Hong Wang[4], Yan Lin[4], Diwakar Davar[2], Hassane M. Zarour[2], John M. Kirkwood[2] and Yana G. Najjar[2]

[1]Department of Hematology/Oncology, Fox Chase Cancer Center, Philadelphia, PA, United States, [2]Department of Medicine, Division of Hematology/Oncology, University of Pittsburgh Medical Center Hillman Cancer Centet, Pittsburgh, PA, United States, [3]Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA, United States, [4]University of Pittsburgh, School of Public Health, Pittsburgh, PA, United States

Background: Preclinical and translational evidence suggest BRAF/MEK inhibitors modulate the tumor microenvironment (TME), providing rationale for combination with immunotherapy.

Methods: This investigator-initiated, phase I trial evaluated pembrolizumab, vemurafenib, and cobimetinib in patients with untreated, BRAFV600E/K mutant advanced melanoma. The first 4 patients received vemurafenib with pembrolizumab, and the next 5 patients received vemurafenib and cobimetinib with pembrolizumab. Primary endpoints: safety and maximum tolerated dose of the triplet.

Secondary endpoints: objective response rate (ORR), progression-free survival (PFS), overall survival (OS), and quality of life (QoL). The trial was closed after enrollment of 9 (planned 30) patients due to dose-limiting toxicity (DLT). Study NCT02818023 was approved by the IRB, and all patients provided informed consent.

Results: Patients received a median of 6 cycles of therapy. 8 of 9 experienced drug-related grade 3/4 AEs. DLTs included dermatitis (n=8), hepatitis (n=1), QTc prolongation (n=1), and arthralgias (n=1 each). QoL assessments identified a clinically significant decrease in self assessed QoL at 1 year compared to baseline (0.38 v 0.43). Median PFS was 20.7 months and median OS was 23.8 months for vemurafenib with pembrolizumab. Median PFS and OS were not reached for patients receiving triple therapy. ORR in the overall cohort was 78% (7/9). 2 patients experienced a complete response, 5 had a partial response, 1 had stable disease, and 1 had progressive disease. 4 patients had ongoing responses at data analysis. Peripheral blood flow cytometry identified significantly decreased PD1 expression on CD4+ T-cells at 3 and 9 weeks compared to baseline, not corresponding to clinical response.

10.3389/fonc.2022.1022496

Conclusions: Triple therapy with vemurafenib, cobimetinib and pembrolizumab is associated with high response rates but significant adverse events, leading to early study closure.

KEYWORDS

immunotherapy, targeted therapy, BRAF, melanoma, metastatic, clinical trial, immune checkpoint inhibitor, triplet therapy

Introduction

The current landscape for management of advanced melanoma includes PD1 inhibitors with or without LAG-3 or CTLA4 inhibitors, and for patients with tumors that harbor BRAFV600E/K mutations, targeted therapy as well. While immunotherapy and targeted therapy each have been shown to improve overall survival in patients with metastatic melanoma (1–3), the recently reported DREAMseq study demonstrated up front combination immunotherapy followed by targeted therapy at the time of progression is associated with a 20% overall survival (OS) benefit compared to up front targeted therapy followed by immunotherapy at progression (4). Preclinical and translational data suggested that BRAF inhibitors (BRAFi) may modulate the tumor microenvironment (TME). After administration of a BRAFi in a BRAF-mutant melanoma model, CD40 ligand and interferon-gamma expression was increased on intratumoral CD4+ tumor-infiltrating lymphocytes (TIL), and regulatory T-cells and myeloid cells were decreased (5), suggesting anti-tumor modulation of the TME. Early data suggested that MEKi may suppress T cell function and RAF/MEKi may inhibit dendritic cell maturation and T cell activation (6, 7). However, further evidence of immune activation was noted when comparing paired patient biopsies at baseline and 10-14 days after treatment with either a BRAFi alone or in combination with a MEK inhibitor (MEKi), which was associated with increased expression of melanoma antigens along with CD8+ TIL (8, 9). Additionally, evidence of immune downregulation has been identified when patients progress on BRAF/MEK inhibition, with a decrease in TIL and antigen expression (10). BRAF inhibition was also associated with an increase in T-cell exhaustion markers TIM-3 and PD1 in tumors of patients with metastatic melanoma (8). Such modification of the TME provided clear rationale for the combination of targeted therapy and immune checkpoint inhibitors.

To date, there have been several reported trials of combinations of checkpoint blockade immunotherapy with targeted therapy. A phase 1 study combining ipilimumab and vemurafenib in patients with metastatic melanoma was closed to accrual due to a high frequency of grade 3 hepatotoxicity (11). Another phase I study evaluated the combination of dabrafenib and ipilimumab, with or without trametinib. The triplet arm of the study closed after 2 of 7 patients experienced colitis complicated by intestinal perforation (12). These early studies highlighted the notable toxicity associated with concurrent administration of immunotherapy and targeted therapy, despite distinct mechanisms of action and individual toxicity profiles that did not otherwise significantly overlap.

In the randomized, phase 2 KEYNOTE-022 trial, patients received either dabrafenib, trametinib and pembrolizumab or dabrafenib, trametinib and placebo, with the primary endpoint of progression-free survival (PFS) (13). Median PFS was higher in the triplet arm (16.0 vs. 10.3 months, HR 0.66 [95% CI 0.4 -1.07], p=0.043), although the trial did not reach the planned benefit for a statistically significant improvement. The investigators speculate this may have been due to differences in the distribution of patients with visceral metastases, of which there were a greater number of patients in the triplet arm. Median duration of response was longer in the triplet arm [18.7 (95% CI 10.1-22.1) vs. 12.5 months (95% CI 6.0-14.1), descriptive HR 0.41]. Grade 3-5 adverse events (AEs) occurred more frequently in the triplet arm (70.0% vs. 45%). The most frequently reported grade 3/4 toxicities were fever (11.7 vs. 5.0%, respectively), increased aspartate aminotransferase (AST; 8.3 vs. 5.0%, respectively), hypertension (8.3 vs. 3.3%, respectively), increased alanine aminotransferase (ALT; 6.7 vs. 5.0%, respectively), increased gamma-glutamyl transferase (GGT; 6.7 vs. 6.7%, respectively), pneumonitis (6.7 vs. 1.7%, respectively), and neutropenia (1.7 vs. 6.7%, respectively). One grade V pneumonitis event was reported in the triplet group.

The randomized, phase 3 COMBI-I trial evaluated the efficacy of spartalizumab, dabrafenib and trametinib compared to placebo, dabrafenib and trametinib as first line treatment of patients with unresectable BRAF mutant melanoma. There was a trend toward improvement in PFS with the triplet combination [16.2 vs. 12 months, HR 0.82 (95% CI, 0.66 to 1.03), one-sided p=0.042], however the study did not meet its primary endpoint. 55% of patients in the triplet arm experienced a treatment-related grade 3 or greater adverse event compared to 33% in the placebo-containing arm. The most frequently reported grade 3/4 events were increased lipase (10 vs. 4%, respectively), increase in

blood creatine phosphokinase (7 vs. 5%, respectively), neutropenia (7 vs. 3%, respectively), and fevers (5 vs. 3%, respectively). The most common events that lead to dose modifications included fever, chills, and diarrhea (14).

IMspire150 is the first phase 3 study evaluating a triplet combination that led to regulatory approval for the treatment of BRAF V600E/K mutant metastatic melanoma. Patients with unresectable stage IIIC/IV BRAF V600E/K mutant melanoma were randomized to treatment with atezolizumab, vemurafenib and cobimetinib or placebo, vemurafenib and cobimetinib (15). This regimen was administered in two phases, the first including only vemurafenib/cobimetinib, and the second adding atezolizumab, with reduction in the dosage of vemurafenib/cobimetinib. PFS was prolonged in the atezolizumab-containing arm (15.1 vs. 10.6 months, HR 0.78 [95% CI 0.63-0.97], p=0.025). At the interim OS analysis, 36% of patients had died in the atezolizumab arm compared to 43% in the control arm [HR 0.85 (95% CI 0.64 -1.11), p=0.23]. ORR was similar between the two groups (66.3% vs. 65%) with 15.7% and 17.1% of patients having a CR, respectively. 79% of patients in the triplet arm and 73% of patients in the placebo arm experienced a grade 3/4 AE. The most common grade 3/4 AEs included increased blood creatinine phosphokinase (20 vs. 15%, respectively), increased lipase (20 vs. 21%, respectively), increased ALT (13 vs. 9%, respectively), maculopapular rash (13 vs. 10%, respectively), increased amylase (10 vs. 7%, respectively), and increased AST (8 vs. 4%, respectively). 13% of patients in the triplet arm (vs. 16% in the control arm) stopped all treatment because of AEs.

Methods

Here, we report the results of a phase 1 study evaluating concurrent pembrolizumab plus vemurafenib and cobimetinib for treatment of advanced BRAF V600E/K mutant melanoma in the first-line setting (NCT02818023). Additional eligibility criteria include, age ≥ 18 years, ECOG 0, 1, or 2, cutaneous or mucosal melanoma, presence of measureable disease, treated and stable brain metastases are permitted, QTc < 480 msThe first four patients received pembrolizumab and vemurafenib (cohort 1), due to early data suggesting that MEKi may be lymphotoxic (16). The protocol was subsequently amended based on emerging data suggesting that MEKi may exert a positive modulatory effect on the TME (17), and the next five patients received pembrolizumab with vemurafenib and cobimetinib (cohort 2). Pembrolizumab was administered at a standard dose of 200 mg every 3 weeks. Patients were enrolled at an initial dose of vemurafenib 720 mg twice daily/cobimetinib 40 mg daily for 21 days in a 28-day cycle. Treatment with pembrolizumab and vemurafenib/cobimetinib began on the same day. The study utilized a modified toxicity probability (mTPI) design. The primary objective was to determine safety and identify the maximum tolerated dose (MTD) of vemurafenib and cobimetinib when administered concurrently with pembrolizumab. MTD was defined as the highest dose with a DLT rate <30%. Patients underwent CT scans at baseline and every 12 weeks to assess treatment response. Secondary endpoints included ORR, PFS, and OS. We planned to accrue 30 patients; however, the trial was closed after enrollment of 9 patients due to an unacceptably high rate of dose-limiting toxicity (DLT). For the mTPI design, the maximum sample size of 30 was determined because it would provide a high probability (>80%) of choosing the correct dose in most likely scenarios. This study was approved by the IRB and all patients provided informed consent.

Results

Patient characteristics can be found in Table 1. Patients received a median of 6 cycles of triplet therapy (range: 1-33). In the overall group, 2 patients experienced a complete response, 5 had a partial response, 1 patient had stable disease, and 1 patient had progressive disease as best response. The overall response rate was 78%. One patient in cohort 1 and 3 patients in cohort 2 had ongoing responses at the time of data analysis. Tumor measurements are plotted in Figure 1. PFS and OS were estimated and plotted with the Kaplan-Meier method and compared between cohort 1 and cohort 2 using the two-sided log-rank test. Median PFS in the overall group was 30.8 months with 95% CI (2.1, NA). Median PFS was 20.7 months in cohort 1 with 95% CI (6.9, NA), and not reached in cohort 2. Median OS in the overall group was 35.3 months with 95% CI (8.2, NA). Median OS was 23.8 months with 95% CI (8.2, NA) in cohort 1 and not reached in cohort 2. Three patients received subsequent systemic therapy after progression, which included: pembrolizumab, encorafenib/binimetinib, and ipilimumab/nivolumab. One patient enrolled in hospice and did not receive a subsequent line of therapy.

Eight of nine patients treated with pembrolizumab plus vemurafenib, with or without cobimetinib, experienced DLT. DLTs were defined as any AE that required a dose reduction or discontinuation in the first 3 weeks of treatment. In the vemurafenib and pembrolizumab group, DLTs included hepatitis (n=1), dermatitis (n=3), and arthralgias (n=1). In the vemurafenib with cobimetinib and pembrolizumab group, DLTs included dermatitis (n=5), QTc prolongation (n=1), and arthralgias (n=1). A complete summary of AEs is reported in Table 2.

Quality of life assessments were collected at baseline, 9 weeks, 6 months, and 1 year. These assessments evaluated patient-reported anxiety, depression, cognitive function, fatigue, pain, physical function, sleep, and social roles using the PROMIS-29 Profile v2.0 and the Cognitive Function short form 4a (18, 19). These PROMIS measures have a mean of 50

TABLE 1 Patient Characteristics (P/V: pembrolizumab/vemurafenib, P/V/C: pembrolizumab/vemurafenib/cobimetinib).

Patient Characteristic	Overall Group (n=9)	Cohort 1(P/V) (n=4)	Cohort 2 (P/V/C) (n=5)
Median age(years)	58	65	57
Male sex	6	4	2
Female sex	3	0	3
White race	9	4	5
ECOG			
0	7	2	5
1	2	2	0
Disease stage			
Stage IIIC	1	1	0
Stage IV	8	3	5
Stage, distant metastases			
M1a	2	0	2
M1b	1	1	0
M1c	5	2	3
M1d	0	0	0
Sum of target lesions			
<60 mm	3	1	2
>60 mm	6	3	3
BRAF V600E/K	9	4	5
Median LDH	214	193	214
Prior adjuvant therapy	2	1	1

with a standard deviation of 10 in the US general population. These assessments identified worsening depression (53.6 vs. 50.6), decreased cognitive function (50.2 vs. 54.4), and increasing fatigue (51.2 vs. 50.3) at 1 year compared to baseline. Anxiety, pain, physical function, sleep, and social roles were not significantly different. A PROPr score of health utility was also calculated in which a value of 1 corresponds to full health and a score of 0 corresponds to dead (20). The assessments identified a clinically significant decrease in average health utility at 1 year compared to baseline (0.38 vs. 0.43). Of note, one patient had evidence of PD at the first scan, and no further assessments were collected for that patient.

Blood samples were collected at baseline, 3 weeks, and 9 weeks, and tumor biopsy samples were collected at baseline and

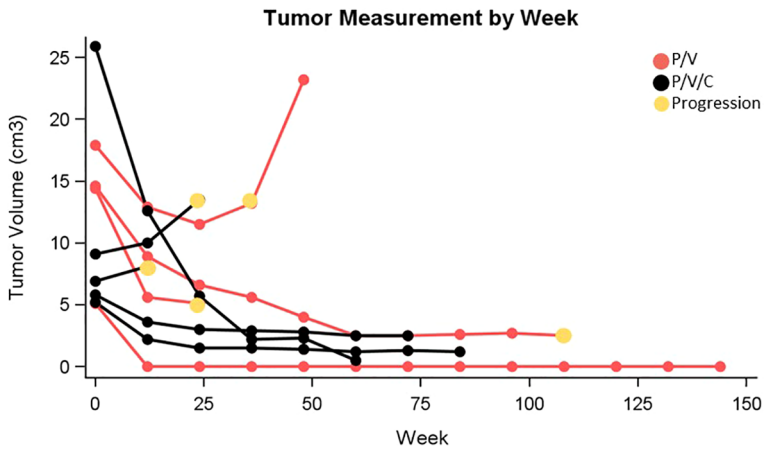


FIGURE 1
Tumor measurement by week (P/V, pembrolizumab/vemurafenib; P/V/C, pembrolizumab/vemurafenib/cobimetinib).

TABLE 2 Adverse events.

Event	Overall (n = 9)		Cohort 1 (n=4)		Cohort 2 (n=5)	
	ALL Grades	Grade ≥ 3	ALL Grades	Grade ≥ 3	ALL Grades	Grade ≥ 3
Any event	8	8	4	3	5	5
Dermatitis	8	7	3	2	5	5
Electrolyte abnormality	7	0	3	2	5	5
Arthralgias	7	2	2	1	5	1
Fatigue	6	0	4	0	2	0
Hepatitis	5	1	2	1	3	0
Fever	4	0	2	0	2	0
Diarrhea	4	2	2	1	2	1
Hypothyroidism	4	0	2	0	2	0
Anemia	4	0	4	0	0	0
Nausea	4	0	4	0	0	0
Burn	3	0	1	0	2	0
Neurotropenia	2	0	1	0	1	0
Thrombocytopenia	2	0	2	0	0	0
Mucositis	2	0	1	0	1	0
Atrial fibrillation	1	0	1	0	0	0
Hypertension	1	0	1	0	0	0
Soft tissue infection	1	0	1	0	0	0
Thrush	1	0	0	0	1	0
Pneumonitis	1	0	1	0	0	0
Adrenal Insufficiency	1	1	0	0	1	1
Edema	1	0	0	0	1	0

at week 3, when feasible. Peripheral blood flow cytometry was performed to assess CD4, CD8, FOXP3, Ki67, PD-1, and LAG3. PD1 expression on the CD4+ T-cells was significantly decreased at 3 weeks compared to baseline (0.9 vs 2.8, p=0.0339 with paired t-test) and remained decreased at 9 weeks (1.1 vs 2.8, p=0.0282) without a significant increase from week 3 (p=0.5574), which may suggest decreased T-cell exhaustion. This did not correspond to clinical response data. The remainder of the flow data did not identify statistically significant differences across the assessed time points. PD-L1 testing was also performed on 6 paired tumor samples, and no significant association was identified between PD-L1 expression at baseline and clinical outcomes.

Discussion

Despite the preclinical and translational evidence for tumor immune modification with BRAF/MEK inhibitors and a PFS of 15.1-16.2 months in the 3 largest reported triplet-therapy trials, the toxicity incurred with triplet therapies has been challenging from a practical standpoint (13–15). Triplet therapies were associated with significant increases in grade 3/4 adverse events compared to doublet therapies in both KEYNOTE-022 (70% vs. 45%) and COMBI-I (55% vs. 33%). There was also a slight increase in grade 3/4 AEs in the triplet arm in IMspire150 (79% vs. 73%). Of note, the control arm in IMspire150 had a higher AE rate compared to the cohort of patients receiving vemurafenib and cobimetinib in coBRIM, in which 60% of patients experienced a grade 3/4 AE (2). Our study adds additional toxicity data for triplet therapy, with 8 of 9 patients experiencing a DLT. Of note, the maximum dose of vemurafenib and cobimetinib administered in this trial was 720 mg twice daily/40 mg daily, which is comparable to the reduced dose of BRAFi in the triplet arm in IMspire150 from cycle 2 onwards. In cycle 1 of IMspire150, pts in the triplet arm received a 3 week-lead in with vemurafenib 960 mg twice-daily and cobimetinib at 60 mg daily, which may have contributed to the difference in adverse event profile compared to our study. The difference may also be related to the differences in immune checkpoint inhibitor. This study utilized pembrolizumab, a PD-1 inhibitor, compared to atezolizumab, a PD-L1 inhibitor. A meta-analysis of 125 clinical trials identified higher rate of grade 3 AEs with PD-1 inhibitors compared to PD-L1 inhibitors, raising the question of interchangeability of these agents with respect to toxicity (21). In addition to significant toxicity, patients reported a significant decrease in overall health utility at 1 year compared to baseline, which may be driven by depression, cognitive function, and fatigue. Here, we report median PFS of 30.8

months in the overall group, which is increased compared to the PFS reported in IMspire 150, which was 15.1 months in the triplet group compared to 10.6 months in control. Of note, the control arm of vemurafenib and cobimetinib had a lower PFS compared to the coBRIM trial, which reported a median PFS of 12.3 months in the doublet arm (vs. 7.2 months, HR 0.58 [95% CI 0.46-0.72], p<0,0001). The PFS benefit seen in IMspire 150 may be related to the increased duration of response in the triplet arm (21.0 vs. 16.0 months) given that the ORR was similar (66% vs. 65%). Notably, IMspire150 compared the triplet regimen to BRAF/MEK inhibition, and how the efficacy data compares to anti-PD1 therapy with or without anti-CTLA4 or anti-LAG3 therapy is not known. Furthermore, encorafenib and binimetinib have since been approved in the metastatic setting, with higher PFS and overall improved tolerability than reported with other targeted therapies (22). This raises the question of whether combination therapy of anti-PD1 and newer BRAF/MEK inhibitors may be better tolerated, and studies of this question are ongoing (NCT04657991, NCT04511013). Our study of vemurafenib and cobimetinib with pembrolizumab had a high ORR but closed early due to high rates of grade 3/4 AEs. In addition, the survival benefit of up-front combination immunotherapy compared to targeted therapy further puts into question which patients would benefit from triplet combination therapy (23). Overall, given the significant toxicities incurred with triplet therapy and modest PFS improvements, physicians must think carefully about which patients are best served with this treatment strategy.

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 studies involving human participants were reviewed and approved by University of Pittsburgh Medical Center IRB. The patients/participants provided their written informed consent to participate in this study.

Author contributions

SS: Data acquisition, analysis and interpretation, manuscript writing. YZ: Data acquisition, analysis and interpretation. JH: Analysis and interpretation, critical manuscript revisions. HW, YL, DD, HZ, JK: Analysis and interpretation, critical manuscript revisions. YN: Conception and study design, analysis and interpretation, manuscript writing. All authors contributed to the article and approved the submitted version.

Funding

This project was funded by Merck. In addition, this project is funded, in part, under a Grant with the Pennsylvania Department of Health. The Department specifically disclaims responsibility for any analyses, interpretations or conclusions.

