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ORIGINAL ARTICLE

PRACTICAL RECOMMENDATIONS FOR THE MANAGEMENT OF SKIN CANCER PATIENTS

Management and treatment of nail melanoma

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ABSTRACT

BACKGROUND: An early and prompt nail apparatus melanoma (NAM) diagnosis is associated with less invasive surgical procedures and a better patient's prognosis. The diagnostic delay may be related both to the clinical misinterpretations and to errors in the diagnosing process. Biopsy techniques have been adequately described by nail experts, but the two main problems in the correct choice of the biopsy are probably related to the difficulty in performing surgery in the nail unit and the risk of permanent nail dystrophy.

METHODS: We retrospectively investigated anamnestic data and diagnostic procedures that all NAM patients referred from 1992 to January 2014, with the following objectives: 1) to evaluate the initial misdiagnoses and quantify the diagnostic delay; 2) to correlate the type of the initial biopsy with the achievement of the correct diagnosis.

RESULTS: In our cases it was easier for a non-dermatologist to misdiagnose NAM for a benign inflammatory disease. Dermatologist instead were easier to refer patients to a tertiary center for nail diseases.

CONCLUSIONS: In the presence of a NAM clinical and dermoscopic suspicion, longitudinal biopsy is recommended in all cases of nail pigmentation (lateral or median), that is estimated in its width as 3-6 mm, or larger than 6 mm. Regarding therapeutic surgery in our experience disarticulation compared to "functional surgical excision" did not correlate with a better prognosis.

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Key words: Nails - Melanoma - Prognosis - Disease management.

Nail apparatus melanoma (NAM) prevalence in Caucasians is estimated to range from 0.3% to 2.8% of all melanomas. NAM clinical presentation might be heterogeneous.[1-6] The first clinical descriptions of this tumor on 1834 by Alexis Boyer, first surgeon to Napoleon, and the further definition of characteristics in 1886 by Sir Jonathan Hutchinson, regarded advanced stage tumors, frequently associated with cutaneous pigmentation also known as Hutchinson sign.[7, 8] Nowadays with the advent of dermoscopy and novel non invasive diagnostic techniques an early diagnosis is the objective of skin cancer screening in the diagnosis of melanomas of all subsets and also of NAM.

An early and prompt NAM diagnosis is associated with less invasive surgical procedures, and a better patient's prognosis. Hence diagnosis is often delayed.[9] The diagnostic delay may be related to the initial confusion with benign inflammatory disease or with benign neoplasms and to the frequent errors in the diagnosing process.

Due to the particular anatomic location and the scarce number of overall cases in the reported series among Caucasians, no absolute consensus exists regarding the optimal diagnostic procedures and surgical treatment of NAM.[10-16]

Biopsy techniques have been adequately described by nail experts,[10-12] but the two main problems in the correct choice and performance of the biopsy are probably related to the difficulty in performing surgery in the nail unit (not enough confidence of the dermatologic surgeons with nail apparatus histopathology) and the risk of permanent nail dystrophy when a portion of nail matrix is excised.

Regarding therapeutic surgery, the current tendency is directed toward a more conservative approach when dealing with early stage NAM. Good outcomes have been reported, though few series are large enough to permit definitive surgical recommendations.[17-22]

We will describe herein our experience in the management of NAM with special regard to the anamnestic data on the most frequent clinical diagnostic errors, the diagnostic procedures and the surgical management.

Materials and methods

We retrospectively investigated anamnestic data and diagnostic procedures that all NAM patients referring to the Skin Cancer Unit of the Dermatology and the Laboratory of Dermatopathology of the University of Bologna, from 1992 to January 2014, had undergone before a definitive diagnosis. Only cases with at least 3 years of follow-up were included.

Anamnestic data achieved from patients' charts and regarding the initial diagnoses were divided in two groups: 1) initial diagnosis performed by a dermatologist; 2) diagnosis performed by a non-dermatologist (general practitioner, orthopedic, or other specialists). Further on, initial diagnoses (misdiagnoses) were subdivided in 1) inflammatory disease; 2) benign tumors; 3) malignant tumors.