Conflict of interest

Author DD reports the following disclosures: Arcus, Checkmate Pharmaceuticals, CellSight Technologies, Immunocore, Merck, Tesaro/GSK research support; Checkmate Pharmaceuticals, Finch, Shionogi, Vedanta Biosciences consulting; Vedanta Biosciences scientific advisory board. CE Speakers' Bureau: Medical Learning Group MLG, Clinical Care Options CCO. Intellectual Property: US Patent 63/124,231, "Compositions and Methods for Treating Cancer", Dec 11, 2020; US Patent 63/208,719, "Compositions and Methods For Determining Responsiveness to Immune Checkpoint Inhibitors ICI, Increasing Effectiveness of ICI and Treating Cancer", June 9, 2021. Author HZ reports the following disclosures: Bristol-Myers Squibb, Checkmate Pharmaceuticals, and GlaxoSmithKline research support and Bristol-Myers Squibb, Checkmate Pharmaceuticals, GlaxoSmithKline, and Vedanta consulting. Author JK reports the following disclosures: Amgen, Bristol-Myers Squibb, Castle Biosciences, Checkmate Pharmaceuticals, Immunocore LLC, Iovance, and Novartis research support and Amgen, BristolMyers Squibb, Checkmate Pharmaceuticals, and Novartis consulting. Author YN reports the following disclosures: Merck, Pfizer, and Bristol-Myers Squibb research support. Array Biopharma, Merck, Novartis, InterVenn Bio consulting/scientific advisory board. Pfizer, Immunocore speaker's bureau. CE Speakers' Bureau: Medical Learning Group MLG.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob J-J, Rutkowski P, Lao CD, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* (2019) 381(16):1535–46. doi: 10.1056/NEJMoa1910836

2. Ascierto PA, McArthur GA, Dréno B, Atkinson V, Liszkay G, Di Giacomo AM, et al. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): Updated efficacy results from a randomised, double-blind, phase 3 trial. *Lancet Oncol* (2016) 17(9):1248–60. doi: 10.1016/s1470-2045(16)30122-x

3. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med* (2015) 372(26):2521–32. doi: 10.1056/NEJMoa1503093

4. Atkins MB, Lee SJ, Chmielowski B, Ribas A, Tarhini AA, Truong T-G, et al. DREAMseq (Doublet, randomized evaluation in advanced melanoma sequencing): A phase III trial–ECOG-ACRIN EA6134. *J Clin Oncol* (2021) 39(36_suppl):356154. doi: 10.1200/JCO.2021.39.36_suppl.356154

5. Ho PC, Meeth KM, Tsui YC, Srivastava B, Bosenberg MW, Kaech SM. Immune-based antitumor effects of BRAF inhibitors rely on signaling by CD40L and IFNγ. *Cancer Res* (2014) 74(12):3205–17. doi: 10.1158/0008-5472.can-13-3461

6. Callahan MK, Masters G, Pratilas CA, Ariyan C, Katz J, Kitano S, et al. Paradoxical activation of T cells *via* augmented ERK signaling mediated by a RAF inhibitor. *Cancer Immunol Res* (2014) 2(1):70–9. doi: 10.1158/2326-6066.cir-13-0160

7. Hoyer S, Eberlein V, Schuler G, Berking C, Heinzerling L, Schaft N, et al. BRAF and MEK inhibitors affect dendritic-cell maturation and T-cell stimulation. *Int J Mol Sci* (2021) 22(21):11951. doi: 10.3390/ijms222111951

8. Frederick DT, Piris A, Cogdill AP, Cooper ZA, Lezcano C, Ferrone CR, et al. BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2013) 19(5):1225–31. doi: 10.1158/1078-0432.ccr-12-1630

9. Wilmott JS, Long GV, Howle JR, Haydu LE, Sharma RN, Thompson JF, et al. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. *Clin Cancer Res an Off J Am Assoc Cancer Res* (2012) 18(5):1386–94. doi: 10.1158/1078-0432.ccr-11-2479

10. Cooper ZA, Reuben A, Spencer CN, Prieto PA, Austin-Breneman JL, Jiang H, et al. Distinct clinical patterns and immune infiltrates are observed at time of progression on targeted therapy versus immune checkpoint blockade for melanoma. *Oncoimmunology*. (2016) 5(3):e1136044. doi: 10.1080/2162402x.2015.1136044

11. Ribas A, Hodi FS, Callahan M, Konto C, Wolchok J. Hepatotoxicity with combination of vemurafenib and ipilimumab. *New Engl J Med* (2013) 368(14):1365–6. doi: 10.1056/NEJMc1302338

12. Minor DR, Puzanov I, Callahan MK, Hug BA, Hoos A. Severe gastrointestinal toxicity with administration of trametinib in combination with dabrafenib and ipilimumab. *Pigment Cell melanoma Res* (2015) 28(5):611–2. doi: 10.1111/pcmr.12383

13. Ferrucci PF, Di Giacomo AM, Del Vecchio M, Atkinson V, Schmidt H, Schachter J, et al. KEYNOTE-022 part 3: A randomized, double-blind, phase 2 study of pembrolizumab, dabrafenib, and trametinib in BRAF-mutant melanoma. *J Immunother Cancer* (2020) 8(2):e001806. doi: 10.1136/jitc-2020-001806

14. Dummer R, Long GV, Robert C, Tawbi HA, Flaherty KT, Ascierto PA, et al. Randomized phase III trial evaluating spartalizumab plus dabrafenib and trametinib for BRAF V600-mutant unresectable or metastatic melanoma. *J Clin Oncol Off J Am Soc Clin Oncol* (2022) 40(13):1428–38. doi: 10.1200/jco.21.01601

15. Gutzmer R, Stroyakovskiy D, Gogas H, Robert C, Lewis K, Protsenko S, et al. Atezolizumab, vemurafenib, and cobimetinib as first-line treatment for unresectable advanced BRAF(V600) mutation-positive melanoma (IMspire150): Primary analysis of the randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet (London England)* (2020) 395(10240):1835–44. doi: 10.1016/s0140-6736(20)30934-x

16. Vella LJ, Pasam A, Dimopoulos N, Andrews M, Puaux A-L, Louahed J, et al. MEK inhibition, alone or in combination with BRAF inhibition, impairs multiple functions of isolated normal human lymphocytes and dendritic cells. *J Immunother Cancer* (2013) 1(Suppl 1):P93–P. doi: 10.1186/2051-1426-1-S1-P93

17. Hu-Lieskovan S, Mok S, Homet Moreno B, Tsoi J, Robert L, Goedert L, et al. Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAF(V600E) melanoma. *Sci Trans Med* (2015) 7(279):279ra41. doi: 10.1126/scitranslmed.aaa4691

18. Hays RD, Spritzer KL, Schalet BD, Cella D. PROMIS((R))-29 v2.0 profile physical and mental health summary scores. *Qual Life Res* (2018) 27(7):1885–91. doi: 10.1007/s11136-018-1842-3

19. Valentine TR, Weiss DM, Jones JA, Andersen BL. Construct validity of PROMIS(R) cognitive function in cancer patients and noncancer controls. *Health Psychol* (2019) 38(5):351–8. doi: 10.1037/hea0000693

20. Dewitt B, Feeny D, Fischhoff B, Cella D, Hays RD, Hess R, et al. Estimation of a preference-based summary score for the patient-reported outcomes measurement information system: The PROMIS((R))-preference (PROPr) scoring system. *Med Decis Making* (2018) 38(6):683–98. doi: 10.1177/0272989X18776637

21. Wang Y, Zhou S, Yang F, Qi X, Wang X, Guan X, et al. Treatment-related adverse events of PD-1 and PD-L1 inhibitors in clinical trials: A systematic review and meta-analysis. *JAMA Oncol* (2019) 5(7):1008–19. doi: 10.1001/jamaoncol.2019.0393

22. Gogas H, Ascierto PA, Flaherty K, Arance A, Mandalà M, Liszkay G, et al. Update on overall survival in COLUMBUS: A randomized phase III trial of encorafenib (ENCO) plus binimetinib (BINI) versus vemurafenib (VEM) or ENCO in patients with BRAF V600-mutant melanoma. *J Clin Oncol* (2020) 38(15_suppl):10012. doi: 10.1200/JCO.2020.38.15_suppl.10012

23. Callahan MK, Chapman PB. PD-1 or PD-L1 blockade adds little to combination of BRAF and MEK inhibition in the treatment of BRAF V600–mutated melanoma. *J Clin Oncol* (2022) 40(13):1393–5. doi: 10.1200/jco.21.02801

frontiers | Frontiers in Oncology

TYPE Case Report
PUBLISHED 12 December 2022
DOI 10.3389/fonc.2022.1032844

OPEN ACCESS

EDITED BY
Selma Ugurel,
Essen University Hospital, Germany

REVIEWED BY
Tapas Ranjan Behera,
Cleveland Clinic, United States
Antonio Giovanni Solimando,
University of Bari Aldo Moro, Italy

*CORRESPONDENCE
Charlée Nardin
charleenardin@hotmail.fr

SPECIALTY SECTION
This article was submitted to Skin Cancer, a section of the journal Frontiers in Oncology

RECEIVED 31 August 2022
ACCEPTED 15 November 2022
PUBLISHED 12 December 2022

CITATION
Neuville C, Aubin F, Puzenat E, Popescu D, Crepin T and Nardin C (2022) Nivolumab-induced capillary leak syndrome associated with chylothorax in a melanoma patient: A case report and review of the literature.
Front. Oncol. 12:1032844.
doi: 10.3389/fonc.2022.1032844

Nivolumab-induced capillary leak syndrome associated with chylothorax in a melanoma patient: A case report and review of the literature

Carole Neuville[1], François Aubin[1,2], Eve Puzenat[1], Dragos Popescu[1], Thomas Crepin[3] and Charlée Nardin[1,2]*

[1]Department of Dermatology, University Hospital, Besançon, France, [2]Univ. Bourgogne Franche-Comté, INSERM, EFS BFC, UMR1098, RIGHT Interactions Greffon-Hôte Tumeur/Ingénierie Cellulaire et Génique, Besançon, France, [3]Department of Nephrology, University Hospital, Besançon, France

Introduction: Adverse events (AEs) of immune checkpoint inhibitors (ICIs) are frequent and mainly due to an overactivity of the immune system leading to excessive inflammatory responses (immune-related AE) that can affect any organ of the body. Beside the most frequent AEs, there are rare AEs whose diagnosis and treatment can be challenging. We report here a singular case of capillary leak syndrome (CLS) associated with chylothorax occurring in a patient who has been treated with adjuvant nivolumab (anti-PD1) for resected AJCC stage IIB primary melanoma.

Case presentation: A 43-year-old woman was diagnosed with a nodular stage IIB melanoma of her left thigh, according to the AJCC 8th edition (T3bN0M0). The woman was treated with adjuvant nivolumab. She stopped the treatment after 4 infusions due to thrombopenia. Three months later, she developed facial and leg edema and ascites due to capillary leak syndrome. The CLS was associated with chylothorax and elevated vascular endothelial growth factor. The patient was initially treated with several pleural puncturing and steroids. CLS and chylothorax progressively decreased with intravenous immunoglobulins and fat-free diet without recurrence of melanoma at one-year follow-up.

Conclusion: CLS is a rare and potentially life-threatening AE of ICIs such as anti-PD1. This AE may be associated with chylothorax probably related to lymphatic permeability induced by anti-PD1.

KEYWORDS

capillary leak syndrome, chylothorax, immune checkpoint inhibitor, anti-PD1, adverse event, VEGF

10.3389/fonc.2022.1032844

Introduction

Immune checkpoint inhibitors (ICIs) such as anti-programmed cell death 1 (anti-PD1) and anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) have revolutionized the prognosis of cancer. Adverse events (AEs) of ICIs are frequent and mainly due to an overactivity of the immune system leading to excessive inflammatory responses (immune-related AE) that can affect any organ of the body. Beside the most frequent AEs, such as thyroid, cutaneous, gastro-intestinal and hepatic AEs, there are rare AEs whose diagnosis and treatment can be challenging. We report here a singular case of capillary leak syndrome (CLS) associated with chylothorax occurring in a patient who had been treated with adjuvant nivolumab (anti-PD1) for resected AJCC stage IIB primary melanoma.

Case report

A 43-year-old woman was diagnosed with a nodular stage IIB NRAS-mutated melanoma of her left thigh, according to the AJCC 8th edition (T3bN0M0). She first underwent a wide resection of the primary lesion and started infusions of anti-PD1 antibodies (nivolumab 480 mg monthly), as part of a therapeutic trial. She developed thyroiditis after 2 infusions of nivolumab with successive phases of hyper- and then hypothyroidism which was treated with thyroid substitution. The occurrence of a grade III immune thrombocytopenia (platelets count: 27 000/mm^3 without autoantibodies) subsequently led to the interruption of nivolumab after 4 infusions and was resolved with systemic corticosteroids (1mg/kg) and eltrombopag olamine (75mg daily). Three months after treatment discontinuation (7 months after initiation of nivolumab), she developed edema of the legs and face, along with a weight gain (+ 8 kgs), asthenia, dyspnea, and cough. Laboratory tests showed a drop of serum-albumin levels from 40g/l to 23g/l, normal hematocrit count without hemodynamics disturbances (122/69mmHg, 100 bpm), without signs of enteropathy, neither heart, kidney or liver failure. All hormonal tests (TSH, cortisol) and serum protein electrophoresis were normal. There was no proteinuria and urinalysis results were normal. The CT-scan revealed mild bilateral pleural effusion and mild ascites due to anasarca (Figure 1). Echocardiography also found a slight pericardial effusion without cardiopathy. Colonoscopy was normal. A scintigraphy with marked albumin (99mTc) showed a capillary hyperpermeability (Landi's test). Thoracentesis allowed a 600cc fluid evacuation. Cytology was normal but triglycerides' levels were elevated at 25g/L (N<1,1 g/L) in favor of chylothorax with normal levels of triglycerides in blood.

The diagnosis of a secondary form of capillary leak syndrome (CLS) with chylothorax induced by nivolumab was made in the absence of infection, vaccine and other inducing

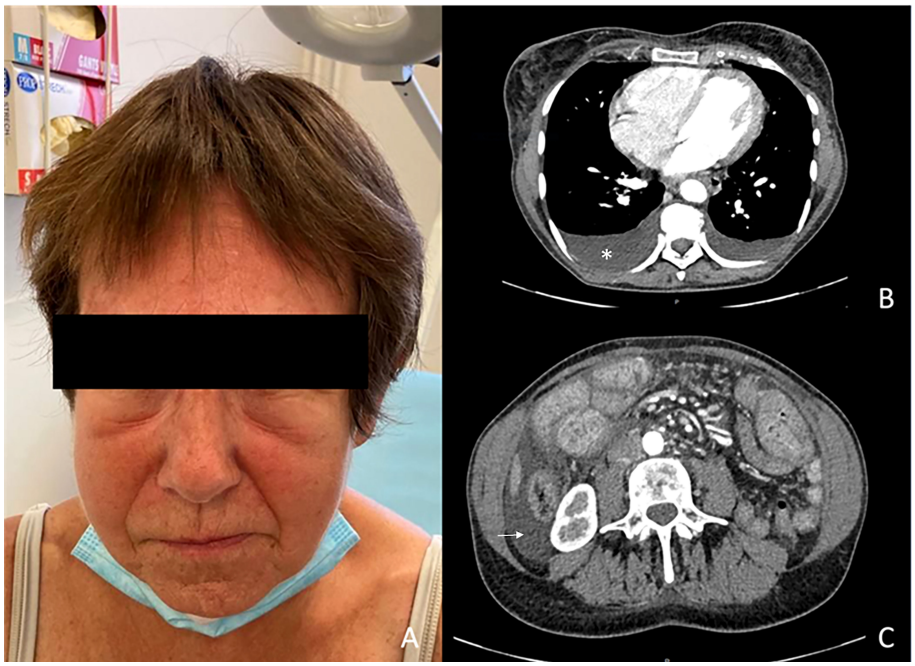


FIGURE 1
Clinical photography **(A)** and CT-scan images **(B, C)**. **(A)** Facial edema **(B)** Pleural effusion (white star). **(C)** Ascites (white arrow).

drugs. Cytokines levels were as follows: normal IL-6 levels (<3,6pg/ml) and elevated vascular endothelial growth factor (VEGF) levels (109 pg/ml, N<60 pg/ml).

High doses of corticosteroids (1mg/kg) and intravenous immunoglobulins (IVIG) (0.4 g/kg for 5 days) followed by IVIG (1g/kg/day for two consecutive days monthly) for 5 months, along with a fat-free diet and iterative pleural puncturing provided the resolution of the edema, the chylothorax and the ascites. At last follow-up (one year after nivolumab discontinuation), there was no evidence of melanoma progression according to CT-scan.

Discussion

CLS is a very rare and potentially life-threatening AE that has been described with different treatments (1, 2).

5 cases of CLS have recently been reported with ICI, particularly anti-PD1 antibodies (nivolumab and pembrolizumab) (3–6).

In the current case, the role of eltrombopag olamine cannot be excluded even if it has never been previously reported.

This case is singular because these rare toxicities (CLS and chylothorax) occurred many months after nivolumab discontinuation. Immune-related adverse events (irAE) can be delayed and occured after the completion of ICI (> 90 days) (7). Indeed, CLS were mostly reported early during anti-PD1 treatment (5)) or soon after their discontinuation (one month after discontinuation of pembrolizumab and nivolumab) (3, 4).

This is the first case of CLS and chylothorax induced by adjuvant nivolumab in a patient with early-stage cancer (resected primary stage IIB melanoma). Until now, CLS induced by ICI has only been reported in patients with advanced cancers. Furthermore, the association of chylothorax with CLS after anti-PD1 treatment has been scarcely reported in the literature. A patient with a stage IV melanoma treated with pembrolizumab and injections of talimogene laherparepvec developed the association of CLS and lymphatic dysfunction with chylous pleural and abdominal effusions (3). Another patient died of a chylothorax related to tumor progression 12 months after initiation of nivolumab for a metastatic pulmonary adenocarcinoma (8). Thus, the association of these two adverse events does not appear coincidental and appear to be irAEs due to the same mechanisms.

In our practice, we lack parameters to identify patients at risk of severe toxicities. In the future, we will evaluate the probability of response and the risk of toxicity to evaluate the benefice/risk balance of ICI treatment, particularly with patients treated at early stage (such as this patient with resected stage II melanoma). Indeed, adjuvant pembrolizumab treatment has demonstrated its efficacy by decreasing the risk of melanoma recurrence in patients with resected stage IIB or IIC melanoma (9). However, there are frequent irAEs including severe irAEs and chronic irAEs (such as endocrine AE reported at 25%). Thus, the benefice/risk balance of ICI treatment should be discussed with the patient.

It will be necessary to stratify the treatment according predictive factors of response such as the TMB, IFN-γ-signature (10) and predictive factors of toxicity such as the fecal microbiote (11) or the diversity of the TCR clones (12).

Regarding toxicity, there are studies evaluating clinical and biological factors associated with toxicities (NCT04871542) and evaluating strategy to avoid treatment toxicity such as fecal microbiota transplantation (NCT04163289). In a phase I study, the role of microbiome modification in preventing immune-related toxicities by adding fecal microbiota transplantation to ICI therapy was associated with a safety profile in unselected metastatic renal cell carcinoma and promising clinical efficacy data (13).

However, most of these biomarkers are not performed in clinical practice (TMB, IFN-γ-signature, TCR clones) and were not available for this patient. Furthermore, there is no tool validated in clinical practice and no recommendation to predict toxicities, prevent toxicities, adapt the treatment according to the risk of toxicities and the probability of response. Therefore, these parameters cannot be use for treatment decision. If predictive factors of response and toxicities are identified and validated in prospective studies, the treatment could be adapted to increase treatment efficacy and avoid toxicity. Thus, further studies are needed to develop individualized approaches to avoid treatments toxicities.

It is remarkable to notice that CLS mainly occurred in patients with a controlled disease suggesting that this AE is associated with a strong anti-tumor immune response as reported with other AEs (14). Although the pathophysiology of CLS is not clear, T-cell activation and the release of cytokines induced by ICIs may be involved. T-CD8 cells surrounding endothelial cells in CLS have been indeed described (15).

The association of CLS with chylothorax suggests a severe endothelial dysfunction, both vascular and lymphatic, induced by ICI, involving the crosstalk between immune cells and endothelial cells (16, 17). It is known that ICI stimulate cytokines secretion by immune cells and it was reported that circulating cytokines levels (including VEGF during treatment with ICI) were increased in patients with severe irAE (18) as found in our case. This supports that the mechanisms of nivolumab-induced CLS and chylothorax involve immunity.

PD-L1 expression was found on endothelial cells and involved in T-cell mediated myocardial injury (19). It is well known that angiogenesis and immunosuppression occurs simultaneously and that there are interactions between angiogenesis and the immune response (17, 20). VEGF promotes the recruitment and proliferation of immunosuppressive cells such as Treg cells, MDSCs, and M2-TAMs, creating a more immunosuppressive environment (21). Indeed, vascular normalization may enhance antitumor immunity and lymphocyte-mediated cancer immunotherapy with immune checkpoint inhibitors (21, 22).

Furthermore, increased levels of VEGF have been reported in CLS and support the hypothesis of endothelial activation and the use of anti-VEGF therapy as found in our patient (23).

Targeting angiogenesis may increase tumor control of cancer such as melanoma (24). Indeed, the combination of anti-angiogenic therapy to ICI can potentiate anti-tumor immune response by regulating the interactions between angiogenesis and the immune response (25, 26). The combination of bevacizumab and ipilimumab was safely administered in patients with metastatic melanoma (27). Finally, strategies combining anti-angiogenic and anti-PD1 agents have been studied and appears to tip the balance of the tumor microenvironment and improve treatment response in advanced melanoma (28, 29). These results support the efficacy of anti-angiogenic agents with ICI and further investigation.

In conclusion, physicians should be aware of the possibility of a CLS associated with lymphatic permeability induced by anti-PD1. Despite the favorable melanoma prognosis, this IRAE may be life-threatening.

Data availability statement

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

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

Substantial contributions to conception and design: ChN, CaN, EP, DP, TC, FA. Acquisition of data: ChN, CaN, EP, DP. Analysis and interpretation of data: ChN, TC, FA. Drafting the article: ChN, CaN, EP and DP. Revising the article: ChN, TC, FA. Final approval of the version to be published: CaN, ChN, EP, DP, TC, FA. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors thank the patient included in this manuscript who has given her oral and written informed consent to the publication.

Conflict of interest

Authors ChN and FA is a consultant for BMS.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Siddall E, Khatri M, Radhakrishnan J. Capillary leak syndrome: etiologies, pathophysiology, and management. *Kidney Int* (2017) 92(1):37–46. doi: 10.1016/j.kint.2016.11.029

2. Bichon A, Bourenne J, Gainnier M, Carvelli J. Capillary leak syndrome: State of the art in 2021. *Rev Med Interne* (2021) 42(11):789–96. doi: 10.1016/j.revmed.2021.05.012

3. Qin H, Vlaminck B, Owoyemi I, Herrmann SM, Leung N, Markovic SN. Successful treatment of pembrolizumab-induced severe capillary leak syndrome and lymphatic capillary dysfunction. *Mayo Clin Proc Innov Qual Outcomes* (2021) 5(3):670–4. doi: 10.1016/j.mayocpiqo.2021.01.004

4. Polishchuk I, Yakobson A, Zemel M, A Sharb A, Shalata W, Rosenberg E, et al. Nivolumab-induced systemic capillary leak syndrome as an ultra rare life-threatening phenomenon of late toxicity and intravenous immunoglobulin efficacy. *Immunotherapy* (2021) 13(10):807–11. doi: 10.2217/imt-2020-0335

5. Percik R, Nethanel A, Liel Y. Capillary-leak syndrome: an unrecognized early immune adverse effect of checkpoint-inhibitors treatment. *Immunotherapy* (2021) 13(8):653–9. doi: 10.2217/imt-2020-0332

6. Lescure C, Lescoat A, Salé A, Bazin Y, Duvergé L, Desrues B, et al. Systemic capillary leak syndrome (Clarkson's disease) as a complication of anti-programmed death 1 immunotherapy. *J Thorac Oncol* (2019) 14(6):e131–2. doi: 10.1016/j.jtho.2019.02.003

7. Couey MA, Bell RB, Patel AA, Romba MC, Crittenden MR, Curti BD, et al. Delayed immune-related events (DIRE) after discontinuation of immunotherapy: Diagnostic hazard of autoimmunity at a distance. *J Immunother Cancer* (2019) 7 (1):165. doi: 10.1186/s40425-019-0645-6

8. Pluchart H, Ferrer L, Giovannini D, Tetaz R, Pinsolle J, Stephanov O, et al. Utilisation concomitante du nivolumab et d'immunosuppresseurs chez un patient greffé rénal. *Rev Mal Respir* (2019) 36(9):1064–8. doi: 10.1016/j.rmr.2019.08.004

9. Luke JJ, Rutkowski P, Queirolo P, Del Vecchio M, Mackiewicz J, Chiarion-Sileni V, et al. Pembrolizumab versus placebo as adjuvant therapy in completely resected stage IIB or IIC melanoma (KEYNOTE-716): A randomised, double-blind, phase 3 trial. *Lancet* (2022) 399(10336):1718–29. doi: 10.1016/S0140-6736(22)00562-1

10. Newell F, Pires da Silva I, Johansson PA, Menzies AM, Wilmott JS, Addala V, et al. Multiomic profiling of checkpoint inhibitor-treated melanoma: Identifying

predictors of response and resistance, and markers of biological discordance. *Cancer Cell* (2022) 40(1):88–102.e7. doi: 10.1016/j.ccell.2021.11.012

11. Dubin K, Callahan MK, Ren B, Khanin R, Viale A, Ling L, et al. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. *Nat Commun* (2016) 7:10391. doi: 10.1038/ncomms10391

12. Lozano AX, Chaudhuri AA, Nene A, Bacchiocchi A, Earland N, Vesely MD, et al. T Cell characteristics associated with toxicity to immune checkpoint blockade in patients with melanoma. *Nat Med* (2022) 28(2):353–62. doi: 10.1038/s41591-021-01623-z

13. Fernandes R, Parvathy SN D, Ernst S, Haeryfar M, Burton J, Silverman, et al. Preventing adverse events in patients with renal cell carcinoma treated with doublet immunotherapy using fecal microbiota transplantation (FMT): Initial results from perform a phase I study. *J Clin Oncol* (2022) 40(16_suppl):4553–3. doi: 10.1200/JCO.2022.40.16_suppl.4553

14. Suo A, Chan Y, Beaulieu C, Kong S, Cheung WY, Monzon JG, et al. Anti-PD1-Induced immune-related adverse events and survival outcomes in advanced melanoma. *Oncologist* (2020) 25(5):438–46. doi: 10.1634/theoncologist.2019-0674

15. Cicardi M, Berti E, Caputo V, Radice F, Gardinali M, Agostoni A. Idiopathic capillary leak syndrome: Evidence of CD8-positive lymphocytes surrounding damaged endothelial cells. *J Allergy Clin Immunol* (1997) 99:417–9. doi: 10.1016/S0091-6749(97)70061-7

16. Liu Z, Zhao Q, Zheng Z, Liu S, Meng L, Dong L, et al. Vascular normalization in immunotherapy: A promising mechanisms combined with radiotherapy. *BioMed Pharmacother* (2021) 139:111607. doi: 10.1016/j.biopha.2021.111607

17. Kim HJ, Ji YR, Lee YM. Crosstalk between angiogenesis and immune regulation in the tumor microenvironment. *Arch Pharm Res* (2022) 45(6):401–16. doi: 10.1007/s12272-022-01389-z

18. Lim SY, Lee JH, Gide TN, Menzies AM, Guminski A, Carlino MS, et al. Circulating cytokines predict immune-related toxicity in melanoma patients receiving anti-PD-1-Based immunotherapy. *Clin Cancer Res* (2019) 25(5):1557–63. doi: 10.1158/1078-0432.CCR-18-2795

19. Grabie N, Gotsman I, DaCosta R, Pang H, Stavrakis G, Butte MJ, et al. Endothelial programmed death-1 ligand 1 (PD-L1) regulates CD8+ T-cell mediated injury in the heart. *Circulation* (2007) 116(18):2062–71. doi: 10.1161/CIRCULATIONAHA.107.709360

20. Chen DS, Mellman I. Oncology meets immunology: The cancer-immunity cycle. *Immunity* (2013) 39(1):1–10. doi: 10.1016/j.immuni.2013.07.012

21. Huang Y, Goel S, Duda DG, Fukumura D, Jain RK. Vascular normalization as an emerging strategy to enhance cancer immunotherapy. *Cancer Res* (2013) 73(10):2943–8. doi: 10.1158/0008-5472.CAN-12-4354

22. Blanchard L, Girard JP. High endothelial venules (HEVs) in immunity, inflammation and cancer. *Angiogenesis* (2021) 24(4):719–53. doi: 10.1007/s10456-021-09792-8

23. Kouadri G, Perzo N, Sauvetre G, Lévesque H, Besnier E. Refractory severe idiopathic systemic capillary leak syndrome successfully treated with bevacizumab: A case report. *Angiogenesis* (2021) 24(3):399–401. doi: 10.1007/s10456-021-09769-7

24. Ribatti D, Solimando AG, Pezzella F. The anti-VEGF(R) drug discovery legacy: Improving attrition rates by breaking the vicious cycle of angiogenesis in cancer. *Cancers (Basel)* (2021) 13(14):3433. doi: 10.3390/cancers13143433

25. Lee WS, Yang H, Chon HJ, Kim C. Combination of anti-angiogenic therapy and immune checkpoint blockade normalizes vascular-immune crosstalk to potentiate cancer immunity. *Exp Mol Med* (2020) 52(9):1475–85. doi: 10.1038/s12276-020-00500-y

26. Fukumura D, Kloepper J, Amoozgar Z, Duda DG, Jain RK. Enhancing cancer immunotherapy using antiangiogenics: Opportunities and challenges. *Nat Rev Clin Oncol* (2018) 15(5):325–40. doi: 10.1038/nrclinonc.2018.29

27. Hodi FS, Lawrence D, Lezcano C, Wu X, Zhou J, Sasada T, et al. Bevacizumab plus ipilimumab in patients with metastatic melanoma. *Cancer Immunol Res* (2014) 2(7):632–42. doi: 10.1158/2326-6066.CIR-14-0053

28. Arance A, de la Cruz-Merino L, Petrella TM, Jamal R, Ny L, Carneiro A, et al. Phase II LEAP-004 study of lenvatinib plus pembrolizumab for melanoma with confirmed progression on a programmed cell death protein-1 or programmed death ligand 1 inhibitor given as monotherapy or in combination. *J Clin Oncol* (2022), JCO2200221. doi: 10.1200/JCO.22.00221

29. Li S, Wu X, Yan X, Zhou L, Chi Z, Si L, et al. Toripalimab plus axitinib in patients with metastatic mucosal melanoma: 3-year survival update and biomarker analysis. *J Immunother Cancer* (2022) 10(2):e004036. doi: 10.1136/jitc-2021-004036

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 08 February 2023
DOI 10.3389/fonc.2022.948472

OPEN ACCESS

EDITED BY
Pamela Bond Cassidy,
Oregon Health and Science University,
United States

REVIEWED BY
Valentin-Florian Rauca,
Technical University of Munich, Germany
Pradipta Ranjan Rauta,
Asian Institute of Public Health, India

*CORRESPONDENCE
Amanda McDaniel
ahm.biosciprof0226@gmail.com
Richard Nuccitelli
rnuccitelli@pulsebiosciences.com

SPECIALTY SECTION
This article was submitted to
Skin Cancer,
a section of the journal
Frontiers in Oncology

RECEIVED 19 May 2022
ACCEPTED 30 December 2022
PUBLISHED 08 February 2023

CITATION
McDaniel A, Freimark B, Navarro C,
Von Rothstein K, Gonzalez D, Linder K
and Nuccitelli R (2023) Nano-pulse
stimulation™ therapy (NPS™) is superior
to cryoablation in clearing murine
melanoma tumors.
Front. Oncol. 13:948472.
doi: 10.3389/fonc.2022.948472

Nano-pulse stimulation™ therapy (NPS™) is superior to cryoablation in clearing murine melanoma tumors

Amanda McDaniel[1]*, Bruce Freimark[1], Cebrina Navarro[1], Kristin Von Rothstein[1], Dacia Gonzalez[1], Keith Linder[2] and Richard Nuccitelli[1]*

[1]Department of Biology, Pulse Biosciences, Hayward, CA, United States, [2]Department of Dermatopathology, Linder Pathology Services, Raleigh, NC, United States

Background: Nano-Pulse Stimulation™ Therapy (NPS™) is a new, bioelectric modality that applies ultrashort pulses of electric energy to trigger regulated cell death in treated tissues. Instead of initiating necrosis by heating or freezing, NPS therapy permeabilizes intracellular organelles to activate the cell's own self-destruct pathway of programmed or regulated cell death. Unlike cryotherapies that can both damage structural tissues and diffuse into the periphery beyond the margins of the lesion, NPS only affects cells within the treated zone leaving surrounding tissue and acellular components unaffected.

Methods: We generated melanoma tumors in mice by injecting B16-F10 cells intradermally and compared the efficacy and resulting skin damage from Nano-Pulse Stimulation Therapy with that of cryoablation in clearing these tumors.

Results: The results of the study demonstrate that NPS is superior at clearing B16-F10 melanoma lesions. NPS permanently eliminated up to 91% of all tumor lesions with a single treatment compared to cryoablation that only eliminated up to 66%. Importantly, NPS permanently eliminated these lesions with no recurrence and with minimal dermal fibrosis, underlying muscle atrophy, permanent hair follicle loss or other markers of permanent skin damage.

Conclusions: These findings suggest that NPS is a promising new modality for the clearance of melanoma tumors and is a more efficacious, less damaging approach than cryoablative methods for the treatment of aggressive malignant tumors.

KEYWORDS

nano-pulse stimulation therapy (NPS), regulated cell death, cryoablation, B16-F10, melanoma, dermal fibrosis and scarring, nanosecond pulsed electric fields (nsPEF)

Introduction

Cutaneous melanoma is the most aggressive and lethal form of skin cancer. Over the past few decades, the incidence of melanoma has been steadily increasing and in 2022 alone there are projected to be nearly 100,000 new cases diagnosed, with over 7% of those cases resulting in mortality (1). Early-stage disease is usually managed with surgical excision alone, but removal of the malignant tissue eliminates further exposure to the immune system. Some other methods to ablate lesions have the additional advantage of initiating an immune response. Two such therapies are cryotherapy (2) and Nano-Pulse Stimulation Therapy (3) so both have been used in this study to compare the efficacy and skin damage resulting from each of them.

Nano-pulse stimulation therapy

Every cell in our bodies contains a fail-safe mechanism called regulated or programmed cell death that allows it to self-destruct when it reaches the end of its useful life, encounters a lethal gene mutation or an injury that it is unable to repair (4–6). Nano-Pulse Stimulation™ Therapy (NPS™) activates this pathway using ultrashort electric pulses. Unlike direct-contact ablation technologies that kill cells by necrosis using heat or cold, NPS is a bioelectric energy modality that triggers the cell's natural self-destruct pathway by initiating a transient permeabilization of the plasma and organelle membranes of targeted cells without causing thermal damage. This alters the function of internal cellular organelles, including the mitochondria and endoplasmic reticulum (7), without disrupting the extracellular tissue, primarily collagen-rich dermal foundation. The current lesion size limitation is 1 cm in diameter for a single treatment, but larger lesions can be treated with multiple applications. Previous published work includes treatments of seborrheic keratosis (8), sebaceous hyperplasia (9), warts (10) and basal cell carcinoma (11). In animal studies, NPS has shown high efficacy in treating a variety of malignant murine tumor types including rat hepatocellular as well as mouse breast, fibrosarcoma, squamous cell carcinoma (SCC), pancreatic, lung and melanoma tumors (12–18).

Cryotherapy

Due to the low cost of cryoablation, it has become an alternative to other more traditional surgical methods for cancer treatment. Cryoablation has been used to treat bone, cervical, eye, kidney, liver, lung, and prostate cancers (19–22). Some of the noted drawbacks to cryoablation have been the potential for scarring and long-term nerve damage caused by the treatment itself (23), as well as a question as to its long-term efficacy and ability to prevent microscopic spread of cancers (24, 25). While cryoablation and NPS are both considered focal therapies, NPS only affects cells between the two sets of microneedles of the applicator while cryoablation spreads beyond the applicator surface due to thermal diffusion.

In this study we demonstrate that NPS has superior efficacy in clearing a B16-F10 murine melanoma tumor, without reoccurrence with minimal dermal fibrosis and tissue damage. Since NPS is highly efficacious while producing less damage to tissue it is a promising minimally invasive physical modality for the treatment of tumors, particularly those that are not surgically resectable.

Materials and methods

In Vivo tumor model

Mice: Female C57BL/6J mice, 6-8 weeks old (Jackson Laboratories, Sacramento, CA) were acclimated for at least 3 days before treatment, housed in groups of 10, and both flanks were shaved before the start of tumor inoculations. Temperature and humidity were monitored daily, and animals were maintained on a 12-hour light/dark cycle. Water (Milli-Q) and food (Pirolab Diet 20 chow) were given *ad libitum*. All experiments were performed in accordance with animal care guidelines set forth by the Pulse Biosciences IACUC.

Tumors: The B16-F10 tumor line was obtained from ATCC (Manasus, VA, cat # CRL-2539) and propagated in tissue culture with DMEM supplemented with 10% v/v fetal bovine serum (FBS), penicillin/streptomycin, and harvested for inoculation between passages 9-12 for all studies. Tumors were initiated in mice by intradermal (i.d.) injection into the right flank with $2x10^5$ cells/30μL in Hank's balanced salt solution (HBSS). Tumor growth was measured twice a week by calipers. The volumes were determined using the formula: volume = length x width^2/2. Tumors were randomized to treatment groups when the largest diameter reached ~5mm on Day 6 post tumor inoculation (PTI). Mice were removed from the study if the animal lost more than 20% of their initial body weight, appeared moribund, the tumor was ulcerated, or the tumor volume exceeded 2000mm^3. The day at which each mouse was sacrificed or found dead was recorded and used to generate a Kaplan-Meier survival curve. Mice who cleared the lesion were continually monitored for tumor regrowth until study completion on Day 65, at which point mice were sacrificed and skin lesions removed for histological analysis.

NPS and cryoablation tumor treatments

Six days post-inoculation, mice that developed tumors were treated with either NPS energy delivered by the CellFX® System (Pulse Biosciences, Hayward, CA) or cryotherapy using a 5mm closed-end conical metal cryoprobe (Brymill, Ellington CT, model CRY-AC-3 B800). Tumors were injected into the intradermal space within the skin so that they could be stretched over a platform designed to isolate them from the body and internal organs (Figure 1). Before treatment, each cage of mice was placed into a chamber containing 2.1% isoflurane in oxygen to induce an anesthetized state. Once mice were recumbent, each mouse was individually placed onto the treatment platform, receiving inhaled isoflurane directly from a nose cone for the duration of the procedure, typically 2-3 minutes. Upon completion of each treatment mice were returned to their home cage for recovery. All procedures were performed according to IACUC-approved protocols.

10.3389/fonc.2022.948472

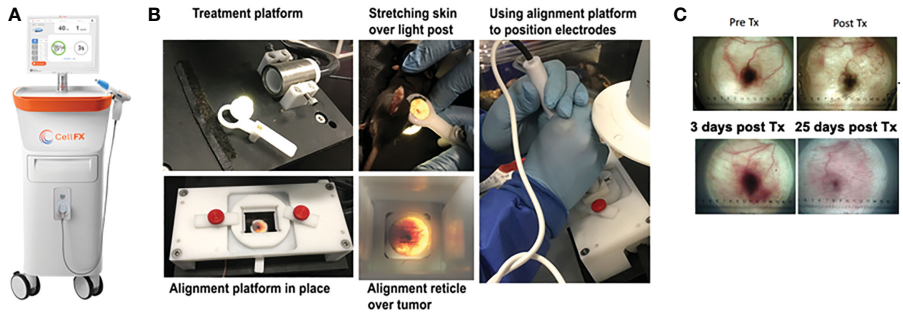


FIGURE 1
Images of the CellFX™ treatment platform and a treated melanoma tumor. **(A)** CellFX Pulse Generator; **(B)** Montage of images illustrating the procedure used to treat the melanoma tumors by stretching the skin containing the tumor over a translucent silicone light post and aligning the tumor with the application electrode followed by treatment with the CellFX system; **(C)** Transillumination images of a typical melanoma over time, Before treatment, immediately after treatment, 3 days post treatment and 25 days after treatment.

Nano-pulse stimulation (NPS) therapy

NPS therapy was delivered using a 5.0 × 5.0 × 3.5mm treatment tip attached to a handpiece plugged directly into the CellFX® device. The treatment tip contained two rows of 5 microneedles 3.5 mm long, spaced 5mm apart. Mouse tumors were treated by stretching the skin containing the tumor over a translucent silicone treatment post and inserting the probe needles to flank the sides of the tumor (Figure 1). A light source housed under the treatment post was employed to illuminate the tumor treatment area to aid in placement of the microneedles around the tumor. Each tumor received either a low-mid dose of 180 mJ/mm^3 or a high dose of 360 mJ/mm^3. The energy doses chosen were selected based on previously performed dose-response tumor clearance studies (Figure 2). The low-mid dose was established as effective at clearing >60-70% of all treated tumors and the second higher dose was capable of clearing >90-100%.

Cryotherapy

Cryotherapy was delivered as a single dose of a cryosurgical system with a 5mm closed-end conical probe cooled with liquid nitrogen applied directly to the tumor (Brymill Corp., Ellington CT). The cryoablation dispenser (model CRY-AC-3 B800) was filled with liquid nitrogen and the probe tip was pre-chilled to -40°C as measured by a thermal imaging camera (FLIR, Estonia; model FLIR-E64501). Mouse skin was stretched across the treatment post and cryotherapy was applied to the tumor for the designated time, during which the temperature was continually monitored, and the probe received a cooling burst every ten seconds to keep the temperature stable at -40°C. Cryoablative temperatures rely on the formation of ice crystals in tissues as the cell death mechanism (26). Durations of exposure, defined as "doses," were chosen based upon previous clinical findings showing that exposures under one minute were less likely to induce complete cell death of all tumor cells, than were exposures lasting longer. Exposure length is thus a critical variable as longer exposures are more likely to permanently eliminate a lesion (21, 27). We chose 45 seconds (45s) as the low-mid dose and 90 seconds (90s) as the high dose.

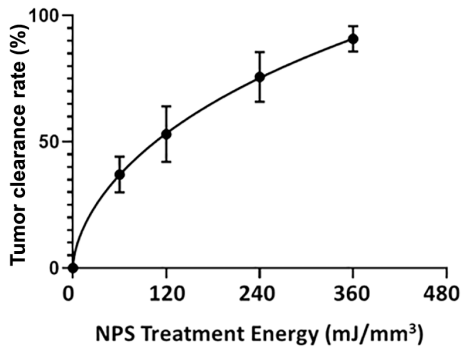


FIGURE 2
The percentage of treated tumors that are completely cleared as a function of the energy applied during treatment. Bars represent the Standard Error of the Mean.

Skin biopsies

After euthanasia, a rectangle of skin (2.5 cm long by 1.5 cm wide) containing the treatment area in the center, was excised andattached flat to paper card stock without stretching, and submersed in 10% neutral-buffered formalin. After 24-48 hours of fixation, each skin sample was bisected in the center of the treatment area, samples were marked with surgical ink to maintain orientation, and both halves were embedded in paraffin along their treatment area cut surfaces. Samples were routinely processed for paraffin histology (AcePix, Hayward, CA), sectioned to 5 micrometers, and stained with hematoxylin and eosin (H&E) or Gomori's trichrome.

Histological analysis of skin samples

Assessment of histopathology was performed by a board-certified veterinary pathologist with expertise in dermatopathology. Skin treatment areas were compared for treatment-related tissue scarring and injury that included dermal fibrosis, width of fibrosis, hair follicle loss, intactness of epidermis, cutaneous trunci muscle atrophy/loss, and inflammation. Dermal fibrosis was identified by linearization,

compactness, and thickness of dermal stroma. Cutaneous trunci muscle atrophy/loss was recognized as segmental muscle thinning or absence in the treatment area. Skin lesions were scored using a standard severity scale: 0 = no change, 1 = mild, 2 = moderate, and 3 = marked (Table 1). All histology slides were randomized and scored in a blinded manner. If lesions differed in sections from the same treatment, then the most severe lesion was scored. Histopathology scores were compared across all groups using a Kruskal-Wallis one-way analysis of variance (ANOVA).

Statistical analysis

Statistical analyses were performed using GraphPad Prism software (v9, La Jolla, CA). Tumor elimination rates were compared between groups using a chi-square contingency test (Figure 3). Kaplan-Meier survival curves were compared by log-rank (Mantel-Cox) test (Figure 4). Kruskal-Wallis non-parametric ANOVA was used to compare histopathological scores generated for each marker of tissue damage for histological samples (Figure 4). A two-tailed p-value of < 0.05 was considered statistically significant (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

Results

Efficacy of tumor clearance

Intradermal B16-F10 murine melanoma tumors grow rapidly in mice and normally reach a size that requires euthanasia within 3 weeks (Figure 3A). However, treatments with both NPS and cryoablation greatly slow this growth and usually result in tumor shrinkage within 2 weeks. Tumors that cleared following NPS treatment remained cleared and did not recur. However, even after initial clearance with cryoablation, tumor growth would resume for many of the tumors within 20-30 days of initial clearance. When mice were treated with a low-mid dose (180 mJ/mm^3) of NPS, tumors were permanently eliminated in 78% of mice (18/23) and when treated with the higher dose (360 mJ/mm^3) of NPS energy the percentage increased to 96% (21/22). In contrast, the lower dose of cryo (45s) eliminated only 58% (14/24) of all tumors and the higher dose (90s) only showed a slight improvement to 66% (16/24). The higher dose of cryo exposure failed to reach the level of efficacy of even the low-mid dose NPS. The rate of complete tumor elimination was significantly greater in the high dose NPS (360 mJ/mm^3) group compared to both the low-mid (**p=0.0096) and high (*p=0.0391) dose cryo groups (Figure 5). The efficacy of each treatment group was also reflected in the survival rate. Mice treated with high-energy NPS (360 mJ/mm^3) were the most likely to survive until the study endpoint (91%) and this was significantly longer than for mice treated with a low dose of cryo (58%) (Figure 4; *p=0.0159).

Histological analysis of post-treatment tissue

Histopathology confirmed the absence of melanoma in treatment areas in all samples evaluated. Evidence of scar in all treatment groups was only mild (Figure 6, 7, S1-4, Table S1). Dermal fibrosis, width of fibrosis, hair follicle loss, and cutaneous trunci muscle atrophy/loss were on average mild or mostly mild. Notably, dermal fibrosis was significantly greater (*p=0.0179) after treatment with a high dose of cryoablation (90s) relative to a low dose (45s) (Figures 6, S1-2, Table S1). However, there were no statistically significant differences between the remainder of the treatment groups for lesion scores. Inflammation was mostly absent in most samples, regardless of treatment and did not differ significantly. The epidermis was intact in all samples and erosions and ulcers were not present.

Discussion

The B16-F10 murine melanoma model was the first used to demonstrate the ability of NPS therapy to permanently eliminate intradermal melanomas (14, 15, 28). It was discovered quite early that the minimum electric field strength required was on the order of 20 kV/cm and that the most likely mechanism involved the formation of pores in lipid membranes (29, 30). The additional discovery that only about 400 mV is required across a lipid membrane to electropermeabilize it (31), suggested that the approximate size of the NPS target must be on the order of 0.2 um, the size of smaller intracellular organelles such as mitochondria. A single NPS pulse has little effect on the tumor but as the pulse number increases, the electropermeabilization effect becomes more evident (Figure 2). However, this response does not depend on any significant

TABLE 1 Severity scoring of markers of tissue damage.

Marker	Definition	Scoring System
Dermal Fibrosis	Linearization and compactness of dermal collagen and thickness of dermis	0=no lesion, 1=mild, 2=moderate, 3-marked
Lesion size	Width of dermal fibrosis was scored	0=no lesion, 1- mild, 2=moderate, 3=marked
Hair follicle loss	Number of follicles missing in area of fibrosis	0=no follicle loss, 1=mild, 2=moderate, 3=marked
Muscle Atrophy/loss	Thinning of panniculus (twitch) muscle, thinning of muscle fibers and loss of muscle fibers	1=Partial loss (thinning), 2=Full-thickness loss of muscle for short distance 5-6 follicles wide or less; 3=Full-thickness loss of muscle greater than 5-6 follicles
Inflammation	Amount of inflammation	0=no inflammation; 1=mild, 2=moderate, 3=marked

10.3389/fonc.2022.948472

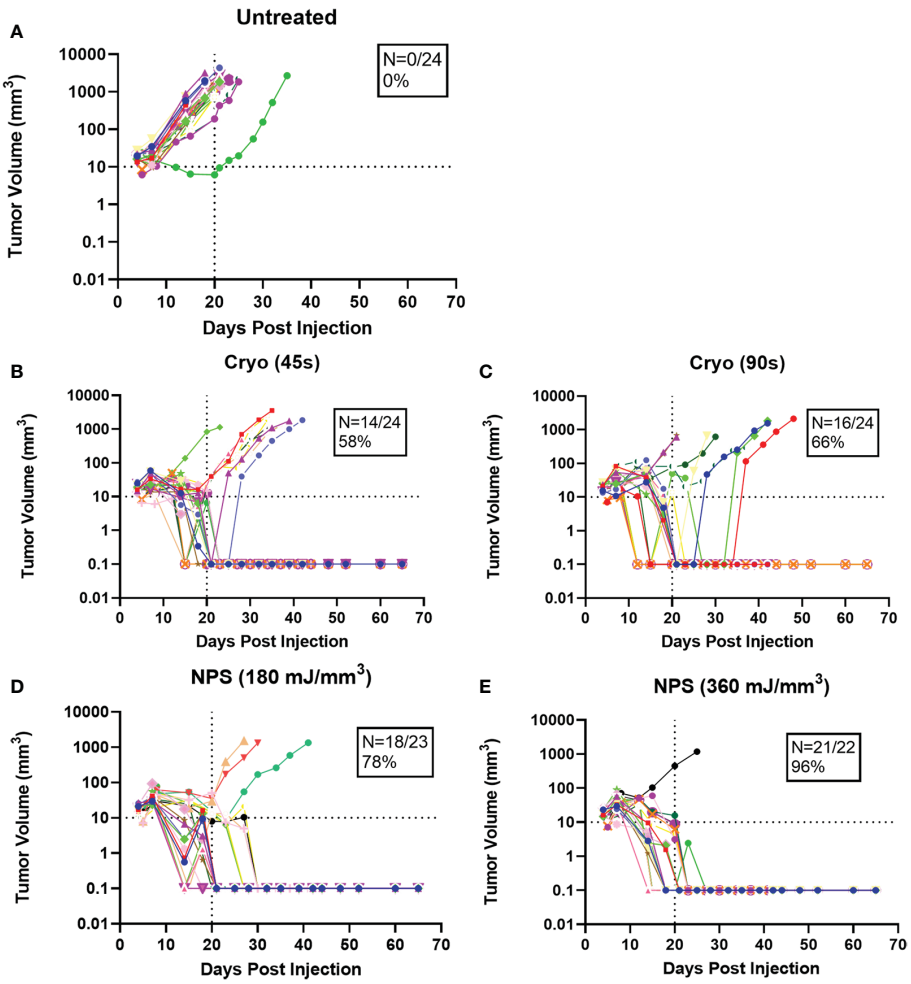


FIGURE 3
Growth rates of individual tumors treated with the treatment indicated above each graph. Each color represents a single tumor. N represents the percentage of tumors completely cleared in each case **(A)** Untreated; **(B)** Cryo (45s); **(C)** Cryo (90s); **(D)** NPS (180 mJ/mm3); **(E)** NPS (360 mJ/mm3).

temperature increase (32, 33) which indicates the cell death mechanism induced by NPS is of a non-thermal nature.