Bioptic procedures considered were: 1) punch biopsy (cases were referred to our Laboratory for histopathologic evaluation); 2) longitudinal biopsy considering both types lateral and median longitudinal nail biopsy; and 3) tangential biopsy (tangential matrix excision). The latter two procedures were performed in our institution in most cases.

Transverse matrix biopsy was not considered among the procedures since it is not widely used by our team of dermatologic surgeons and further on, there were no cases referred with this procedure.[23-27]

Finally surgical procedures were: 1) functional surgery (functional surgical excision), consisting in an *en bloc* NA excision (including nail bed, hyponychium, proximal fold, matrix, with a 6 mm lateral margin around the anatomic delineating structures and down to the bone);[28] and 2) phalanx disarticulation. The analyses obtained from each case were correlated to literature data. Histopathologic specimens were collected from the Laboratory of Dermatopathology and assessed respecting the final AJCC guidelines.[29]

This study objectives were:

— endpoint 1: to evaluate the initial misdiagnoses and quantify the diagnostic delay. "Delay" was defined as more than 6 months from the time a patient first noticed an abnormality on the involved nail (*e.g.* discoloration, non-healing ulcer, nail plate splitting) until NAM diagnosis;

— endpoint 2: to correlate the type of the initial biopsy with the achievement of the correct diagnosis.

Results

NAM was diagnosed in 46 patients (3.5%) out of 1640 melanomas diagnosed from 1992 until 2014. All the patients were Caucasian. Five patients with insufficient medical records were excluded. Forty-one cases entered this study: 26 women and 15 men. The mean age at diagnosis of NAM was 61.2 (range 29-88 years).

Endpoint 1

Previous misdiagnoses were frequently referred in our series and are shown in Table I.

The misdiagnoses performed by non dermatologist were inflammatory diseases (75% of cases) such as onychomycosis, a subungual hematoma, ulcer. Dermatologist most frequent misdiagnoses were: squamous cell carcinoma (approximately 30% of cases) and acquired nevus (20-25% of cases).

The diagnostic delay was considered in 35 out of 41 patients; the other patients' anamnestic data were not reliable. Almost 90% of patients had a delayed diagnosis. The median delay time was 48 months (range 16-72 months).

Endpoint 2

A punch biopsy was performed in 5 cases referred

TABLE I.—*Anamnestic data on initial diagnoses and estimated diagnostic delay.*

Profession/diagnosis	Non-dermatologist initial diagnosis	Dermatologist initial diagnosis
Inflammatory disease	75%	23%
Benign neoplasms	14%	37%
Malignant neoplasm	11%	40%
Delay on diagnosis (months)	34-72	16-24