NPS has also demonstrated high efficacy in the treatment of tumors in other murine models of cancer. The tumor elimination rate is typically around 75-100% dependent upon the model and treatment energy used (34). NPS demonstrated 100% efficacy in eliminating 4T1 murine breast cancer in one study (35), in another study a 75% elimination rate was observed after NPS treatment of mouse hepatocellular carcinomas (36) and a 80-90% response rate was noted in the treatment of rat hepatocellular carcinomas (37). Within our laboratory alone, we have shown that an energy of 360 mJ/mm^3 eliminates between 90-100% of tumors across several murine tumor types, including B16-F10 melanoma (Figure 2). Response rates are related to tumor type and size as well as

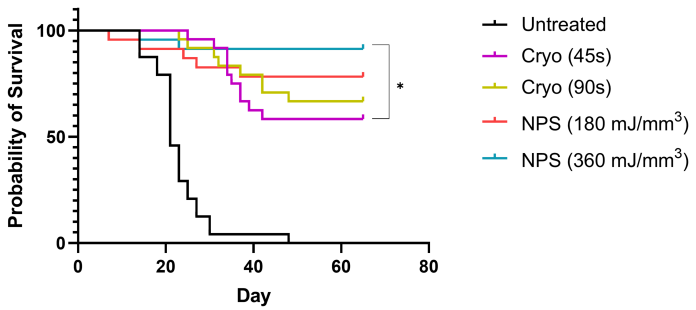


FIGURE 4
Kaplan-Meier Survival data to day 65 for each treatment. The high dose NPS group survival is significantly better than the 45s cryo treatment group. Both treatment does of NPS had higher rates of survival than either cryo group (log-Rank Mantel-Cox test, *p<0.05).

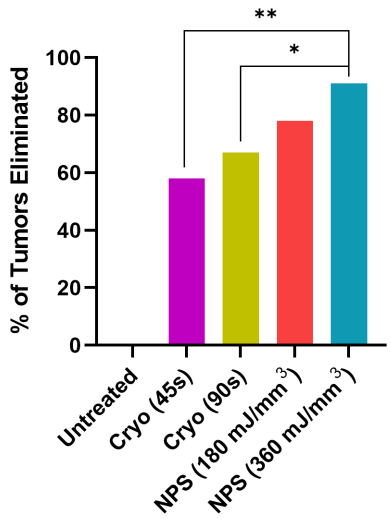


FIGURE 5
The percentage of tumors that were completely eliminated by the indicated treatments. Both low-mid (180 mJ/mm^3) and high (360 mJ/mm^3) NPS treatment groups exhibited higher rates of complete tumor elimination than either the low (45 s) or high (90 s) cryoablation treatment groups. (Chi square test: *p<0.05, **p<0.01).

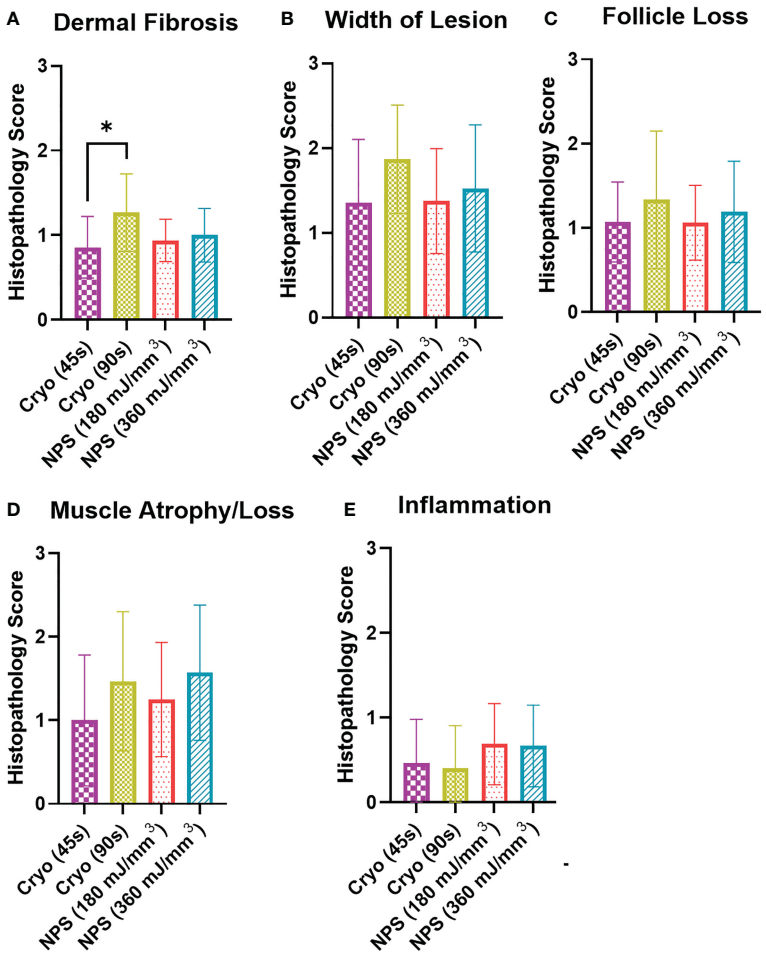


FIGURE 6
Histopathology scoring of histology sections collected on day 65 from each tumor treatment. Scale: 0=no lesion, 1=mild, 2=moderate, 3= marked. **(A)** Dermal fibrosis was significantly higher for the 90s cryo treatment than the 45s treatment (Kruskal-Wallis ANOVA, *p<0.05); **(B)** Lesion width showed no significant differences between the different treatments; **(C)** Hair follicle loss was similar for all treatments; **(D)** Muscle atrophy/loss: 1=partial loss; 2= full-thickness loss of muscle for short distance; 3=extension of atrophy beyond full-thickness loss. There was no significant difference in atrophy among the four treatments; **(E)** Inflammation score indicated only very minor inflammation at 65 days for the four treatments.

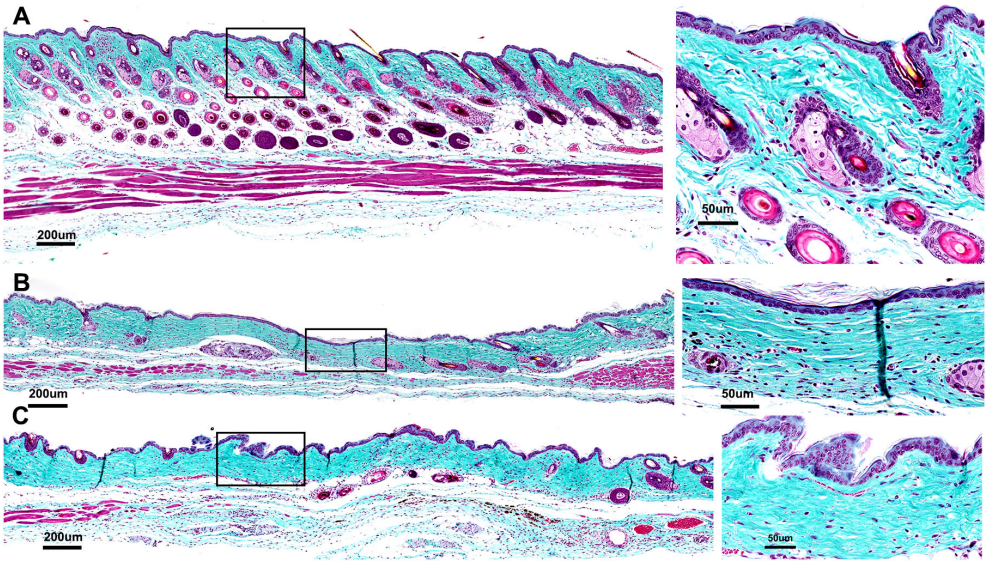


FIGURE 7
Histological sections of skin regions where the tumor had been treated stained with Gomori's trichrome to assess collagen linearization and compaction (inset 4X greater magnification of black box region marked on the left). **(A)** Section showing no damage in which collagen and muscle are structurally intact with no loss of hair follicles; **(B)** Mild-moderate damage indicated by slight linearization and compaction of collagen indicating dermal fibrosis and loss of hair follicles; **(C)** Moderate-Marked damage indicated by moderate linearization and compaction of dermal collagen, clear loss of hair follicles and significant muscle atrophy across entire treatment site.

treatment energy. NPS has also typically exhibited an ability to induce an immune response after treatment, likely due to the immunogenic nature of the RCD process triggered by NPS (3, 37–40).

In addition to the high efficacy in treating murine tumors, NPS exhibits similar high levels of efficacy in treating human skin lesions while producing limited damage to the skin itself (8, 41, 42). Histologic examination has shown only a minimal degree of epidermal and dermal inflammation associated with NPS treatments and this was less than typically observed in skin treated with cryoablative and other physical methods of lesion removal. These low levels of inflammation lead to less abnormal collagen deposition resulting in less dermal fibrosis and therefore less permanent scaring (43). Clinical trials utilizing NPS in the treatment of seborrheic keratosis, sebaceous gland hyperplasia, non-genital warts and basal cell carcinoma have all shown successful treatment outcomes (8, 11, 41, 42). Based on the results of these trials the CellFX® device, used to deliver NPS energy, recently attained medical device clearance for the treatment of human benign skin lesions in the USA (FDA 510(k)), Canada (Health Canada) and the EU (CE mark).

While NPS and cryoablation share treatment similarities, the mechanism each uses to destroy cells is quite different. NPS uses ultrashort, high voltage electric pulses that generate transient nanopores in cell and organelle membranes, leading to the initiation of a regulated cell death process in the exposed cells while leaving acellular tissue components unharmed (43, 44). It can also treat tissues with more precise boundaries than thermal-based treatment modalities, ensuring the treatment zone is highly focal to the lesion (3). In contrast, the cryoablation mechanism of cell death involves the quick drastic cooling of the tissue to -40°C which leads to the formation of ice (26, 45). Ice formation causes immediate cell shrinkage and damage to intracellular proteins and membranes. Over time the continued exposure to extremely low temperatures causes thrombosis, tissue hypoxia and eventual necrosis. Cryo exposure also causes cell death indirectly, as the formation of ice within tissues can destroy supporting structural tissue and vasculature that is required for the survival of cells. Vascular endothelial cells can be significantly damaged, and as the tissues gradually thaw, reperfusion draws in platelets that can cause significant clotting and blockage of blood vessels (46–48). This ischemic outcome serves to starve the treated tissue of needed blood supply. The ischemia can also cause hyperemia, erythema, and edema through the production of molecules that cause vasodilation and inflammation (47, 49). While these effects are critical to the mechanism of cryo-induced cell death they also have the potential to significantly damage surrounding tissues due to thermal diffusion, particularly if longer exposure times and multiple cycles are being utilized for treatment.

One of the biggest potential drawbacks to the use of cryosurgical techniques, is that regimens aggressive enough to completely eliminate tumors without recurrence are also highly damaging to other tissues. When cryotherapy is used to ablate cancerous lesions in the clinic it requires the use of multiple cycles and longer freeze times, which increases the likelihood of scarring and damage to underlying structures and peripheral tissues (50, 51). This has kept cryotherapy from being a recommended first line therapy for most malignant lesions and tumors and only remains an option when surgical excision is not (24). The current strategy for elimination of cancerous lesions with cryo therapies such as non-melanoma skin cancers (NMSCs), prostate, kidney or hepatic lesions (52) is typically

to overtreat and extend the margins into the periphery to ensure complete elimination of all fast growing malignant cells to prevent reccurrence or even metastasis (52). Obviously, the complete elimination of all tumor cells is imperative, and the primary endpoint of any treatment used to eliminate a malignant lesion. However, minimizing the destruction of normal cells and tissues is also vital and an obvious objective in any clinical trial. Thus, one of the largest potential benefits of NPS treatment over cryoablation therapies is its high rate of tumor clearance at a dose that demonstrates very minimal damage to surrounding tissues.

Additionally, when cryoablative treatments are used to treat melanomas, they are typically used in combination with immunotherapies, other surgical procedures and/or to debulk non-surgically accessible metastatic lesions (53, 54). Trials are currently being conducted to investigate the use of therapies that combine immune adjuvants and/or immune checkpoint blockade with cryotherapy to treat melanoma and other aggressive cancers (55–57). These studies are intended to harness the immune response that is induced by the release of antigens after treatment and direct it towards an adaptive CD8+ memory response that has the ability to target and destroy tumor cells left over after the primary mechanism of cell death has ceased (57). The potential for abscopal effects that may target metastatic sites is also being investigated (58).

Although we only examined the single-agent efficacy of NPS on primary tumor elimination in this study, previous published studies have documented the ability of NPS to inhibit both the growth of a tumor cell rechallenge and prevent metastasis in a CD8-dependent manner (13, 15, 17, 18, 34, 38). The RCD process induced by NPS is likely responsible for priming this $CD8^+$ T cell- mediated immune response (44). The combination of NPS with immune adjuvants appears to have an additive effect that boosts treatment efficacy and prevents the growth of a tumor cell rechallenge as evidenced in studies conducted within our laboratory. In the future, we may plan studies to compare the immune responses induced by NPS with those induced by cryoablation.

Conclusion

NPS displayed superior efficacy over cryoablation with negligible impact to the skin tissue in our side-by-side preclinical comparison. Although NPS has not yet been used in the human clinic to treat aggressive malignant tumors such as melanoma, it has displayed a high rate of efficacy in the treatment of murine tumor types that are typically difficult to kill, without reoccurrence. NPS shares many of the features that make cryotherapies attractive, such as the ability to target hard-to-access lesions and tumors that are untreatable with surgical means, without the associated thermal tissue damage characteristic of cryotherapy.

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 authors.

Ethics statement

The animal study was reviewed and approved by Pulse Biosciences IACUC.

Author contributions

AM, BF, CN, KVR, DG and RN conducted all the animal treatments. KL evaluated and scored the histological sections. AM wrote the first draft of the manuscript and RN revised to incorporate the reviewer's suggestions. All authors contributed to the article and approved the submitted version.

Conflict of interest

AM, BF, CN, KVR, DG and RN were on the payroll by Pulse Biosciences. Pulse Biosciences funded this study and was involved in the data analysis and writing of this article. KL is the owner of Linder Pathology Services.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.948472/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Representative composite image based on average severity scoring for Cryo (45s) condition, shown at 10X and 40X magnification. Severity scoring for all metrics of tissue damage were as follows: Dermal Fibrosis = 1; Lesion Width = 1; Follicle Loss = 1; Muscle Atrophy = 1; Inflammation = 1.

SUPPLEMENTARY FIGURE 2
Representative composite image based on average severity scoring for Cryo (90s) condition, shown at 10X and 40X magnification. Severity scoring for all metrics of tissue damage were as follows: Dermal Fibrosis = 1; Lesion Width = 2; Follicle Loss = 2; Muscle Atrophy = 2; Inflammation = 0.

SUPPLEMENTARY FIGURE 3
Representative composite image based on average severity scoring for NPS (180 mJ/mm3) condition, shown at 10X and 40X magnification. Severity scoring for all metrics of tissue damage were as follows: Dermal Fibrosis = 1; Lesion Width = 1; Follicle Loss = 1; Muscle Atrophy = 1; Inflammation = 1.

SUPPLEMENTARY FIGURE 4
Representative composite image based on average severity scoring for NPS (360 mJ/mm3) condition, shown at 10X and 40X magnification. Severity scoring for all metrics of tissue damage were as follows: Dermal Fibrosis = 1; Lesion Width = 1; Follicle Loss = 1; Muscle Atrophy = 2; Inflammation = 0.

References

1. Society AC. Survival rates for melanoma skin cancer. In: *Cancer statistics* (2022). Available at: http://cancerstatisticscenter.cancer.org.

2. Hamad GG, Neifeld JP. Biochemical, hematologic, and immunologic alterations following hepatic cryotherapy. *Semin Surg Oncol* (1998) 14:122–8. doi: 10.1002/(SICI)1098-2388(199803)14:2<122::AID-SSU5>3.0.CO;2-7

3. Nuccitelli R, McDaniel A, Anand S, Cha J, Mallon Z, Berridge JC, et al. Nano-pulse stimulation is a physical modality that can trigger immunogenic tumor cell death. *J immunotherapy Cancer* (2017) 5:32. doi: 10.1186/s40425-017-0234-5

4. Tang D, Kang R, Berghe TV, Vandenabeele P, Kroemer G. The molecular machinery of regulated cell death. *Cell Res* (2019) 29:347–64. doi: 10.1038/s41422-019-0164-5

5. Flores-Romero H, Ros U, Garcia-Saez AJ. Pore formation in regulated cell death. *EMBO J 39* (2020). doi: 10.15252/embj.2020105753

6. Wang JYJ. Cell death response to DNA damage. *Yale J Biol Med* (2019) 92:771–9.

7. Nuccitelli R, McDaniel A, Connolly R, Zelickson B, Hartman H. Nano-pulse stimulation induces changes in the intracellular organelles in rat liver tumors treated in situ. *Lasers Surg Med* (2020) 52:882–9. doi: 10.1002/lsm.23239

8. Hruza GJ, Zelickson BD, Selim MM, Rohrer TE, Newman J, Park H, et al. Safety and efficacy of nanosecond pulsed electric field treatment of seborrheic keratoses. *Dermatol Surg* (2020) 46:1183–9. doi: 10.1097/DSS.0000000000002278

9. Munavalli GS, Zelickson BD, Selim MM, Kilmer SL, Rohrer TE, Newman J, et al. Safety and efficacy of nanosecond pulsed electric field treatment of sebaceous gland hyperplasia. *Dermatologic Surg Off Publ Am Soc Dermatologic Surg* (2019) 46:803–9.

10. Nuccitelli R, LaTowsky BM, Lain E, Munavalli G, Loss L, Ross EV, et al. Safety and efficacy of nano-pulse stimulation treatment of non-genital, cutaneous warts (Verrucae). *Lasers Surg Med* (2021) 53(10):1301–6. doi: 10.1002/lsm.23423

11. Ross AS, Schlesinger T, Harmon CB, Moy RL, Rohrer TE , Mehregan DR, et al. Multicenter, prospective feasibility study of nano-pulse stimulation™ technology for the treatment of both nodular and superficial low-risk basal cell carcinoma. *Front Oncol* (2022) 12:1044694. doi: 10.3389/fonc.2022.1044694

12. Nuccitelli R. Application of pulsed electric fields to cancer therapy. *Bioelectricity* (2019) 1:30–4. doi: 10.1089/bioe.2018.0001

13. Nuccitelli R, Berridge JC, Mallon Z, Kreis M, Athos B, Nuccitelli P. Nanoelectroablation of murine tumors triggers a CD8-dependent inhibition of secondary tumor growth. *PloS One* (2015) 10:e0134364. doi: 10.1371/journal.pone.0134364

14. Nuccitelli R, Pliquett U, Chen X, Ford W, James Swanson R, Beebe SJ, et al. Nanosecond pulsed electric fields cause melanomas to self-destruct. *Biochem Biophys Res Commun* (2006) 343:351–60. doi: 10.1016/j.bbrc.2006.02.181

15. Nuccitelli R, Tran K, Lui K, Huynh J, Athos B, Kreis M, et al. Non-thermal nanoelectroablation of UV-induced murine melanomas stimulates an immune response. *Pigment Cell Melanoma Res* (2012) 25:618–29. doi: 10.1111/j.1755-148X.2012.01027.x

16. Beebe SJ, Lassiter BP, Guo S. Nanopulse stimulation (NPS) induces tumor ablation and immunity in orthotopic 4T1 mouse breast cancer: A review. *Cancers* (2018) 10(4):97. doi: 10.3390/cancers10040097

17. Guo S, Jing Y, Burcus NI, Lassiter BP, Tanaz R, Heller R, et al. Nano-pulse stimulation induces potent immune responses, eradicating local breast cancer while reducing distant metastases. *Int J Cancer* (2018) 142:629–40. doi: 10.1002/ijc.31071

18. Lassiter BP, Guo S, Beebe SJ. Nano-pulse stimulation ablates orthotopic rat hepatocellular carcinoma and induces innate and adaptive memory immune mechanisms that prevent recurrence. *Cancers (Basel)* (2018) 10(3):69. doi: 10.3390/cancers10030069

19. Hurtado-Roca Y, Becerra-Chauca N, Malca M. Efficacy and safety of cryotherapy, cold cone or thermocoagulation compared to LEEP as a therapy for cervical intraepithelial neoplasia: Systematic review. *Rev Saúde Pública* (2020) 54:27. doi: 10.11606/s1518-8787.2020054001750

20. Mahnken A, König A, Figiel J. Current technique and application of percutaneous cryotherapy. *RöFo - Fortschr auf dem Gebiet der Röntgenstrahlen und der bildgebenden Verfahren* (2018) 190:836–46. doi: 10.1055/a-0598-5134

21. Baust JG, Gage AA, Bjerklund Johansen TE, Baust JM. Mechanisms of cryoablation: Clinical consequences on malignant tumors. *Cryobiology* (2014) 68:1–11. doi: 10.1016/j.cryobiol.2013.11.001

22. Jung JH, Risk MC, Goldfarb R, Reddy B, Coles B, Dahm P. Primary cryotherapy for localised or locally advanced prostate cancer. *Cochrane Database Systematic Rev 2018* (2018) 5(5). doi: 10.1002/14651858.CD005010.pub3

23. Zhou JT, Fang DM, Xia S, Li T, Liu RL. The incidence proportion of erectile dysfunction in patients treated with cryotherapy for prostate cancer: a meta-analysis. *Clin Transl Oncol* (2019) 21:1152–8. doi: 10.1007/s12094-019-02036-8

24. Bahner JD, Bordeaux JS. Non-melanoma skin cancers: photodynamic therapy, cryotherapy, 5-fluorouracil, imiquimod, diclofenac, or what? *Facts controversies. Clin Dermatol* (2013) 31:792–8. doi: 10.1016/j.clindermatol.2013.08.020

25. Ahdoot M, Lebastchi AH, Turkbey B, Wood B, Pinto PA. Contemporary treatments in prostate cancer focal therapy. *Curr Opin Oncol* (2019) 31:200–6. doi: 10.1097/CCO.0000000000000515

26. Baust JG, Gage AA. Progress toward optimization of cryosurgery. *Technol Cancer Res Treat* (2004) 3:95–101. doi: 10.1177/153303460400300202

27. Klossner DP, Robilotto AT, Clarke DM, VanBuskirk RG, Baust JM, Gage AA, et al. Cryosurgical technique: assessment of the fundamental variables using human prostate cancer model systems. *Cryobiology* (2007) 55:189–99. doi: 10.1016/j.cryobiol.2007.07.003

28. Nuccitelli R, Chen X, Pakhomov AG, Baldwin WH, Sheikh S, Pomicter JL, et al. A new pulsed electric field therapy for melanoma disrupts the tumor's blood supply and causes complete remission without recurrence. *Int J Cancer* (2009) 125:438–45. doi: 10.1002/ijc.24345

29. Vernier PT, Sun Y, Gundersen MA. Nanoelectropulse-driven membrane perturbation and small molecule permeabilization. *BMC. Cell Biol* (2006) 7:37.