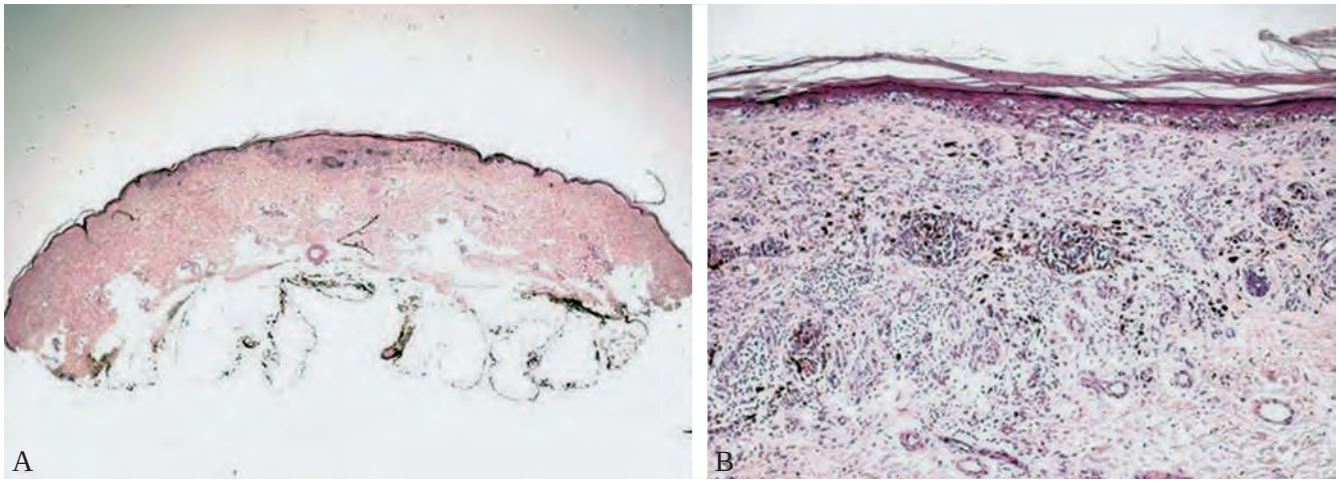

Figure 1.—A, B) Histopathologic aspects of a nail matrix biopsy (H&E Original magnification 10X; 25X) showing proliferation of atypical melanocytes in the basal and suprabasal layers of the nail matrix.

to our laboratory for histopathologic evaluation at the nail bed level and in one case performed at the matrix level. Only the latter case of NAM out of the 6 cases, was diagnosed by the biopsy (Figure 1A, B). Histopathologist required clinical patient evaluation and a second longitudinal biopsy was performed in 4 cases and *en bloc* tumor excision in two cases (the clinical features of these latter cases were strongly suggestive for melanoma).

The longitudinal incisional biopsy was performed in 29 patients and was diagnostic in all cases (Figure 2A, B).

A tangential biopsy was performed in 3 cases. The diagnosis of melanoma could be performed in each case, but the determination of Breslow thickness was evaluated as difficult and imprecise by histopathologists.

Three patients underwent NAM *en bloc* surgical excision at first approach as clinical features and additional clues such as Hutcinson sign were suggestive for melanoma.

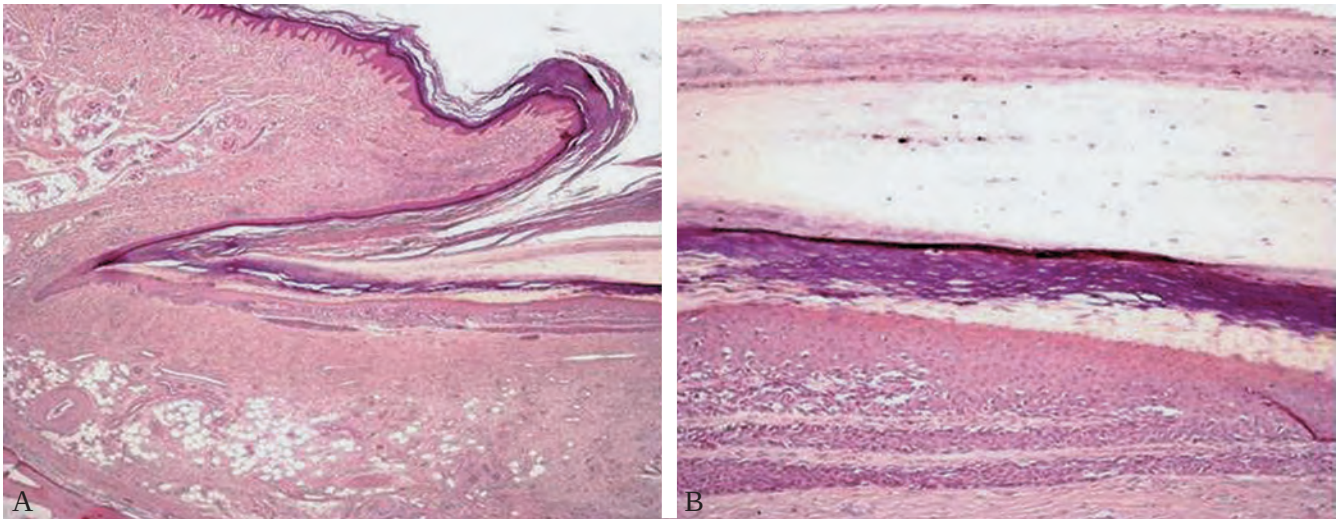

Figure 2.—A, B) Histopathologic aspects of a longitudinal nail biopsy (H&E Original magnification 20X; 25X) showing proliferation of atypical melanocytes in the nail matrix and nail bed.