30. Vernier PT, Sun Y, Wang J, Mya TM, Garon E, Valderrabano M, et al. Nanoelectropulse intracellular perturbation and electropermeabilization technology: phospholipid translocation, calcium bursts, chromatin rearrangement, cardiomyocyte activation, and tumor cell sensitivity. *Conf Proc IEEE Eng Med Biol Soc* (2005) 6:5850–3. doi: 10.1109/IEMBS.2005.1615820

31. Robello M, Gliozzi A. Conductance transition induced by an electric field in lipid bilayers. *Biochim Biophys Acta* (1989) 982:173–6. doi: 10.1016/0005-2736(89)90189-2

32. Pliquett U, Nuccitelli R. Measurement and simulation of joule heating during treatment of b-16 melanoma tumors in mice with nanosecond pulsed electric fields. *Bioelectrochemistry* (2014) 100:62–8. doi: 10.1016/j.bioelechem.2014.03.001

33. Nuccitelli R, Pliquett U, Chen X, Ford W, James SR, Beebe SJ, et al. Nanosecond pulsed electric fields cause melanomas to self-destruct. *Biochem Biophys Res Commun* (2006) 343:351–60. doi: 10.1016/j.bbrc.2006.02.181

34. Beebe S, Lassiter B, Guo S. Nanopulse stimulation (NPS) induces tumor ablation and immunity in orthotopic 4T1 mouse breast cancer: A review. *Cancers* (2018) 10:97. doi: 10.3390/cancers10040097

35. Guo S, Jing Y, Burcus NI, Lassiter BP, Tanaz R, Heller R, et al. Nano-pulse stimulation induces potent immune responses, eradicating local breast cancer while reducing distant metastases. *Int J Cancer* (2018) 142:629–40. doi: 10.1002/ijc.31071

36. Chen X, Zhuang J, Kolb JF, Schoenbach KH, Beebe SJ. Long term survival of mice with hepatocellular carcinoma after pulse power ablation with nanosecond pulsed electric fields. *Technol Cancer Res Treat* (2012) 11:83–93. doi: 10.7785/tcrt.2012.500237

37. Chen R, Sain NM, Harlow KT, Chen YJ, Shires PK, Heller R, et al. A protective effect after clearance of orthotopic rat hepatocellular carcinoma by nanosecond pulsed electric fields. *Eur J Cancer* (2014) 50:2705–13. doi: 10.1016/j.ejca.2014.07.006

38. Skeate JG, Da Silva DM, Chavez-Juan E, Anand S, Nuccitelli R, Kast WM. Nano-pulse stimulation induces immunogenic cell death in human papillomavirus-transformed tumors and initiates an adaptive immune response. *PloS One* (2018) 13:e0191311. doi: 10.1371/journal.pone.0191311

39. Nuccitelli R, McDaniel A, Connolly R, Zelickson B, Hartman H. Nano-pulse stimulation induces changes in the intracellular organelles in rat liver tumors treated in situ. *Lasers Surg Med* (2020) 52:882–9. doi: 10.1002/lsm.23239

40. Nuccitelli R, Berridge JC, Mallon Z, Kreis M, Athos B, Nuccitelli P. Nanoelectroablation of murine tumors triggers a CD8-dependent inhibition of secondary tumor growth. *PLoS. One* (2015) 10:e0134364. doi: 10.1371/journal.pone.0134364

41. Munavalli GS, Zelickson BD, Selim MM, Kilmer SL, Rohrer TE, Newman J, et al. Safety and efficacy of nanosecond pulsed electric field treatment of sebaceous gland hyperplasia. *Dermatol Surg* (2020) 46:803–9. doi: 10.1097/DSS.0000000000002154

42. Nuccitelli R, LaTowsky BM, Lain E, Munavalli G, Loss L, Ross EV, et al. Safety and efficacy of nano-pulse stimulation treatment of non-genital, cutaneous warts (Verrucae). *Lasers Surg Med* (2021) 53:1301–6. doi: 10.1002/lsm.23423

43. Kaufman D, Martinez M, Jauregui L, Ebbers E, Nuccitelli R, Knape WA, et al. A dose-response study of a novel method of selective tissue modification of cellular structures in the skin with nanosecond pulsed electric fields. *Lasers Surg Med* (2020) 52:315–22. doi: 10.1002/lsm.23145

44. Nuccitelli R, McDaniel A, Anand S, Cha J, Mallon Z, Berridge JC, et al. Nano-pulse stimulation is a physical modality that can trigger immunogenic tumor cell death. *J ImmunoTherapy Cancer 5* (2017) 5:32. doi: 10.1186/s40425-017-0234-5

45. Gage AA, Baust J. Mechanisms of tissue injury in cryosurgery. *Cryobiology* (1998) 37:171–86. doi: 10.1006/cryo.1998.2115

46. Finelli A, Rewcastle JC, Jewett MA. Cryotherapy and radiofrequency ablation: pathophysiologic basis and laboratory studies. *Curr Opin Urol* (2003) 13:187–91. doi: 10.1097/00042307-200305000-00003

47. Baust JG, Snyder KK, Santucci KL, Robilotto AT, Van Buskirk RG, Baust JM. Cryoablation: physical and molecular basis with putative immunological consequences. *Int J Hyperthermia* (2019) 36:10–6. doi: 10.1080/02656736.2019.1647355

48. Weber SM, Lee FTJr., Chinn DO, Warner T, Chosy SG, Mahvi DM. Perivascular and intralesional tissue necrosis after hepatic cryoablation: results in a porcine model. *Surgery* (1997) 122:742–7. doi: 10.1016/S0039-6060(97)90082-9

49. Baust JG, Gage AA. The molecular basis of cryosurgery. *BJU Int* (2005) 95:1187–91. doi: 10.1111/j.1464-410X.2005.05502.x

50. Mazur P. Freezing of living cells: mechanisms and implications. *Am J Physiology-Cell Physiol* (1984) 247:C125–42. doi: 10.1152/ajpcell.1984.247.3.C125

51. Muldrew K, McGann LE. The osmotic rupture hypothesis of intracellular freezing injury. *Biophys J* (1994) 66:532–41. doi: 10.1016/S0006-3495(94)80806-9

52. Prohaska J, Jan AH. Cryotherapy. In: *StatPearls*. Treasure Island (FL: , StatPearls Publishing LLC. (2022). StatPearls Publishing Copyright © 2022.

53. Woon WW, Haghighi KS, Zuckerman RS, Morris DL. Liver resection and cryotherapy for metastatic melanoma. *Int Surg* (2008) 93:274–7.

54. Parker W, Sunna R. Endobronchial malignant melanoma debrided by cryotherapy. *Chest* (2013) 144:20A. doi: 10.1378/chest.1703075

55. Aarts BM, Klompenhouwer EG, Rice SL, Imani F, Baetens T, Bex A, et al. Cryoablation and immunotherapy: an overview of evidence on its synergy. *Insights into Imaging* (2019) 10(1):53. doi: 10.1186/s13244-019-0727-5

56. Yakkala C, Chiang CL, Kandalaft L, Denys A , Duran R. Cryoablation and immunotherapy: An enthralling synergy to confront the tumors. *Front Immunol* (2019) 10:2283. doi: 10.3389/fimmu.2019.02283

57. Yakkala C, Dagher J, Sempoux C, Chiang CL, Denys A , Kandalaft LE, et al. Rate of freeze impacts the survival and immune responses post cryoablation of melanoma. *Front Immunol* (2021) 12:695150. doi: 10.3389/fimmu.2021.695150

58. Abdo J, Cornell DL, Mittal SK, Agrawal DK. Immunotherapy plus cryotherapy: Potential augmented abscopal effect for advanced cancers. *Front Oncol* (2018) 8:85. doi: 10.3389/fonc.2018.00085

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 14 March 2023
DOI 10.3389/fonc.2023.1032300

OPEN ACCESS

EDITED BY
Sapna Patel,
University of Texas MD Anderson Cancer Center, United States

REVIEWED BY
Enrica Teresa Tanda,
San Martino Hospital (IRCCS), Italy
Juan Esteban Garcia-Robledo,
Mayo Clinic Arizona, United States
José Manuel Lopes,
University of Porto, Portugal

*CORRESPONDENCE
Pamela Salman
pvsalman@gmail.com

SPECIALTY SECTION
This article was submitted to Skin Cancer, a section of the journal Frontiers in Oncology

RECEIVED 12 October 2022
ACCEPTED 27 February 2023
PUBLISHED 14 March 2023

CITATION
Salman P, de Melo AC, Rico-Restrepo M, Rodriguez J, Russi A, Schmerling RA, Zambrano A and Cinat G (2023) Addressing the unmet needs of patients with BRAF-mutated melanoma in Latin America: Expert perspective.
Front. Oncol. 13:1032300.
doi: 10.3389/fonc.2023.1032300

Addressing the unmet needs of patients with BRAF-mutated melanoma in Latin America: Expert perspective

Pamela Salman[1]*, Andreia Cristina de Melo[2], Mariana Rico-Restrepo[3], Jeronimo Rodriguez[4], Andrea Russi[5], Rafael Aron Schmerling[6], Angela Zambrano[7] and Gabriela Cinat[8]

[1]Oncology Department, Oncovida Cancer Center, Santiago, Chile, [2]Brazilian National Cancer Institute, Grupo Oncoclínicas, Rio de Janeiro, Brazil, [3]Public Health Department, Americas Health Foundation, Bogota, Colombia, [4]Centro Oncológico, Hospital Médica Sur, Mexico City, Mexico, [5]Departamento de Oncología, Hospital Universitario San Ignacio, Centro Javeriano de Oncología, Bogotá, Colombia, [6]Hospital do Coracao (HCor), Oncology Department, São Paulo, SP, Brazil, [7]Departamento de Oncología, Fundación Valle del Lili, Cali, Colombia, [8]Instituto de Oncología Ángel Roffo, Universidad de Buenos Aires, Fundación CIDEA, Buenos Aires, Argentina

Melanoma represents an increasing public health burden with extensive unmet needs in Latin America (LA). A mutation in the *BRAF* gene is present in approximately 50% of all melanomas in White populations and is a target of precision medicine, with the potential to dramatically improve patient outcomes. Thus, increased access to *BRAF* testing and therapy is LA must be explored. At a multi-day conference, a panel of Latin American experts in oncology and dermatology were provided with questions to address the barriers limiting access to testing for *BRAF* mutation in patients with melanoma in LA, who may be eligible for targeted therapy to improve their prognosis. During the conference, responses were discussed and edited until a consensus on addressing the barriers was achieved. Identified challenges included ignorance of *BRAF*-status implications, limited human and infrastructural resources, affordability and reimbursement, fragmented care delivery, pitfalls in the sample journey, and lack of local data. Despite the clear benefits of targeted therapies for *BRAF*-mutated melanoma in other regions, there is no clear path to prepare LA for a sustainable personalized medicine approach to this disease. Due to melanoma's time-sensitive nature, LA must aim to provide early access to *BRAF* testing and consider mutational status within treatment decision making. To this end, recommendations are provided and include establishing multidisciplinary teams and melanoma referral centers and improving access to diagnosis and treatment.

KEYWORDS

*BRAF-**mutated melanoma, Latin America, melanoma, V600E, access, health policy***

Introduction

Melanoma represents an increasing public health burden with extensive unmet needs in Latin America (LA). Globally, melanoma incidence has risen and accounts for most skin cancer-related mortality (1), with 324,635 new cases in 2020 and 57,000 deaths worldwide (2) Moreover, studies report that patients with skin melanoma in low-and-middle-income countries, such as those in LA, are more likely to present with advanced disease and have poorer survival when compared to high-income countries (3–5).

Nevertheless, the prognosis of patients with advanced melanoma has dramatically improved in recent years. Before immunotherapy and targeted therapy emerged, the average five-year survival for patients with stage IV melanoma was 2·3%, and the median survival was eight to ten months (6). More recently, the advent of precision medicine has leveraged the increased understanding of tumor biology and the immune system's role in developing personalized cancer therapies. One target of precision medicine is the BRAF protein, encoded by *BRAF*.

BRAF is a potent oncogene, present in approximately 50% of all melanomas in the White population, that plays a critical role in the Ras-Raf-mitogen-activated protein kinase/extracellular signal-related kinase (MEK) cell-signaling pathway (7, 8). People with melanoma who possess *BRAF* gene variants exhibit distinctive clinical features, with particularly aggressive biological behavior; patients are often younger and have tumors in areas without chronic sun exposure, with superficial spreading or nodular histology, and have an increased nevus count (9, 10). Additionally, *BRAF*-mutated tumors are more likely to metastasize to the brain (11, 12).

Data regarding melanoma mutations primarily comes from high-income countries (HIC), likely due to greater access to testing. Unlike in Europe and the US (13), data on melanoma incidence in LA is scarce. The available reports are mainly based on hospital records or private institutions that do not represent the general population, likely leading to underestimating the burden of this disease in the region (3, 14, 15). Additionally, the available data regarding mutational status has not been collected prospectively, thus having limited accuracy. Therefore, increased epidemiologic and data collection efforts are necessary to characterize the different populations and perform improved clinicopathologic correlation studies.

Melanoma epidemiology and *BRAF*-mutation frequency are heterogeneous and affected by ethnicity. For instance, a meta-analysis comparing the incidence rates of Asians and Whites found 19.5% and 40.3%, respectively (9). *BRAF*-mutation prevalence in LA has been found to be lower than in predominantly White populations. This is potentially a result of higher proportions of indigenous heritage and higher rates of acral-lentiginous melanoma in LA. However, ethnic variations among and within countries in LA are wide (9), and prior genomic knowledge of country-specific populations key to mutation screenings is largely lacking.[7]

Still, a few studies have documented *BRAF*-related melanoma in LA. In a single-institution cohort of 459 patients with melanoma in Barretos, Brazil, 34% carried a *BRAF* mutation (16). Another cohort showed V600 *BRAF* mutations in around 40% of cases (17, 18). However, this data is from a private institution and may not represent the general population (17–19). In Argentina, *BRAF* V600 mutation was reported in 50·2% of 354 patients (20).. Most of this sample is from patients from either the city or province of Buenos Aires, where most residents are White. In Mexico, studies identified *BRAF* V600E variant frequencies in primary melanoma studies ranging from 6·4% (3/47 patients in Mexico City) (21) to 73·0% (24/33 in Northeast Mexico) (22). In Chile, there is a complete lack of epidemiologic data.

Patients with melanoma with *BRAF* V600E and V600K mutations respond to clinically available BRAF inhibitors. Targeted treatment for patients with *BRAF* melanoma has reversed the poor prognosis associated with this molecular alteration (10). The success of targeted therapies in *BRAF*-mutated melanoma has led to the recommendation that patients with advanced disease and at high relapse risk be screened for V600 mutations to help guide therapeutic decision making (23).

Despite these advances, unmet needs remain, especially in regions such as LA, where determining mutational status and access to timely diagnosis and treatment are challenging. This review discusses the unmet needs of patients with *BRAF*-mutated melanoma in LA, including molecular testing strategies for detecting the mutation and their appropriate use within the regional context. The content is from the literature and panelists' experience and opinion. The challenges to providing adequate and effective diagnosis and treatment for patients with *BRAF*-mutated melanoma are discussed, and recommendations on overcoming these barriers will be provided.

Methods

Study design and panelists

Americas Health Foundation (AHF) identified seven experts in oncology and dermatology from Argentina, Brazil, Chile, Colombia, and Mexico who have published in *BRAF*-mutated, melanoma, or health economics since 2016. As it was not practical to gather panelists from all the countries in Latin America together for a conference, the panel was chosen to provide a perspective of oncologists and dermatologists from countries across Latin America. The panel convened for a three-day virtual meeting on October 26-29, 2021, to discuss the need for region-specific recommendations. To identify the panel, AHF conducted a literature review to identify scientists and clinicians from the above countries who have publications relating to *BRAF*-mutated melanoma since 2016. Augmenting this search, AHF contacted opinion leaders from LA's medical field to corroborate the list of individuals who adequately represented the necessary fields of study. All the experts who attended the meeting are named authors of this paper. An AHF staff member moderated the discussion. The authors retain complete control over the content of the paper.

Search strategy AHF conducted a literature review using PubMed, MEDLINE, and EMBASE. The following search terms

were used: "BRAF," "melanoma treatment," and "cancer," in combination with "Latin America," "Mexico," "Colombia," "Argentina," "Brazil," and "Chile," "molecular testing," from 01/01/2016 until 04/10/2021. The articles identified were in English, Portuguese, and Spanish. Particular attention was paid to identifying literature and research in LA.

AHF developed specific questions to address barriers limiting access to testing *BRAF* variants in LA and assigned one to each panel member (Supplementary Table 1). A written response to each question was drafted by individual panel members based on the literature review and personal expertise. Each narrative was reviewed and edited by the entire panel during the three-day conference through numerous rounds of discussion until a complete agreement was reached. For issues where there was disagreement among the panel, additional dialogues took place until all panel members agreed to the content included in this paper. The recommendations developed were based on the evidence gathered, expert opinion, and personal experience and were approved by the entire panel. After the conference, the final manuscript was distributed by email to the panel for review and approval.

Role of the funding source

This manuscript was supported by an unrestricted grant given to AHF, a 501(c)3 nonprofit organization dedicated to improving health care throughout LA, by Novartis. The funder had no role in the study design, data collection, data analysis, interpretation, and writing of the report.

BRAF mutation testing

Patient selection and timing

International clinical practice guidelines (CPG) suggest that *BRAF* mutation testing be mandatory in patients with stage III or stage IV melanoma and high-risk resected disease (24). When metastases occur, it is recommended to use the metastatic sample. If it is unavailable, the analyses may be performed on lymph node metastases or the primary tumor, as there is a high degree of concordance between the *BRAF* status of primary melanomas and their metastatic lesions (25).

In the appropriate clinical context, initiating reflex testing at an earlier stage (IIB, IIC) for patients with limited access to frequent visits and specialist care can prevent unnecessary delays in targeted BRAF/MEK inhibitor therapy (26). They may prove to have a similar benefit for *BRAF*-mutated melanoma, as earlier treatment initiation may improve clinical outcomes for patients.

Testing methods

Different methods for *BRAF* testing exist and can be considered based on their utility in screening, confirmatory, and reference testing (27). Testing decisions often depend upon available methods and infrastructure, specificity and sensitivity, and variable cost and access throughout the region (Table 1) (28). Primary cutaneous melanomas, metastatic lymph nodes, or radiologically detected lesions are fixed with formaldehyde and paraffin-embedded in blocks that can be used for testing (29). Essential to accurate molecular testing, the tissue sample journey is complicated and involves prefixation, fixation, and post-fixation processes. The specific procedures of each step must be optimized to achieve preservation and ensure a high-quality sample.

Tumor heterogeneity in advanced-stage melanoma must be considered as it may have implications for molecular testing and, thus, treatment (30). To mitigate the risk of misinterpreting *BRAF* mutational status due to intratumor heterogeneity, testing should always be conducted on metastatic lesions when available (31, 32). Sequential analysis using confirmatory methods for detecting *BRAF* mutations is usually performed in high income countries (HIC). Nevertheless, this approach is not always feasible or cost-effective in limited-resource contexts. Ideally, confirmatory testing with real-time polymerase chain reaction (PCR), Sanger sequencing, or Next-Generation Sequencing (NGS) should be conducted in all patients with a negative test result through immunohistochemistry (IHC). Alternatively, a blood sample may be assayed for circulating tumor DNA, but its lower sensitivity compared with a tissue-based biopsy must be considered (33). Figure 1 proposes a suggested pathway for *BRAF* testing in limited-resource settings.

Immunohistochemistry (IHC)

IHC is relatively simple and inexpensive, providing rapid results. *BRAF* V600E is the only variant that can be detected through this method (26, 34). Of note, IHC is not the best method regarding sensitivity: mutations may not be detected in some patients (31). However, employing IHC can be a cost-effective tool and a valuable supplement to conventional mutation testing to allow patients with V600E-variant metastatic melanoma to be triaged rapidly into appropriate treatment pathways (31, 32).

Real-time PCR-based techniques

Various real-time PCR-based methods are available, including Cobas 4800 and THxID-BRAF. Although these methods offer the advantages of a relatively quick turnaround time, they do not allow direct identification of the specific nucleotide sequence. These are commercially available companion diagnostic kits, each targeted to identify mainly V600E and V600K mutations (8).

Next-generation sequencing (NGS)

Despite NGS time, cost, and data-analysis demands, it can provide robust data on all *BRAF* and other actionable variants and quantify allele frequency. High specificity and sensitivity can be achieved with a small proportion of tumor DNA from the tissue sample. Its role is especially prominent in research, large mutation analysis, clinical trials, therapeutics, and confirmatory testing.

Sanger sequencing

Sanger sequencing, long known as the gold standard for reference testing in somatic variants, is 100% specific, providing quality sample preparation (8). It primarily serves as a confirmatory test in the case of inconclusive PCR methods, given its relatively higher costs and turnaround time (35).

TABLE 1 Sensitivity, specificity, and use in LA clinical practice for common *BRAF* diagnostic techniques.

Diagnostic Technique	Sensitivity (%)	Specificity (%)	Limit of Detection (%)	Use in clinical practice
IHC	93-97	92-98	5	🇦🇷 🇧🇷 🇨🇱 🇨🇴 * 🇲🇽 *
RT-PCR *(Cobas 4800 BRAF p.V600 and THxID -BRAF)*	98-100	98-100	0.5-5	🇦🇷 🇧🇷 🇨🇱 🇨🇴 🇲🇽
NGS	97.5	100	5	🇦🇷 * 🇧🇷 * 🇨🇱 * 🇨🇴 * 🇲🇽 *
Sanger sequencing	92-98	100	20-25	🇦🇷 * 🇧🇷 * 🇨🇱 * 🇨🇴 * 🇲🇽 *
Pyrosequencing	>98	90-100	5-10	Only for research purposes
dPCR	100	95	0.001	Only for research purposes
MALDI-TOF MS	97.5	100	1-5	Only for research purposes

IHC, immunohistochemistry; RT-PCR, reverse transcription polymerase chain reaction, NGS, next generation sequencing; dPCR, digital polymerase chain reaction; MALDI-TOF MS, Matrix-assisted laser desorption/ionization- time of flight mass spectrometry.
* Only available in select highly specialized institutions.

Other methods

Several other methods for *BRAF* testing that offer high sensitivity and specificity exist but are generally not available in LA outside of the research context, primarily due to high costs and technological requirements. These methods include pyrosequencing, matrix-assisted laser desorption ionization time of flight mass spectrometry, allele-specific PCR, and droplet digital PCR.

Systemic treatment for *BRAF*-mutated melanoma

Targeted therapy defied the conventional thinking in the United States, Europe, and Australia that patients in poor clinical condition due to advancing disease should not be treated. The uptake of these therapies throughout LA has been slower than in other regions due to access limitations, infrastructure issues, and cost constraints. Nevertheless, healthcare systems in the region could benefit from increasing the use of a precision medicine approach regarding patient outcomes, cost-effectiveness, and resource allocation.

Despite the fact that targeted therapy and immunotherapy have been highly effective in treating advanced *BRAF*-mutated melanoma (36–38), data comparing the two treatments revealed that targeted therapy has a greater response relative to immunotherapy. In contrast, immunotherapy confers longer-lasting results (39). Therefore, disease features, safety profiles, medical history, patient preferences, and access must be considered when making treatment decisions.

The first BRAF inhibitor developed, vemurafenib, surprised the oncological community with its Phase-I trial results showing rapid and profound responses that had never been seen in melanoma, though short-lived (40). The combination of MEK and BRAF inhibitors improved response rates, progression-free survival, and overall survival (OS) compared to monotherapy with BRAF inhibitors, as demonstrated with dabrafenib + trametinib (38); vemurafenib + cobimetinib (36); and encorafenib + binimetinib (37). For these three therapies, five-year progression-free and OS results were almost 20% and 35%, respectively. Some advanced

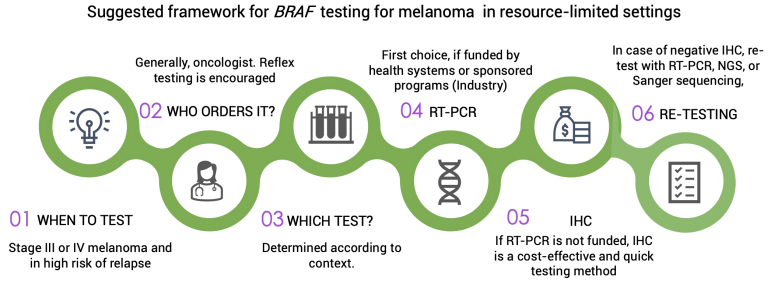


FIGURE 1
IHC, immunohistochemistry; RT-PCR, reverse transcription polymerase chain reaction; NGS, next generation sequencing.

melanoma characteristics associated with long-term responses to these drugs are normal lactate dehydrogenase (LDH) levels, less than three metastatic sites, and a good Eastern Cooperative Oncology Group performance status (38). These clinical features may help select patients with *BRAF*-mutated melanoma that would benefit most from targeted therapy in the long term. Patients with a high tumor burden, rapid progression, or poor performance status represent an unmet need for currently available drugs; reasonable access to target therapy for these patients is vital because of the rapidly progressing disease.

Combination therapy with immunotherapy (nivolumab/ipilimumab) followed by target therapy (dabrafenib/trametinib) is emerging as an option that may yield greater overall survival in patients with *BRAFV600*-mutated advanced melanoma (38, 39, 41, 42). The CheckMate 067 is a phase III trial which randomized previously untreated unresectable stage III or stage IV melanoma patients to receive nivolumab plus ipilimumab (four doses) followed by nivolumab; or nivolumab alone; or ipilimumab alone. The 6.5-year overall survival rates were respectively 57%, 43%, and 25% in patients with *BRAF*-mutant tumors and 46%, 42%, and 22% in those with *BRAF*-wild-type tumors, and the median overall survival is the longest in a phase III melanoma trial reported to date (43).

Few prospective data are available on sequential immunotherapy and BRAF/MEK inhibition for *BRAF*-mutant metastatic melanoma. The SECOMBIT is a noncomparative phase II trial which randomized patients with untreated, metastatic *BRAF*-mutant melanoma to receive A) encorafenib plus binimetinib until progressive disease followed by ipilimumab plus nivolumab; or B) ipilimumab plus nivolumab until progressive disease followed by encorafenib plus binimetinib; or C) encorafenib plus binimetinib for 8 weeks followed by ipilimumab plus nivolumab until progressive disease followed by encorafenib plus binimetinib. At a median follow-up of 32.2 months, the median overall survival was not reached in any arm. However, the 2 and 3-year OS rates showed that sequential immunotherapy and targeted therapy provide clinically meaningful survival benefits (44).

Access to BRAF testing and treatment in LA

Although the proportion of patients with a melanoma diagnosis that undergoes *BRAF*-mutation testing in LA is unknown, it is likely lower than that of HIC due to restricted access. Access to diagnostic methods and treatments varies widely among and within countries in LA. Moreover, vast inequities exist in access between the regional private and public healthcare systems. Molecular testing demands an infrastructure comprising technological, financial, and human resources, which few institutions in LA possess (45). The complex technologies and processes are mostly only available in highly specialized centers, usually concentrated in major cities, leaving large populations underserved. In general, public hospitals do not offer this testing.

In countries in LA where targeted therapies are approved, *BRAF* testing is sometimes provided cost-free by the pharmaceutical industry, making it available to a large portion of the population for as long as the sponsored programs exist (45). Although this offers a short-term solution to access, it is not without problems. Logistical issues arise in the sample-handling journey, often resulting in diagnostic and treatment initiation delays. Further, these programs displace the government's responsibility to provide reimbursement for testing, which is an undesirable situation.

Ideally, access to *BRAF* testing must be accompanied by access to targeted therapies. This is often not the case in LA, mainly due to economic constraints. Although at least one targeted therapy for *BRAF*-mutated melanoma is approved by regulatory agencies and available in many countries in LA, including Argentina, Brazil, Chile, Colombia, Mexico, and Uruguay, a lack of reimbursement, particularly within the public healthcare systems, continues to limit drug access (46). Of note, since advanced melanoma is a time-sensitive malignancy, access to both testing and therapy must be prompt.

Discussion

Challenges to *BRAF*-mutated melanoma management in LA

A precision medicine approach to *BRAF*-mutated melanoma has demonstrated potential to improve health outcomes; however, factors inherent to precision medicine and LA's healthcare systems create significant implementation obstacles. Given molecular pathology's technical considerations and complexities, achieving precision medicine's potential requires overcoming these hurdles. The barriers to quality management of *BRAF*-mutated melanoma in LA throughout the patient journey are depicted in Figure 2.

Affordability/Reimbursement

Precision medicine approaches demand high up-front investments in infrastructure, personnel training, and funding for molecular testing and targeted therapies. Although access to testing methods is not uncommon in the region, the corresponding targeted therapies are not always reimbursed or available. Additionally, anti-cancer drugs are proportionally much more expensive in LA when compared with higher income regions (47) because drugs are acquired with weaker currencies, and procurement strategies are not optimized. In the authors'

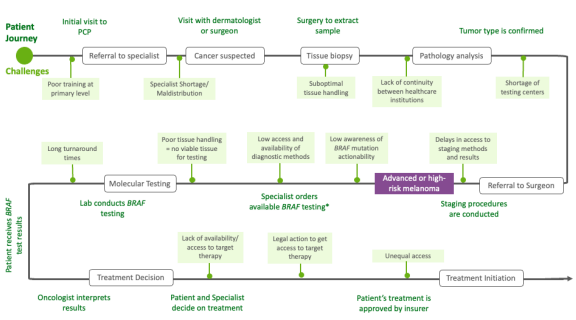


FIGURE 2
Patient journey of people with BRAF melanoma in Latin America. PCP, primary care physician.

experience, the extent to which financing for testing limits access varies from not being an issue to affecting 75% of the time. The majority believe that financing for therapy is a limiting factor 25% of the time, although some report it is a factor 50-75% of the time.

Limited infrastructure

Healthcare centers with the capacity to provide melanoma care in LA are disproportionately located in major cities, leaving large areas underserved with unequal access levels. Even in major cities, pathology laboratories that meet the infrastructural demands of molecular tests used to diagnose *BRAF* mutations are scarce, resulting in long turnaround times for testing or unreliable test results.

Fragmented care

From diagnosis to treatment, melanoma care in LA is generally fragmented, a stark contrast to the current standard of care that involves a multidisciplinary team approach. Delays occur at virtually every step of the patient journey due to miscoordinations in care efforts at the primary level, during the diagnostic phase, and in treatment decision making. Tumor boards for melanoma, which can aid in overcoming diagnostic and management barriers, are not widely implemented.

Lack of human resources

There is a generalized shortage of medical personnel involved in melanoma care, including dermatologists, clinical oncologists, and pathologists, leading to high workloads and delays in diagnosis and treatment. This shortage may be related to the relatively small number of training opportunities for residencies and fellowships in these specialties within the region. Furthermore, a lack of resources in smaller cities or rural areas disincentivizes specialists from practicing in these areas, creating severe access gaps due to geographic resource maldistribution (48). At the primary care level, medical personnel lack training in identifying suspicious lesions and appropriate referral, which may also result in more advanced stages at diagnosis.

Lack of awareness of *BRAF*-status implications

Despite *BRAF* testing for advanced melanoma being compulsory based on unanimous international CPG, a generalized lack of awareness among stakeholders involved in melanoma decision making exists. Treating physicians do not always adhere to CPG, and similar treatment approaches are often taken for all or most patients with melanoma in LA, without regard to mutational status. Likewise, government, regulatory agencies, and payers do not always make evidence-based decisions to approve and reimburse targeted therapies and their corresponding diagnostic methods.

Pitfalls in the sample journey/quality assurance

Adequate institutional protocols and quality-control standards to regulate sample preparation are not standard across laboratories (49). Suboptimal practices within the tissue sample journey, including insufficient sample quantities, create technical challenges to *BRAF* testing. Because of limited infrastructural resources, there is a lack of continuity across the different healthcare institutions which the sample must pass in its journey, causing further delays.

Lack of local data

Region- and country-specific data to characterize the *BRAF*-variation prevalence in each population, enable accurate treatment decisions, and guide public policy are severely deficient. Global data corroborate the positive impact of introducing precision medicine strategies for melanoma treatment; however, the lack of local data hinders advancing this approach in the region. It is also necessary to define adequate metrics and indicators to track patient outcomes and determine the cost-effectiveness of targeted therapies for melanoma. Most decisions on healthcare resource allocation in LA are primarily based on cost. Yet coordinated (international) efforts to negotiate more accessible prices are not initiated by the governing bodies.

Recommendations

This panel has addressed the lack of access to diagnosis and treatment for *BRAF*-mutated melanoma. With increasing health care costs and limited resources, a critical need exists to understand the root causes of these technologies' underuse in the population for which they were developed. Additionally, efforts to increase access should be a collaborative, multi-stakeholder endeavor. The recommendations below address the challenges to widespread access to these diagnostic tools and, as a result, adequate treatments. Because these access issues are not exclusive to this region or this cancer, these recommendations may be tailored on a country-by-country and cancer-by-cancer basis.

1. **Improve affordability and reimbursement**

   Governments must work toward achieving a sustainable approach to sourcing high-cost cancer diagnostic methods and therapies as a region by:

   - Implementing procurement and contracting strategies such as managed entry agreements or risk-sharing strategies (50).
   - Leveraging negotiation power using pooled procurement, several countries can unite as a single buying bloc by combining their resources and requesting tests and doses.
   - Improving the coherence of approval and reimbursement for both pieces of the companion diagnostic (i.e., testing and therapy). Ideally, these regulatory pathways should provide an aligned channel for co-developed products to ensure innovation is not stifled.

2. **Improve testing infrastructure**

   Stakeholders must develop high-quality laboratories that can perform the molecular testing required for *BRAF* detection by:

- Increasing investment in pathology departments and laboratories to meet the technological and training demands of high-quality molecular testing.
- Establishing adequate quality-control standards, accreditation programs, and institutional protocols that regulate sample preparation and the quality of *BRAF* testing (51).
- Creating centralized laboratories to perform the tests throughout the countries may help optimize turnaround times and save costs.

3. **Establish multidisciplinary teams and melanoma referral centers**

   Healthcare institutions should provide a *multidisciplinary approach* to melanoma management by:

   - Establishing multidisciplinary teams that include primary care physicians, dermatologists, clinical oncologists, surgeons, pathologists, geneticists, radiation oncologists, palliative care doctors, oncology nurses, and social workers.
   - Providing oncology navigators to guide and support patients through the medical and administrative process complexities related to cancer care.
   - Increasing referral centers for skin cancer care to promote care continuity and reduce delays (52–55).

4. **Raise awareness of melanoma and the actionability of *BRAF* variants**

   Medical societies and healthcare professionals must engage in continuous medical education at every level of care on the importance of determining *BRAF* mutational status.

   - Primary care physicians must be trained to recognize suspicious lesions and understand appropriate referral situations.
   - Specialists must understand the importance of determining *BRAF* mutations and facilitate diagnostic testing. Leveraging the concept of reflex testing may help reduce diagnostic delays.
   - Pathologists and molecular biologists must be adequately trained to conduct and interpret tests reliably and accurately.

5. **Increase BRAF testing**

   When indicated, healthcare professionals, medical societies, government, and patient organizations should promote testing for *BRAF* mutations.

   - All patients with metastatic or unresectable melanoma or those at high risk of relapse should be screened for *BRAF* V600 mutations, preferably in a metastatic lesion, and for adjuvant therapy in the primary tumor to guide therapeutic decision making (56).

6. **Address shortage and maldistribution of specialists**

   Governments, medical societies, and academic institutions must address the shortage and maldistribution of specialists by:

   - Increasing training opportunities in residency and fellowship programs in dermatology, surgical and clinical oncology, and pathology to address the lack of specialists in these fields.
   - Implementing virtual tumor boards or second opinion networks to bridge the gaps created by geographical disparities.

7. **Increase data collection and outcomes tracking**

   All stakeholders must prioritize funding for melanoma research to:

   - Establish national disease-specific registries to generate local data on which to base health policies tailored to the national context (50).
   - Define and quantify quality metrics, including indicators for access to diagnostic tools and therapies, to monitor patient outcomes and the impact of precision medicine strategies.

Conclusion

There are wide opportunities in LA to improve the landscape of melanoma prevention, early detection, characterization, and management. Strategies to bolster primary and secondary prevention of skin melanomas must be prioritized as essential steps to improving the OS of patients with skin melanoma and also considering the economic implications this may have for health systems by avoiding disease evolution to more complex stages (57–60). That said, healthcare systems in LA must be better equipped to adapt to the complexities of the advanced stages of melanoma. Delaying diagnosis and treatment initiation negatively impacts progression-free survival and OS. In HIC, broad access to effective therapy for advanced disease has led to reductions in mortality. Similar results can be expected in LA if access is improved (55, 61). Despite the clear outcome benefits from targeted therapies for *BRAF*-mutated melanoma in other regions, there is no clear path to prepare LA for a sustainable personalized medicine approach to this disease. Due to melanoma's time-sensitive nature, LA countries must provide early access to *BRAF* testing in appropriate situations and for mutational status to be considered within treatment decision making.

Author contributions

PS and AM Writing-original draft, investigation, formal analysis, validation. MR-R Writing-review and editing, visualization, conceptualization, methodology, project administration. JR, AR, RS, AZ, and GC Writing-original draft, investigation, formal analysis, validation. All authors contributed to the article and approved the submitted version.

Funding

This manuscript was supported by an unrestricted grant given to AHF, a 501(c)3 nonprofit organization dedicated to improving health care throughout LA, by Novartis. The funder had no role in the study design, data collection, analysis, interpretation, and report writing.

Conflict of interest

GC received: grants or had contracts from Novartis, Merck Sharp & Dohme, Pfizer, Roche/Genentech, Bristol Myers Squibb, Array, Springer, Grupo Español de Melanoma; consulting fees from Merck Sharp & Dohme, Novartis, Bristol Myers Squibb, Roche/Genentech; honoraria for presentations from Novartis, Merck Sharp & Dohme, Pfizer, Roche, Bristol Myers Squibb, Array, Springer, and Grupo Español de Melanoma; support for attending meetings from Novartis, Merck Sharp & Dohme, Bristol Myers Squibb, Roche; payment for board participation from Novartis, Merck Sharp & Dohme, Bristol Myers Squibb, Roche/Genentech. AM's institution received grants from Clovis Oncology, Regeneron, Merck Sharp & Dohme, Bristol Myers Squibb, GlaxoSmithKline, AstraZeneca, Novartis, Amgen, and Roche for clinical trials. AM received payment for lectures from Merck Sharp & Dohme, Bristol Myers Squibb, GlaxoSmithKline, AstraZeneca, Novartis, Roche, Sanofi. Advisory Board participation from Merck Sharp & Dohme, Bristol Myers Squibb, GlaxoSmithKline, AstraZeneca, Novartis, Roche. RS received: consulting fees from Merck Sharp & Dohme and L'Oreal; payment for presentations from Merck Sharp & Dohme, Bristol Myers Squibb, Sanofi Genzyme, Pfizer, Merck Serono, Novartis; support from Bristol Myers Squibb for expert testimony; support for attending meetings from Sanofi Genzyme and Merck Sharp & Dohme.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1032300/full#supplementary-material

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* (2017) 67(1):7–30. doi: 10.3322/caac.21387

2. *Melanoma of skin*. International Agency for Research on Cancer (2121). Available at: https://gco.iarc.fr/today/data/factsheets/cancers/16-Melanoma-of-skin-fact-sheet.pdf.

3. de Vries E, Sierra M, Piñeros M, Loria D, Forman D. The burden of cutaneous melanoma and status of preventive measures in central and south America. *Cancer Epidemiol* (2016) 44:S100–S9. doi: 10.1016/j.canep.2016.02.005

4. Schmerling RA, Loria D, Cinat G, Ramos WE, Cardona AF, Sánchez JL, et al. Cutaneous melanoma in Latin America: The need for more data. *Rev Panam Salud Publica* (2011) 30(5):431–8. doi: 10.1590/S1020-49892011001100005

5. Sortino-Rachou AM, Curado MP, de Camargo Cancela M. Cutaneous melanoma in Latin America: A population-based descriptive study. *Cad Saúde Pública* (2011) 27(3). doi: 10.1590/S0102-311X2011000300016

6. Korn EL, Liu PY, Lee SJ, Chapman JA, Niedzwiecki D, Suman VJ, et al. Meta-analysis of phase II cooperative group trials in metastatic stage IV melanoma to determine progression-free and overall survival benchmarks for future phase II trials. *J Clin Oncol* (2008) 26(4):527–34. doi: 10.1200/JCO.2007.12.7837

7. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. *Nature* (2002) 417(6892):949–54. doi: 10.1038/nature00766

8. Vanni I, Tanda ET, Spagnolo F, Andreotti V, Bruno W, Ghiorzo P. The current state of molecular testing in the BRAF-mutated melanoma landscape. *Front Mol Biosci* (2020) 7. doi: 10.3389/fmolb.2020.00113

9. Kim S, Kim S, Hahn H, Lee Y, Choe Y, Ahn K. Metaanalysis of BRAF mutations and clinicopathologic characteristics in primary melanoma. *J Am Acad Dermatol* (2015) 72(6):1036–46.e2. doi: 10.1016/j.jaad.2015.02.1113

10. Long GV, Menzies AM, Nagrial AM, Haydu LE, Hamilton AL, Mann GJ, et al. Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. *J Clin Oncol* (2011) 29(10):1239–46. doi: 10.1200/JCO.2010.32.4327

11. O'Shea PJ, Tatineni V, Rauf Y, Jia X, Murphy ES, Chao ST, et al. Outcomes of BRAF mutated vs. wild type tumors in melanoma brain metastasis. *Int J Radiat Oncol Biol Phys* (2021) 111(3, Supplement):e576. doi: 10.1016/j.ijrobp.2021.07.1551

12. Venur VA, Kotecha R, Chen Z, Chao ST, Elson P, Suh JH, et al. Impact of BRAF mutation in patients with brain metastasis from melanoma. *J Clin Oncol* (2015) 33 (15_suppl):e13016-e. doi: 10.1200/jco.2015.33.15_suppl.e13016

13. Bauer J, Büttner P, Murali R, Okamoto I, Kolaitis NA, Landi MT, et al. BRAF mutations in cutaneous melanoma are independently associated with age, anatomic site of the primary tumor, and the degree of solar elastosis at the primary tumor site. *Pigment Cell Melanoma Res* (2011) 24(2):345–51. doi: 10.1111/j.1755-148X.2011.00837.x

14. Reyes E, Uribe C, de Vries E. Population-based incidence and melanoma-specific survival of cutaneous malignant melanoma in a Colombian population 2000–2009. *Int J Dermatol* (2018) 57(1):21–7. doi: 10.1111/ijd.13839

15. dos Santos CA, Souza DLB. Melanoma mortality in Brazil: Trends and projections (1998-2032). *Ciênc saúde Colet* (2019) 24(4). doi: 10.1590/1413-81232018244.13932017

16. da Costa LMM, Crovador CDS, de Carvalho CEB, Vazquez VDL. Characteristics of Brazilian melanomas: Real-world results before and after the introduction of new therapies. *BMC Res Notes* (2019) 12(1):296. doi: 10.1186/s13104-019-4336-7

17. Fouto Matias JE, Jung JE, Böer A, Bresch M, Falk TM. BRAF mutations in cutaneous melanoma: No correlation with histological prognostic factors or overall survival. *Jornal Brasileiro Patologia e Medicina Laboratorial* (2010) 46(6):487–93. doi: 10.1590/S1676-24442010000600009

18. Carranza H, Cardona AF, Vargas CA, Archila P, Otero JM, Bernal L, et al. Genotyping melanoma in Colombia. *J Clin Oncol* (2014) 32(15_suppl):e20054-e. doi: 10.1200/jco.2014.32.15_suppl.e20054

19. Vicente A, Crovador CS, Macedo G, Scapulatempo-Neto C, Reis RM, Vazquez VL. Mutational profile of driver genes in Brazilian melanomas. *J Glob Oncol* (2019) 5:1–14. doi: 10.1200/JGO.19.00169

20. Prost D, llanos A, Bence P, Boixadera L, Krasnapolski M, Bilbao E, et al. Frequency of BRAF V600 mutation in 354 locallyadvanced or metastatic melanoma patients froman academic center in Argentina: The apple does not fall very far from the tree. *Pigment Cell Melanoma Res* (2017) 31:5. doi: 10.1016/j.jmoldx.2012.10.002

21. Zepeda-Lopez PD, Salas-Alanis JC, Toussaint-Caire S, Gutierrez-Mendoza D, Vega-Memije E, Silva SL, et al. BRAF mutation (V600E) prevalence in Mexican patients diagnosed with melanoma. *Case Rep Oncol* (2016) 9(1):241–5. doi: 10.1159/000445939

22. Fajardo Ramírez OR, Salas Alanis JC, Guzmán Huerta E, Martínez U, Barbosa A, Scott SP, et al. BRAF mutations among patients from the northeast of Mexico with malignant melanoma. *Clin Res J* (2014) 66(3):2.

23. Basurto-Lozada P, Molina-Aguilar C, Castaneda-Garcia C, Vázquez-Cruz ME, Garcia-Salinas OI, Álvarez-Cano A, et al. Acral lentiginous melanoma: Basic facts, biological characteristics and research perspectives of an understudied disease. *Pigment Cell Melanoma Res* (2021) 34(1):59–71. doi: 10.1111/pcmr.12885

24. Michielin O, van Akkooi ACJ, Ascierto PA, Dummer R, Keilholz U. Cutaneous melanoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up†. *Ann Oncol* (2019) 30(12):1884–901. doi: 10.1093/annonc/mdz411

25. Godoy-Gijón E, Yuste-Chaves M, Santos-Briz Á. BRAF mutation status concordance between primary cutaneous melanomas and corresponding metastases: A review of the latest evidence. *Actas Dermosifiliogr* (2017) 108(10):894–901. doi: 10.1016/j.ad.2016.12.025

26. Colomba E, Hélias-Rodzewicz Z, Von Deimling A, Marin C, Terrones N, Pechaud D, et al. Detection of BRAF p.V600E mutations in melanomas: Comparison of four methods argues for sequential use of immunohistochemistry and pyrosequencing. *J Mol Diagn* (2013) 15(1):94–100. doi: 10.1016/j.jmoldx.2012.09.001

27. *List of cleared or approved companion diagnostic devices*. US Food and Drug Administration (2021) Washington, D.C., USA. Available at: https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools.

28. Ihle MA, Fassunke J, König K, Grünewald I, Schlaak M, Kreuzberg N, et al. Comparison of high resolution melting analysis, pyrosequencing, next generation sequencing and immunohistochemistry to conventional Sanger sequencing for the detection of p.V600E and non-p.V600E BRAF mutations. *BMC Cancer* (2014) 14:13. doi: 10.1186/1471-2407-14-13

29. Cheng L, Lopez-Beltran A, Massari F, MacLennan GT, Montironi R. Molecular testing for BRAF mutations to inform melanoma treatment decisions: A move toward precision medicine. *Mod Pathol* (2018) 31(1):24–38. doi: 10.1038/modpathol.2017.104

30. Grzywa TM, Paskal W, Włodarski PK. Intratumor and intertumor heterogeneity in melanoma. *Transl Oncol* (2017) 10(6):956–75. doi: 10.1016/j.tranon.2017.09.007

31. Manfredi L, Meyer N, Tournier E, Grand D, Uro-Coste E, Rochaix P, et al. Highly concordant results between immunohistochemistry and molecular testing of mutated V600E BRAF in primary and metastatic melanoma. *Acta Derm Venereol* (2016) 96(5):630–4. doi: 10.2340/00015555-2326

32. Long GV, Wilmott JS, Capper D, Preusser M, Zhang YE, Thompson JF, et al. Immunohistochemistry is highly sensitive and specific for the detection of V600E BRAF mutation in melanoma. *Am J Surg Pathol* (2013) 37(1):61–5. doi: 10.1097/PAS.0b013e31826485c0

33. Santiago-Walker A, Gagnon R, Mazumdar J, Casey M, Long GV, Schadendorf D, et al. Correlation of BRAF mutation status in circulating-free DNA and tumor and association with clinical outcome across four BRAFi and MEKi clinical trials. *Clin Cancer Res* (2016) 22(3):567–74. doi: 10.1158/1078-0432.CCR-15-0321

34. Pearlstein MV, Zedek DC, Ollila DW, Treece A, Gulley ML, Groben PA, et al. Validation of the VE1 immunostain for the BRAF V600E mutation in melanoma. *J Cutan Pathol* (2014) 41(9):724–32. doi: 10.1111/cup.12364

35. McEvoy AC, Wood BA, Ardakani NM, Pereira MR, Pearce R, Cowell L, et al. Droplet digital PCR for mutation detection in formalin-fixed, paraffin-embedded melanoma tissues: A comparison with Sanger sequencing and pyrosequencing. *J Mol Diagn* (2018) 20(2):240–52. doi: 10.1016/j.jmoldx.2017.11.009

36. Ascierto PA, Dreno B, Larkin J, Ribas A, Liszkay G, Maio M, et al. 5-year outcomes with cobimetinib plus vemurafenib in BRAFV600 mutation–positive advanced melanoma: Extended follow-up of the coBRIM study. *Clin Cancer Res* (2021) 27:10. doi: 10.1158/1078-0432.CCR-21-0809

37. Dummer R, Flaherty K, Robert C, Arance AM, de Groot JW, Garbe C, et al. Five-year overall survival (OS) in COLUMBUS: A randomized phase 3 trial of encorafenib plus binimetinib versus vemurafenib or encorafenib in patients (pts) with BRAF V600-mutant melanoma. *J Clin Oncol* (2021) 39(15_suppl):9507. doi: 10.1200/JCO.2021.39.15_suppl.9507

38. Robert C, Grob JJ, Stroyakovskiy D, Karaszewska B, Hauschild A, Levchenko E, et al. Five-year outcomes with dabrafenib plus trametinib in metastatic melanoma. *N Engl J Med* (2019) 381(7):626–36. doi: 10.1056/NEJMoa1904059

39. Lelliott EJ, McArthur GA, Oliaro J, Sheppard KE. Immunomodulatory effects of BRAF, MEK, and CDK4/6 inhibitors: Implications for combining targeted therapy and immune checkpoint blockade for the treatment of melanoma. *Front Immunol* (2021) 12. doi: 10.3389/fimmu.2021.661737

40. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* (2010) 363(9):809–19. doi: 10.1056/NEJMoa1002011

41. National Library of Medicine (US). *Dabrafenib and trametinib followed by ipilimumab and nivolumab or ipilimumab and nivolumab followed by dabrafenib and trametinib in treating patients with stage III-IV BRAFV600 melanoma* (2022). Available at: https://www.clinicaltrials.gov/ct2/show/NCT02224781.