Regarding therapy and the correlation of the latter with patients' prognosis, in our experience all patients underwent surgery. Thirty-two patients underwent a conservative surgery with *en bloc* NA excision. Two out of 31 patients had a local recurrence, respectively after 12 and 20 months. A disarticulation of the last phalanx was further performed.

Nine patients the disarticulation of the last phalanx was performed at first approach.

Histopathology showed a median Breslow thickness of 2.6 mm (range 0.4 to 6).

No correlation was found between surgical procedures and patients outcome:[26] disarticulation compared to "functional surgical excision" did not correlate with a better prognosis.[30]

Finally on staging,[27] sentinel lymph node positivity was retrived in 4/41 patients. The latter underwent complete lymph node dissection; 8/41 (19.5%) patients

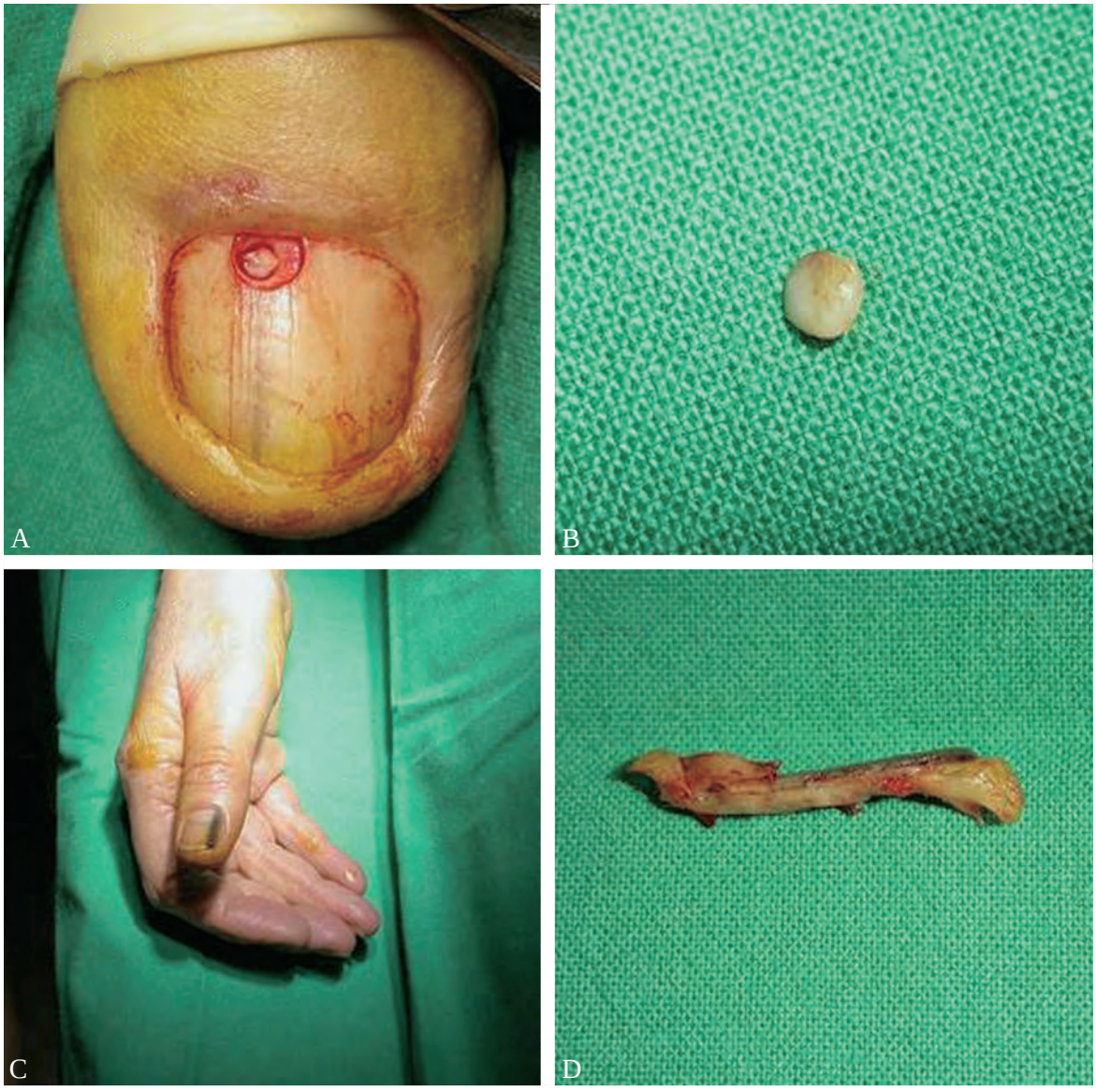

Figure 3.—A, B) Surgical aspects of nail matrix punch biopsy and C, D) nail longitudinal biopsy.

deceased for the development of multiple visceral metastases (median Breslow thickness 2.7 mm). The mean time for disease recurrences in these cases was 3 years (range 2-8). 33/41 (80.4%) of patients are alive and disease-free. Literature data report survival rates at 5 years that range from 18% to 58%.[23-27] Comparison of our data with literature data and conclusions on a possible ameliorated prognosis cannot be performed, since the different staging of the disease in diverse series and the shorter follow-up herein described.

Discussion

Since the first description of the ABCD rule in the diagnosis of NAM, named also the alphabet of nail melanoma, misdiagnoses of this entity are continuously reported in the scientific literature.[8] A delayed diagnosis is also repeatedly emphasized.

In our cases it was easier for a non dermatologist to misdiagnose NAM for a benign inflammatory disease (study endpoint 1). The dermatologist instead referred patients to a tertiary center for nail diseases more frequently, in order to perform a biopsy or for a re-evaluation. A delay in the diagnostic process was associated with a more advanced stage disease in the first group of clinicians (non dermatologists).