42. Schummer P, Schilling B, Gesierich A. Long-term outcomes in BRAF-mutated melanoma treated with combined targeted therapy or immune checkpoint blockade: Are we approaching a true cure? *Am J Clin Dermatol* (2020) 21(4):493–504. doi: 10.1007/s40257-020-00509-z

43. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Lao CD, et al. Long-term outcomes with nivolumab plus ipilimumab or nivolumab alone versus ipilimumab in patients with advanced melanoma. *J Clin Oncol* (2022) 40(2):127–37. doi: 10.1200/JCO.21.02229

44. Ascierto PA, Mandalà M, Ferrucci PF, Guidoboni M, Rutkowski P, Ferraresi V, et al. Sequencing of ipilimumab plus nivolumab and encorafenib plus binimetinib for untreated BRAF-mutated metastatic melanoma (SECOMBIT): A randomized, three-arm, open-label phase II trial. *J Clin Oncol*. 2022, 41(2):212-21. doi: 10.1200/JCO.21.02961

45. da Cunha IW, de Almeida Coudry R, de Macedo MP, de Assis EACP, Stefani S, Soares FA. A call to action: Molecular pathology in Brazil. *Surg Exp Pathol* (2021) 4(1):15. doi: 10.1186/s42047-021-00096-1

46. Valdes A. *Access to high-cost medicines in the americas*. Pan American Health Organization / World Health Organization (2021) Washington, D.C., USA. Available at: https://www.paho.org/hq/index.php?option=com_content&view=article&id=2149%3A2008-el-acceso-medicamentos-alto-costo-americas&catid=1266%3Amedicines-access-innovation&Itemid=1178&lang=en. PAHO/WHO.

47. Morel CM, McGuire A, Mossialos E. The level of income appears to have no consistent bearing on pharmaceutical prices across countries. *Health Aff (Millwood)* (2011) 30(8):1545–52. doi: 10.1377/hlthaff.2010.0317

48. Maceira D, Paraje G, Aramayo F, Masi SD, Sánchez YD. Financiamiento público de la investigación en salud en cinco países de américa latina. *Rev Panam Salud Publica* (2010) 27(6):10.

49. da Cunha IW, de Almeida Coudry R, de Macedo MP, de Assis EACP, Stefani SD, Soares FA. A call to action: Molecular pathology in Brazil. *Surg Exp Pathol* (2021) 4:1–27. doi: 10.1186/s42047-021-00096-1

50. Wouters MW, Michielin O, Bastiaannet E, Beishon M, Catalano O, Del Marmol V, et al. ECCO essential requirements for quality cancer care: Melanoma. *Crit Rev Oncol Hematol* (2018) 122:164–78. doi: 10.1016/j.critrevonc.2017.12.020

51. Volger S, Paris V, Panteli D. *Ensuring access to medicines: how to redesign pricing, reimbursement and procurement*. Copenhagen: WHO Regional Office for Europe (2018). Available at: https://www.euro.who.int/:data/assets/pdf_file/0009/379710/PolicyBrief_AUSTRIA_PB30_web_13082018.pdf.

52. Cornelius LA, Fields RC, Tarhini A. Multidisciplinary care of BRAF-mutant stage III melanoma: A physicians perspective review. *Oncologist* (2021) 26(9):e1644–e51. doi: 10.1002/onco.13852

53. Denton E, Conron M. Improving outcomes in lung cancer: The value of the multidisciplinary health care team. *J Multidiscip Healthc* (2016) 9:137–44. doi: 10.2147/JMDH.S76762

54. Kesson EM, Allardice GM, George WD, Burns HJ, Morrison DS. Effects of multidisciplinary team working on breast cancer survival: Retrospective, comparative, interventional cohort study of 13 722 women. *Bmj* (2012) 344:e2718. doi: 10.1136/bmj.e2718

55. Macdermid E, Hooton G, Macdonald MR, McKay G, Grose D, Mohammed N, et al. Improving patient survival with the colorectal cancer multi-disciplinary team. *Colorectal Dis* (2008) 11(3):4. doi: 10.1111/j.1463-1318.2008.01580.x

56. Dummer R, Hauschild A, Guggenheim M, Keilholz U, Pentheroudakis G. Cutaneous melanoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* (2012) 23(Suppl 7):vii86–91. doi: 10.1093/annonc/mds229

57. Perez M, Abisaad JA, Rojas KD, Marchetti MA, Jaimes N. Skin cancer: Primary, secondary, and tertiary prevention. part I. *J Am Acad Dermatol* (2022) 87(2):255–68. doi: 10.1016/j.jaad.2021.12.066

58. Rojas KD, Perez ME, Marchetti MA, Nichols AJ, Penedo FJ, Jaimes N. Skin cancer: Primary, secondary, and tertiary prevention. part II. *J Am Acad Dermatol* (2022) 87(2):271–88. doi: 10.1016/j.jaad.2022.01.053

59. Trager MH, Queen D, Samie FH, Carvajal RD, Bickers DR, Geskin LJ. Advances in prevention and surveillance of cutaneous malignancies. *Am J Med* (2020) 133(4):417–23. doi: 10.1016/j.amjmed.2019.10.008

60. Gordon L, Shih S, Watts C, Goldsbury D, Green A. The economics of skin cancer prevention with implications for Australia and new Zealand: Where are we now? *Public Health Res Pract*. 2022, 32(1):e31502119. doi: 10.17061/phrp31502119

61. Cornelissen JJ, van der Holt B, Verhoef GEG, van 't Veer MB, van Oers MHJ, Schouten HC, et al. Myeloablative allogeneic versus autologous stem cell transplantation in adult patients with acute lymphoblastic leukemia in first remission: A prospective sibling donor versus no-donor comparison. *Blood* (2009) 113(6):1375–82. doi: 10.1182/blood-2008-07-168625

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 16 June 2023
DOI 10.3389/fonc.2023.1075823

OPEN ACCESS

EDITED BY
Selma Ugurel,
Essen University Hospital, Germany

REVIEWED BY
Elizabeth Gaughan,
University of Virginia, United States
Rosario Le Moli,
University of Catania, Italy
Zhifang Zhai,
Army Medical University, China

*CORRESPONDENCE
Yana G. Najjar
najjaryg@upmc.edu

†These authors have contributed equally to this work

RECEIVED 21 October 2022
ACCEPTED 27 March 2023
PUBLISHED 16 June 2023

CITATION
Augustin RC, Huang Z, Ding F, Zhai S, McArdle J, Santisi A, Davis M, Sander C, Davar D, Kirkwood JM, Delgoffe GM, Warner AB and Najjar YG (2023) Metformin is associated with improved clinical outcomes in patients with melanoma: a retrospective, multi-institutional study. *Front. Oncol.* 13:1075823. doi: 10.3389/fonc.2023.1075823

Metformin is associated with improved clinical outcomes in patients with melanoma: a retrospective, multi-institutional study

Ryan C. Augustin[1,2], Ziyu Huang[3], Fei Ding[3], Shuyan Zhai[3], Jennifer McArdle[2], Anthony Santisi[1], Michael Davis[4], Cindy Sander[2], Diwakar Davar[2], John M. Kirkwood[1,2], Greg M. Delgoffe[5], Allison Betof Warner[6†] and Yana G. Najjar[1,2*†]

[1]Department of Medicine, University of Pittsburgh, Pittsburgh, PA, United States, [2]UPMC Hillman Cancer Center, Pittsburgh, PA, United States, [3]Department of Biostatistics, UPMC Hillman Cancer Center, Pittsburgh, PA, United States, [4]Department of Biomedical Informatics, University of Pittsburgh, Pittsburgh, PA, United States, [5]Department of Immunology, University of Pittsburgh, Pittsburgh, PA, United States, [6]Memorial Sloan Kettering Cancer Center, New York, NY, United States

Background: Pre-clinical studies have shown that metformin reduces intratumoral hypoxia, improves T-cell function, and increases sensitivity to PD-1 blockade, and metformin exposure has been associated with improved clinical outcomes in various types of cancer. However, the impact of this drug in diabetic melanoma patients has not yet been fully elucidated.

Methods: We reviewed 4,790 diabetic patients with stage I-IV cutaneous melanoma treated at the UPMC-Hillman Cancer Center and Memorial Sloan Kettering Cancer Center between 1996-2020. The primary endpoints included recurrence rates, progression free survival (PFS), and overall survival (OS) with and without metformin exposure. Tabulated variables included BRAF mutational status, immunotherapy (IMT) by type, and incidence of brain metastases.

Results: The five-year incidence of recurrence in stage I/II patients was significantly reduced with metformin exposure (32.3% vs 47.7%, p=0.012). The five-year recurrence rate for stage III patients was also significantly reduced (58.3% vs 77.3%, p=0.013) in the metformin cohort. OS was numerically increased in nearly all stages exposed to metformin, though this did not reach statistical significance. The incidence of brain metastases was significantly lower in the metformin cohort (8.9% vs 14.6%, p=0.039).

Conclusion: This is the first study to demonstrate significantly improved clinical outcomes in diabetic melanoma patients exposed to metformin. Overall, these results provide further rationale for ongoing clinical trials studying the potential augmentation of checkpoint blockade with metformin in advanced melanoma.

KEYWORDS

tumor microenvironment (TME), melanoma, checkpoint blockade, oxidative phosphorylation, tumor infiltrating lymphocyte, progression-free survival, metformin

Background

Melanoma is the fifth most common cancer diagnosed in the United States, with an incidence that continues to rise (1). Most patients have localized disease with excellent survival outcomes; however, five-year survival rates dramatically decrease for patients with locoregionally advanced or metastatic disease (1). The current era of immunotherapy (IMT) has revolutionized the treatment of advanced melanoma in both the adjuvant and metastatic setting (2–5), though validated predictive biomarkers are lacking to date (6). Metformin, a commonly utilized type II diabetes drug, has been shown to metabolically reprogram the tumor microenvironment (TME) (7), and in pre-clinical models, to augment the effectiveness of anti-programmed cell death protein 1 (PD1) IMT (8). The purpose of this study is to examine the impact of metformin on the clinical outcomes of diabetic patients with melanoma.

Several retrospective studies of various cancer types have demonstrated improved clinical outcomes in patients taking metformin (9). An analysis of over 300 diabetic patients with endometrial cancer revealed improved progression free survival (PFS) (HR 0.59, p=0.01) and overall survival (OS) (HR 0.43, p=0.005) in patients taking metformin versus those not exposed to the drug (10). A study of 302 diabetic patients with pancreatic cancer also demonstrated improved OS when comparing patients with and without metformin exposure (HR 0.68, p=0.003) (11). A 2018 retrospective study of 55 patients with unresectable stage IIIC and stage IV melanoma patients treated with IMT showed improved overall response rate (ORR) and PFS in patients exposed to metformin (12), though these results were not statistically significant. Furthermore, this study only included patients with advanced stage disease, and compared diabetic patients treated with metformin with non-diabetic patients who were thus not exposed to metformin. We therefore sought to analyze a large cohort of diabetic melanoma patients across all stages of disease to assess the association between metformin exposure and recurrence rates, PFS, OS, and incidence of brain metastases, among other variables.

A variety of mechanisms have been proposed to explain the improved clinical outcomes in cancer patients with metformin exposure. Many of these hypotheses center around metabolic alterations of the TME, which is understood to have important implications for tumor infiltrating lymphocytes (TIL), and thus, clinical outcomes with IMT (13). Metformin has been shown to activate a variety of both AMP-activated protein kinase (AMPK)-dependent and independent cellular signaling pathways that may alter the metabolic milieu of the TME and corresponding T-cell function (14). Metformin alters pro-inflammatory cytokine signaling in the TME, thereby rescuing exhausted CD8+ TILs and promoting anti-tumor effects (7, 15). Additionally, metformin was found to inhibit PD-L1 signaling *via* endoplasmic reticulum associated degradation; an effectual checkpoint blockade further enhancing TIL function (16). Metformin-mediated metabolic shifts have also been shown to inhibit regulatory T cells (Treg) in the TME and may also enhance immunity *via* alteration in the microbiome (17, 18). Furthermore, the association between metformin, a known complex I inhibitor, and hypoxia reversal in the TME is of significant interest, as it is now understood that the impact of hypoxia on immune function is largely detrimental. Hypoxia can induce an immunosuppressive state *via* enhanced Treg function and inhibitory T-cell receptor signaling (19). Hypoxic signaling can also lead to TIL dysfunction and phenotypic exhaustion, with important clinical implications for immune-based therapy (20, 21).

Notably, the related and previously studied biguanide, phenformin, has been shown to be a more potent mitochondrial inhibitor as compared to metformin (22). While both biguanides have a similar mechanism of action related to AMPK activation and oxidative phosphorylation (ox-phos) inhibition, phenformin has been shown to reduce tumorigenesis to a greater degree in some pre-clinical murine models (23). Additional pre-clinical work suggests that phenformin mediated AMPK activation can directly inhibit the mitogen-activated protein kinase (MAPK) pathway and provide a synergistic effect in combination with BRAF/MEK inhibitors in melanoma tumors with BRAF V600E/K mutations (24). Given these findings, an ongoing phase I clinical trial (NCT03026517) aims to assess the safety and efficacy of phenformin plus BRAF and MEK inhibitors in patients with BRAF mutant advanced melanoma. Apart from this trial, phenformin has been largely withdrawn from clinical use based on prior reports linking phenformin with higher rates of lactic acidosis and thus was not included in this retrospective study.

We and others have reported that the metabolic landscape of the TME is innately immunosuppressive (25, 26). Deregulated metabolism of tumor cells results in both a lack of nutrients such as glucose and oxygen, and buildup of toxic byproducts such as lactic acid. We previously demonstrated that melanoma patient tumor cells can be metabolically profiled directly from biopsies, and that deregulation of metabolism in tumor cells reveals insight into the status of the antitumor immune response to checkpoint blockade (27). Specifically, we showed that oxidative tumor cell metabolism is linked to resistance to anti-PD1 IMT; TIL isolated from melanoma tumors with high oxidative metabolism are more exhausted and less functional. High oxidative metabolism in tumor cells and the consequent generation of tumor hypoxia is associated with resistance to anti-PD1 and worse clinical outcomes, including decreased PFS, duration of response (DOR), and OS (27). We therefore developed a murine melanoma model to evaluate the impact of complex I inhibition on intra-tumoral hypoxia and T-cell function (27). Tumors from mice with complex I knock-down showed reduced ox-phos, improved T cell function, and decreased T cell exhaustion. Furthermore, only the complex I knock-down models showed response to anti-PD1, whereas GLUT1 knock down did not impact responses to IMT, suggesting that oxidative metabolism is implicated in resistance to anti-PD1 based IMT (27). Given these findings, we sought to evaluate actual clinical outcomes *via* this large retrospective analysis of diabetic melanoma patients with and without metformin exposure.

Study methods

A retrospective cohort study was conducted at the UPMC-Hillman Cancer Center (HCC) and Memorial Sloan Kettering

Cancer Center (MSKCC). With IRB approval from each institution, 4,790 charts were reviewed from patients seen between 1996-2020. Relevant charts were identified based on coding for the diagnoses of cutaneous melanoma and type 2 diabetes (T2DM). After initial review, 668 patients were found to have T2DM, with stage identified at initial diagnosis (Figure 1). This final subset was further categorized into metformin versus no metformin exposure and the key variables were tabulated for each patient, including: stage at initial diagnosis (AJCC 7 criteria), age at diagnosis, sex, BMI, performance status (Eastern Cooperative Oncology Group, ECOG), ulceration, BRAF mutational status, metformin exposure (yes/no), metformin dose, recurrence status, time to recurrence, use of IMT and indication (adjuvant/systemic), type of IMT (high dose interferon alpha-2b (HDI), high-dose IL-2, anti-PD1, anti-CTLA4, anti-PD1 plus anti-CTLA4), presence of brain metastases, date of last follow up and vital status. Histologic subtype was also collected but consistent data was not available in a large portion of our early patient records. Nondiabetic patients were not included in order to reduce confounding variables between metformin exposed and non-exposed patients.

The primary objectives of this study included recurrence rates with and without metformin in patients with stage I-III melanoma, and PFS and OS in stage I-IV patients (with and without metformin). Secondary outcomes included recurrence and survival outcomes stratified by BMI, IMT treatment, and presence of brain metastases with or without metformin exposure. Recurrence rates were defined as the proportion of patients with documented recurrence (stage I-III) or disease progression (PD) (stage IV). PFS was defined as time in months from the diagnosis of melanoma to PD or death or being censored at the last follow-up. OS was defined as time in months from the diagnosis of melanoma to death or being censored at the last follow-up.

Comparison of recurrence and survival rates was performed using the Cox proportional hazards model. Normal continuous variables were tested using the t-test. Non-normal continuous variables were tested using the Wilcoxon rank-sum test. Categorical variables were tested using the Chi-squared test, with Fisher's exact test being used for small counts. Crude recurrence rates between metformin groups were compared using the Fisher's exact test. All analyses were performed using R version 4.1.1.

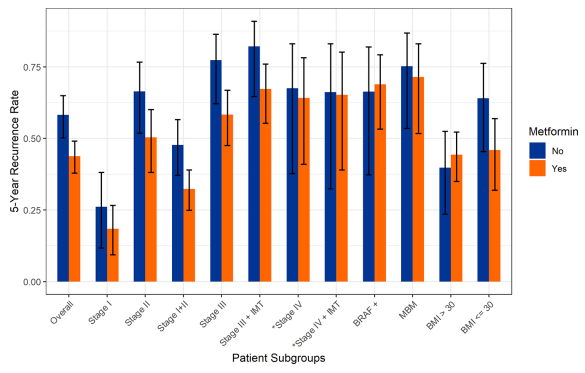


FIGURE 1
Five-year recurrence rates stratified by stage and metformin exposure. *Rates of disease progression.

Results

Baseline characteristics

Patient demographics (n=668) are listed in Table 1. In total, 422 patients were treated with metformin and 246 patients were not. The mean age of the entire cohort was 64.2 years, and 68.2% were male. At initial diagnosis, 210 patients had stage I, 183 stage II, 195 stage III, and 80 patients had stage IV disease (AJCC 7). BRAFV600E/K mutation was found in 33.6% of the entire cohort, and this was not significantly different between the two groups. Most patients had an ECOG performance status of 0 or 1, and there was a higher proportion of ECOG 0 patients in the metformin cohort (55.8% vs 37.9%, p=0.001). Ulceration was present in 34.3% (n=169) of patients at diagnosis; this was not significantly different between the two groups. LDH levels at diagnosis were similar between the cohorts.

Adjuvant therapy was reported in 36.7% (n=112) of eligible patients; this was similar between the two groups, with HDI being most common. 52.4% (n=350) of patients were exposed to IMT, with the most common being HDI (n=108), anti-PD1 therapy (n=100), and anti-CTLA4 (n=44); this was similar between the metformin and no metformin groups.

The median metformin dose was 1000 mg daily (range 250-2000 mg; dose reported in 269 of 422 patients). Of 436 patients with BMI data, 39.7% were obese (BMI >30) with 60.3% non-obese (BMI ≤30). Other variables including years since T2DM diagnosis, mean Hgb A1C, utilization of other hypoglycemic agents, and duration of metformin therapy were not consistently available.

Recurrence rates

For the overall cohort (n=668), the five-year incidence of recurrence was significantly lower in patients exposed to metformin (43.8% vs 58.2, p=0.002). In a pooled cohort of patients with stage I or II melanoma (n=393), we again note that the five-year incidence of recurrence was significantly lower in patients exposed to metformin (32.3% vs 47.7%, p=0.012); this was still significant after adjusting for age, gender, and BMI (Figure 1 and Table 2). Evaluating individual stage cohorts for stage I and II, we find a consistent numerical increase in 5-year recurrence rates for patients not exposed to metformin, though this did not reach statistical significance (Table 2).

Similarly, the five-year recurrence rate in all stage III patients (n=195) was lower with metformin compared to no metformin exposure (58.3% vs 77.3%, p=0.013) (Table 2). In patients treated with adjuvant therapy (stage IIB-IIIC, n=112), the recurrence rate was lower 59.3% vs 67.6% with metformin exposure, though this did not reach statistical significance (p=0.42). The overall incidence of brain metastases (MBM) was significantly lower in the metformin cohort (8.9% vs 14.6%, p=0.039) (Table 1), though

10.3389/fonc.2023.1075823

TABLE 1 Demographics and baseline characteristics.

	Total n=668	Metformin n=422	No Metformin n=246	p-value
Age at diagnosis (years)				
Mean(Sd)	64.2(12.3)	63.9(11.74)	64.7(13.2)	0.32
Gender, n(%)				
M	455(68.2)	286(67.9)	169(69.6)	0.91
F	212(31.8)	70(31.2)	77(30.4)	
BMI at diagnosis, median(IQR)				
	31.6(28, 36.3)	31.65 (28.0, 37.1)	31.1 (27.8, 35.7)	0.22
Stage at diagnosis, n				
I	210	138	72	0.33
II	183	109	74	0.24
III	195	129	66	0.33
IV	80	46	34	0.27
LDH at diagnosis, >ULN(%)				
	48.4	44.0	58.3	0.139
ECOG Performance Status, n(%)				
0	207(49.3)	149(55.8)	58(37.9)	**0.001**
1	187(44.5)	106(39.7)	81(52.9)	**0.03**
2	26(6.2)	12(4.5)	14(9.2)	0.08
Brain metastases, n(%)				
Yes	68(11.0)	35(8.9)	33(14.6)	**0.039**
No	552(89.0)	359(91.1)	193(85.4)	
Ulceration, n(%)				
Ulcerated	169(25.3)	108(25.6)	61(24.8)	0.07
Not ulcerated	323(48.4)	215(50.9)	108(43.9)	
NA	176(26.3)	99(23.5)	77(31.3)	
Number of metastases, n(%)				
0	189(39.3)	129(41.9)	60(34.7)	0.062
1	66(13.7)	49(15.9)	17(9.8)	0.059
2 or 2+	48(10.0)	29(9.4)	19(11.0)	0.76
3 or 3+	100(20.8)	56(18.2)	44(25.4)	0.12
4 or 5	27(5.6)	13(4.2)	14(8.1)	0.11
5+	51(10.6)	32(10.4)	19(11.0)	0.9
Prior adjuvant, n(%)				
Yes	112(36.7)	78(42.2)	34(28.3)	0.06
No	163(53.4)	95(51.4)	68(56.7)	
Type of adjuvant, n(%)				
HDI	60(53.6)	42(53.8)	18(52.9)	0.9
anti-PD1	32(28.6)	23(29.5)	9(26.5)	0.82

(Continued)

TABLE 1 Continued

	Total n=668	Metformin n=422	No Metformin n=246	p-value
anti-CTLA4	5(4.5)	4(5.1)	1(2.9)	0.9
Other	15(13.4)	9(11.5)	6(17.6)	0.4
BRAF V600E/K mutations, n(%)				
Yes	86(33.6)	60(37.0)	26(27.7)	0.163
No	170(66.4)	102(63.0)	68(72.3)	
Metformin median dose				
Median(median IQR)		1000 mg daily (1000-2000)		
Systemic Therapy, n(%)				
Yes	350(52.4)	226(53.6)	124(50.4)	0.42
No	156(23.4)	94(22.2)	61(24.8)	
Type of therapy, n(%)				
anti-CTLA4	44(12.6)	27(11.9)	17(13.7)	0.62
anti-PD1	100(28.6)	69(17.3)	31(25)	0.33
anti-CTLA4/anti-PD1	70(20)	45(20)	25(120.2)	0.9
HDI	108(30.9)	76(33.6)	42(33.9)	0.10
anti-TIM3	1(0.3)	0(0)	1(0.8)	–
IL-2	5(1.4)	2(0.9)	3(2.4)	-
Oncolytic viral therapy	10(2.9)	5(2.2)	5(4.0)	–

significant changes in survival were not seen in the MBM subgroup. A low BMI (<30) was marginally protective against recurrence in the metformin group (45.9% vs 64.0%, p=0.068) (Table 2). Regardless of metformin exposure, BRAF mutation was not associated with any difference in recurrence. Additionally, higher BMI was associated with a nonsignificant reduction in recurrence regardless of metformin status (36.5% vs 44.7%, p=0.089).