Regarding the correct choice and execution of the bioptic procedure and its correlation to the definitive diagnosis (endpoint 2), we can state that in our experience in the presence of a NAM clinical and dermoscopic suspicion, a longitudinal biopsy is recommended in all cases of a nail pigmentation (lateral or median), estimated as 3-6 mm in its width, or larger than 6 mm. On longitudinal biopsy correct information is provided regarding melanocytes morphology and distribution in the various components of the nail unit (Figure 3A, B). A punch biopsy should be avoided since it can often lead to misdiagnoses of NAM.

Tangential biopsy, recently encouraged by expert authors due to the optimal surgical outcome (less dystrophy of the nail plate), in our experience gives incomplete information on tumor extention. Breslow thickness evaluation could be a concerning issue since it represents one of the most important predictive factors on patients prognosis. We believe the technique could be proposed when dealing with highly collaborative and compliant patients.

Controversies regarding NAM treatment have persisted for decades. The surgical management of NAM has been influenced by studies on CM. In CM, wide local excision (WLE) is the treatment of choice, consequently early recommendations for NAM promoted amputation of the affected finger or toe. Recent series reported that the resection level of amputations does not influence the prognosis.[20-23, 26, 27] The conservative approach, performing the so called "functional surgical excision" has shown a good outcome with low rates of recurrences, especially in cases of NAM *in situ* or NAM with Breslow thickness <1 mm.

Conclusions

Regarding NAM prognosis, most studies refer to single center's experiences, reporting relatively small series if compared to CM.[27] We believe that the correct evaluation of the patients is crucial for patients' outcome in terms of prognosis. Though, similar to melanomas of other subsets, a lot has still to be explained in NAM regarding the biologic behavior and prognosis, considering that the latter might be influenced by intrinsic genetic characteristics.[30-32]

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ORIGINAL ARTICLE

PRACTICAL RECOMMENDATIONS FOR THE MANAGEMENT