Survival outcomes

There was no significant difference in PFS between the metformin cohorts (Table 3 and S1). Patients with lower stage disease (I/II) were noted to have an OS hazard ratio of 0.563 (p=0.084) in the metformin group (Table 4 and S2). When stratified by higher BMI, patients exposed to metformin had an OS hazard ratio of 0.598 (p=0.076) (Table 4). All survival data were adjusted for age, sex, and BMI at diagnosis. Other covariates including BRAF mutation, ulceration, performance status, and adjuvant therapy were not included in the Cox regression models due to the relatively large proportion of missing data.

Discussion

Over the past decade, IMT has led to a groundbreaking improvement in clinical outcomes of patients with advanced melanoma, with 6.5 year OS for ipilimumab plus nivolumab of 49% (95% CI 44-55%) (28). However, a significant subset of patients develop primary or secondary resistance (29). Considerable effort is underway to better understand the mechanisms of resistance to IMT. It is now well understood that immunosuppressive and metabolically hostile TME, including decreased pH, altered amino acid metabolism, mitochondrial dysfunction, and hypoxia have substantial effects on the phenotype and function of TIL (8). This altered metabolic milieu of the TME may help to explain why only half of patients ultimately benefit from checkpoint blockade.

Seahorse cell analysis has been used to quantitatively measure oxidative phosphorylation (OCR) and glycolytic metabolism (ECAR) in melanoma tumor cells, and baseline tumor cell metabolism has implications on the TME, TIL function and clinical outcomes (8). Metformin reduced OCR in B16 bearing mice, and resulted in decreased intratumoral hypoxia. Furthermore, when mice were inoculated with a PD1 resistant melanoma cell line, the combination of metformin with anti-PD1 led to tumor regression in 80% of mice, whereas metformin or anti-PD1 monotherapy showed minimal anti-tumor efficacy, suggesting a synergistic effect on T-cell function in the TME (8). We have also shown that high oxidative metabolism in patient derived melanoma tumor cells is associated with decreased function and increased exhaustion of TIL, with significantly worse clinical outcomes, suggesting that high oxidative metabolism in melanoma tumor cells is associated with resistance to anti-PD1 (27). Furthermore, an experimental ox-phos inhibitor, IACS-010759, has shown improved survival in a pre-clinical murine MBM model (30).

TABLE 2 Recurrence rates stratified by stage/subgroup and metformin exposure.

Time to recurrence	Stratification	Metformin	No Metformin	p-value
Crude	Overall	42.4% (37.8%, 47.2%)	55.3% (49%, 61.4%)	**0.002**
	Stage I and II	32% (26.5%, 38.1%)	49.3% (41.3%, 57.4%)	**0.001**
	Stage I	18.8% (13.2%, 26.2%)	31.9% (22.2%, 43.5%)	**0.04**
	Stage II	48.6% (39.4%, 57.9%)	66.2% (54.8%, 76%)	**0.023**
	Stage III	58.9% (50.2%, 67.1%)	71.2% (59.2%, 80.8%)	0.117
	Stage III with IMT	69.7% (60%, 77.9%)	84.1% (70.2%, 92.2%)	*0.097*
	BRAF+	80% (68%, 88.3%)	76.9% (57.2%, 89.2%)	0.777
	BMI>30	37.1% (30.6%, 44.1%)	34.7% (24.7%, 46.4%)	0.775
	BMI<=30	38.4% (29.9%, 47.7%)	56.9% (44%, 68.9%)	**0.024**
5-Year	Overall	43.8% (37.9%, 49.1%)	58.2% (50.2%, 65%)	**0.002**
	Stage I and II	32.3% (24.9%, 39%)	47.7% (37.1%, 56.6%)	**0.012**
	Stage I	18.4% (9.4%, 26.6%)	26.1% (11.7%, 38.2%)	0.338
	Stage II	50.3% (38.1%, 60.1%)	66.4% (51.8%, 76.6%)	0.053
	Stage III	58.3% (47.6%, 66.8%)	77.3% (62.1%, 86.4%)	**0.013**
	Stage III with IMT	67.3% (55.3%, 76%)	82.1% (64.6%, 91%)	*0.067*
	BRAF +	68.9% (53.3%, 79.2%)	66.4% (37.3%, 81.9%)	0.84
	BMI > 30	44.3% (35%, 52.2%)	39.7% (23.5%, 52.5%)	0.597
	BMI <= 30	45.9% (31.9%, 57%)	64% (45.4%, 76.3%)	0.068

While these pre-clinical models show that metformin has a beneficial impact on TIL function, our present clinical data shows a notable improvement in clinical outcomes for diabetic melanoma patients taking metformin. In the metformin group, we note a significant reduction in 5-year recurrence rates (stages I-III) and rates of PD (stage IV) for patients in the overall cohort, and also for patients with stage I/II and stage III disease. The finding of a numerical increase in 5-year recurrence in patients not on metformin may not have reached statistical significance in the individual stage I and II cohorts due to a smaller sample size. The significant reduction in recurrence rates noted here for the overall cohort, and for stage I/II patients taking metformin is certainly of interest, albeit difficult to attribute to any single metabolic change that might derive from metformin's multiple downstream signaling effects. Based on pre-clinical data (8, 27), one may hypothesize that patients with early stage melanoma taking metformin have a more favorable TME and more efficient TIL anti-tumor activity at a critically early stage of pathogenesis. A similar study of 242 diabetic gastric cancer patients with or without metformin exposure showed significantly improved survival in only the localized (N0) subgroup, promoting this hypothesis in early stage disease (31). Our study also showed decreased 5-year recurrence in patients with stage III melanoma who took metformin. Overall, these findings in the context of our pre-clinical work suggest a more concerted and targeted metabolic effect secondary to metformin exposure (e.g. reduction in tumor cell oxidative metabolism improved TIL function, and reversal of hypoxia) (8, 25), though definitive correlation would require tumor-derived biomarker assessment. The decrease in recurrence rates for metformin-exposed stage III patients treated with or without IMT was not significantly improved with IMT, potentially due to the relatively small number of patients in this subgroup. Additionally, while there was an overall trend towards improved survival across all stages, patients with metastatic disease at diagnosis (n=80) did not exhibit any difference in survival with metformin exposure, though this was relatively small cohort.

TABLE 3 Progression free survival hazard ratios (HR) for each stage and subgroup by metformin exposure.

Stratification	HR (CI)	p-value
All	0.883 (0.64-1.22)	0.447
I	1.368 (0.42-4.5)	0.603
II	0.682 (0.39-1.19)	0.181
I+II	0.757 (0.46-1.24)	0.269
III	0.785 (0.47-1.30)	0.349
III+IMT	0.881 (0.49-1.60)	0.676
IV	1.866 (0.78-4.44)	0.159
IV+IMT	1.519 (0.60, 3.87)	0.381
BRAF+ (all)	1.385 (0.68-2.82)	0.37
MBM (all)	1.315 (0.63-2.76)	0.468
BMI >30 (all)	1.058 (0.67-1.68)	0.81
BMI <30 (all)	0.76 (0.48-1.21)	0.249

TABLE 4 Overall survival hazard ratios (HR) for each stage and subgroup by metformin exposure.

Stratification	HR (CI)	p-value
All	0.744 (0.495-1.119)	0.156
I	0.53 (0.144-1.954)	0.34
II	0.561 (0.262-1.2)	0.136
I+II	0.563 (0.294-1.08)	0.084
III	0.907 (0.434-1.895)	0.795
III + IMT	0.856 (0.395-1.855)	0.69
IV	1.005 (0.43-2.35)	0.99
IV + IMT	0.723 (0.291-1.797)	0.485
BRAF+	1.149 (0.388-3.405)	0.802
MBM (all)	1.119 (0.476-2.629)	0.796
BMI >30 (all)	0.598 (0.338-1.056)	0.076
BMI <30 (all)	0.916 (0.507-1.654)	0.772

Further, patients may be less likely to derive benefit from metformin at more advanced stages of disease, when the drug is less able to significantly impact the TME due to nutrient competition, restricted drug delivery, and multiple acquired mechanisms of resistance.

Metformin exposure was associated with reduced incidence of brain metastases in this study. Our data aligns with previous work demonstrating improved OS in a MBM murine model after administration of an ox-phos inhibitor (30). In correlation, a recent gene expression analysis of intracranial and extracranial melanoma metastases revealed a significant increase in ox-phos expression along with immunosuppression in melanoma brain metastases (30), suggesting a selective pressure favoring the outgrowth of highly oxidative hypermetabolic clones. Given the biochemical properties permitting metformin to cross the blood-brain barrier, high prevalence of brain metastases in melanoma, and association with increased morbidity and mortality, these studies provide grounds for ongoing translational research in the field of immunometabolism (32, 33).

Notably, our data showing significantly improved recurrence rates with metformin exposure did not correlate with survival outcomes. Given the retrospective nature of this study with some of the patient data originating from over two decades ago, there are significant confounding factors that likely affected differential survival in these patient cohorts. Namely, improvements in SOC treatments leading to improved overall survival may have over time diluted metformin-mediated survival advantages. Regardless, after adjusting for various baseline characteristics, patients with stage I-II melanoma who were exposed to metformin had reduced recurrence rates. Though tissue biomarkers were not available in this retrospective study, our prior data suggests a biological shift towards an anti-tumor phenotype of more functional TILs with critical relevance towards combatting the immunosuppressive nature of the TME (8, 25).

Hahn et al. have shown that obesity is associated with both improved survival and reduced ox-phos in metastatic melanoma (34), and we therefore assessed obesity related outcomes in our patient population. While lower BMI was marginally protective against recurrence in the metformin cohort, survival outcomes suggested the alternative. Patients with an elevated BMI and metformin exposure had a lower OS hazard ratio. Further, a nonsignificant reduction in recurrence was seen in patients with higher BMI, regardless of metformin exposure. Taken together, these data suggest reduced ox-phos, potentially *via* metabolic changes induced through obesity or metformin, could lead to improved outcomes in patients with melanoma (35).

Our retrospective study has several limitations inherent to its nature. Conclusions drawn are based on data available in the electronic medical record. However, all the patients included in this cohort were seen at regular intervals for routine follow-up, and information on demographics and clinical outcomes such as response rates, treatment utilized, and recurrence was readily available. Furthermore, data on response to therapy was based on investigator assessment, which may increase variability with regards to this outcome. Our sample size for patients with stage III treated with adjuvant immunotherapy precludes our ability to draw clear conclusions in this patient population. Similarly, our cohort size for stage IV was smaller than for patients with earlier stage melanoma, as would be expected. Most covariates were well balanced between the two cohorts. However, a significantly higher proportion of patients with an ECOG 0 or 1 were found in the metformin exposed group, a potential confounding variable. Further, more patients in the metformin cohort had received adjuvant therapy, though this did not reach statistical significance. Despite its limitations, this analysis generates a hypothesis of clinically improved outcomes in diabetic melanoma patients treated with metformin, compared to diabetic melanoma patients not exposed to this drug. This is in line with reports of metformin in other malignancies and is confirmatory of our pre-clinical findings. In addition, we screened nearly 5,000 patients for inclusion in this study, resulting in a considerable cohort size of diabetic melanoma patients treated across two tertiary medical centers.

Overall, these data provide further rationale for prospectively investigating the role of metformin in the treatment of advanced melanoma and/or prevention of recurrence in the adjuvant setting. A prospective translational trial investigating anti-PD1 with or without metformin is underway (NCT03311308) and will assess the role of decreasing tumor cell ox-phos and reversing hypoxia in the TME together with checkpoint inhibition for the treatment of advanced melanoma.

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

The studies involving human participants were reviewed and approved by University of Pittsburgh IRB Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

RA: Data collection, analysis, manuscript writing ZH, FD, SZ, JM, AS, MD, CS: Data collection, statistical support DD, JM, GD: Editorial support AW, YN: project design, analysis, editorial oversight. All authors contributed to the article and approved the submitted version.

Funding

This project used the UPMC Hillman Cancer Center Bioinformatics Core that is supported in part by award P30CA047904. ABW is supported in part by P30CA08747. This project was supported in part by the Hillman Fellows for Innovative Cancer Research Program funded by the Henry L. Hillman Foundation. This research was supported in part by the University of Pittsburgh UPMC Hillman Cancer Center award P30CA04904.

Conflict of interest

DD: Grant/research support: Bristol Meyers Squibb, Checkmate, Merck, GlaskoSmith Kline, Tesaro, Amgen

Honoraria: GlaxoSmith Kline, Tesaro, Instil Bio, Array Biopharma. Advisory board: GlaxoSmith Kline. JK: Grant/research support and/or consultant: BMS, Merck, Novartis, Roche, Genentech, EMD, Serono, Array Biopharma, Prometheus. GD: Grant/research support: Pfizer, Bluebirdbio, TCR2 Therapeutics. Consultant: TTMS, Pieris Pharmaceuticals. AW: Consulting/advisory: Nanobiotix, Iovance, Novartis, Shanghai Jo'Ann Medical Technology, BluePath Solutions, Pfizer, Instil Bio. YGN: Grant/research support: Merck, Bristol Meyers Squibb, Pfizer, Replimune. Consulting/Advisory board: Array Biopharma, InterVenn Bio, Merck, Novartis, Pfizer, BMS and Immunocore. Non-CE Honoraria: Pfizer, Immuncore.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1075823/full#supplementary-material

References

1. *Melanoma of the skin {[/amp]]mdash; cancer stat facts*. Available at: https://seer.cancer.gov/statfacts/html/melan.html (Accessed October 22, 2021).

2. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob J-J, Rutkowski P, Lao CD, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* (2019) 381(16):1535–46. doi: 10.1056/NEJMoa1910836

3. Robert C, Ribas A, Schachter J, Arance A, Grob J-J, Mortier L, et al. Pembrolizumab versus ipilimumab in advanced melanoma (KEYNOTE-006): post-hoc 5-year results from an open-label, multicentre, randomised, controlled, phase 3 study. *Lancet Oncol* (2019) 20(9):1239–51. doi: 10.1016/S1470-2045(19)30388-2

4. Eggermont AMM, Blank CU, Mandalà M, Long GV, Atkinson VG, Dalle S, et al. Adjuvant pembrolizumab versus placebo in resected stage III melanoma (EORTC 1325-MG/KEYNOTE-054): distant metastasis-free survival results from a double-blind, randomised, controlled, phase 3 trial. *Lancet Oncol* (2021) 22(5):643–54. doi: 10.1016/S1470-2045(21)00065-6

5. Weber J, Mandala M, Del Vecchio M, Gogas HJ, Arance AM, Cowey CL, et al. Adjuvant nivolumab versus ipilimumab in resected stage III or IV melanoma. *N Engl J Med* (2017) 377(19):1824–35. doi: 10.1056/NEJMoa1709030

6. Nowicki TS, Hu-Lieskovan S, Ribas A. Mechanisms of resistance to PD-1 and PD-L1 blockade. *Cancer J* (2018) 24(1):47–53. doi: 10.1097/PPO.0000000000000303

7. Eikawa S, Nishida M, Mizukami S, Yamazaki C, Nakayama E, Udono H. Immune-mediated antitumor effect by type 2 diabetes drug, metformin. *Proc Natl Acad Sci USA* (2015) 112(6):1809–14. doi: 10.1073/pnas.1417636112

8. Scharping NE, Menk AV, Whetstone RD, Zeng X, Delgoffe GM. Efficacy of PD-1 blockade is potentiated by metformin-induced reduction of tumor hypoxia. *Cancer Immunol Res* (2017) 5(1):9–16. doi: 10.1158/2326-6066.CIR-16-0103

9. Dulskas A, Patasius A, Linkeviciute-Ulinskiene D, Zabuliene L, Urbonas V, Smailyte G. Metformin increases cancer specific survival in colorectal cancer patients-national cohort study. *Cancer Epidemiol* (2019) 62:101587. doi: 10.1016/j.canep.2019.101587

10. Ko EM, Walter P, Jackson A, Clark L, Franasiak J, Bolac C, et al. Metformin is associated with improved survival in endometrial cancer. *Gynecol Oncol* (2014) 132 (2):438–42. doi: 10.1016/j.ygyno.2013.11.021

11. Sadeghi N, Abbruzzese JL, Yeung S-CJ, Hassan M, Li D. Metformin use is associated with better survival of diabetic patients with pancreatic cancer. *Clin Cancer Res* (2012) 18(10):2905–12. doi: 10.1158/1078-0432.CCR-11-2994

12. Afzal MZ, Mercado RR, Shirai K. Efficacy of metformin in combination with immune checkpoint inhibitors (anti-PD-1/anti-CTLA-4) in metastatic malignant melanoma. *J Immunother Cancer* (2018) 6(1):64. doi: 10.1186/s40425-018-0375-1

13. Allison KE, Coomber BL, Bridle BW. Metabolic reprogramming in the tumour microenvironment: a hallmark shared by cancer cells and T lymphocytes. *Immunology* (2017) 152(2):175–84. doi: 10.1111/imm.12777

14. Verdura S, Cuyàs E, Martin-Castillo B, Menendez JA. Metformin as an archetype immuno-metabolic adjuvant for cancer immunotherapy. *Oncoimmunology* (2019) 8(10):e1633235. doi: 10.1080/2162402X.2019.1633235

15. Hirsch HA, Iliopoulos D, Struhl K. Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth. *Proc Natl Acad Sci USA* (2013) 110(3):972–7. doi: 10.1073/pnas.1221055110

16. Cha J-H, Yang W-H, Xia W, Wei Y, Chan L-C, Lim S-O, et al. Metformin promotes antitumor immunity *via* endoplasmic-Reticulum-Associated degradation of PD-L1. *Mol Cell* (2018) 71(4):606–620.e7. doi: 10.1016/j.molcel.2018.07.030

17. Kunisada Y, Eikawa S, Tomonobu N, Domae S, Uehara T, Hori S, et al. Attenuation of $CD4^+CD25^+$ regulatory T cells in the tumor microenvironment by metformin, a type 2 diabetes drug. *EBioMedicine* (2017) 25:154–64. doi: 10.1016/j.ebiom.2017.10.009

18. Pollak M. The effects of metformin on gut microbiota and the immune system as research frontiers. *Diabetologia* (2017) 60(9):1662–7. doi: 10.1007/s00125-017-4352-x

19. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and treg cells. *J Exp Med* (2011) 208(7):1367–76. doi: 10.1084/jem.20110278

20. Phan AT, Goldrath AW. Hypoxia-inducible factors regulate T cell metabolism and function. *Mol Immunol* (2015) 68(2 Pt C):527–35. doi: 10.1016/j.molimm.2015.08.004

21. Rivadeneira DB, Delgoffe GM. Antitumor T-cell reconditioning: improving metabolic fitness for optimal cancer immunotherapy. *Clin Cancer Res* (2018) 24 (11):2473–81. doi: 10.1158/1078-0432.CCR-17-0894

22. Janzer A, German NJ, Gonzalez-Herrera KN, Asara JM, Haigis MC, Struhl K. Metformin and phenformin deplete tricarboxylic acid cycle and glycolytic intermediates during cell transformation and NTPs in cancer stem cells. *Proc Natl Acad Sci USA* (2014) 111(29):10574–9. doi: 10.1073/pnas.1409844111

23. Appleyard MVCL, Murray KE, Coates PJ, Wullschleger S, Bray SE, Kernohan NM, et al. Phenformin as prophylaxis and therapy in breast cancer xenografts. *Br J Cancer* (2012) 106(6):1117–22. doi: 10.1038/bjc.2012.56

24. Yuan P, Ito K, Perez-Lorenzo R, Guzzo CD, Lee JH, Shen C-H, et al. Phenformin enhances the therapeutic benefit of BRAF(V600E) inhibition in melanoma. *Proc Natl Acad Sci USA* (2013) 110(45):18226–31. doi: 10.1073/pnas.1317577110

25. Scharping NE, Menk AV, Moreci RS, Whetstone RD, Dadey RE, Watkins SC, et al. The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. *Immunity* (2016) 45 (2):374–88. doi: 10.1016/j.immuni.2016.07.009

26. Jiang Y, Li Y, Zhu B. T-Cell exhaustion in the tumor microenvironment. *Cell Death Dis* (2015) 6:e1792. doi: 10.1038/cddis.2015.162

27. Najjar YG, Menk AV, Sander C, Rao U, Karunamurthy A, Bhatia R, et al. Tumor cell oxidative metabolism as a barrier to PD-1 blockade immunotherapy in melanoma. *JCI Insight* (2019) 4(5):e124989. doi: 10.1172/jci.insight.124989

28. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Grob J-J, Rutkowski P, Lao CD, et al. CheckMate 067: 6.5-year outcomes in patients (pts) with advanced melanoma. *JCO* (2021) 39(15_suppl):9506–6. doi: 10.1200/JCO.2021.39.15_suppl.9506

29. Kluger HM, Tawbi HA, Ascierto ML, Bowden M, Callahan MK, Cha E, et al. Defining tumor resistance to PD-1 pathway blockade: recommendations from the first meeting of the SITC immunotherapy resistance taskforce. *J Immunother Cancer* (2020) 8(1):e000398. doi: 10.1136/jitc-2019-000398

30. Fischer GM, Jalali A, Kircher DA, Lee W-C, McQuade JL, Haydu LE, et al. Molecular profiling reveals unique immune and metabolic features of melanoma brain metastases. *Cancer Discovery* (2019) 9(5):628–45. doi: 10.1158/2159-8290.CD-18-1489

31. Seo HS, Jung YJ, Kim JH, Lee HH, Park CH. The effect of metformin on prognosis in patients with locally advanced gastric cancer associated with type 2 diabetes mellitus. *Am J Clin Oncol* (2019) 42(12):909–17. doi: 10.1097/COC.0000000000000627

32. Davies MA, Liu P, McIntyre S, Kim KB, Papadopoulos N, Hwu W-J, et al. Prognostic factors for survival in melanoma patients with brain metastases. *Cancer* (2011) 117(8):1687–96. doi: 10.1002/cncr.25634

33. Łabuzek K, Suchy D, Gabryel B, Bielecka A, Liber S, Okopień B. Quantification of metformin by the HPLC method in brain regions, cerebrospinal fluid and plasma of rats treated with lipopolysaccharide. *Pharmacol Rep* (2010) 62(5):956–65. doi: 10.1016/S1734-1140(10)70357-1

34. Hahn AW, Xu M, Li J, Haydu LE, Khan MAW, Gide TN, et al. Abstract LB208: obesity is associated with lower?tumor oxidative phosphorylation (OXPhos) in metastatic melanoma (MM). In:?*Molecular and cellular Biology/Genetics*. Philadelphia (PA): American Association for Cancer Research (2021). p. LB208–8. doi: 10.1158/1538-7445.AM2021-LB208

35. Hahn AW, Menk AV, Rivadeneira DB, Augustin RC, Xu M, Li J, et al. Obesity is associated with altered tumor metabolism in metastatic melanoma. *Clin Cancer Res* (2023) 29(1):154–64. doi: 10.1158/1078-0432.CCR-22-2661

Frontiers in Oncology

Advances knowledge of carcinogenesis and tumor progression for better treatment and management

The third most-cited oncology journal, which highlights research in carcinogenesis and tumor progression, bridging the gap between basic research and applications to imrpove diagnosis, therapeutics and management strategies.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact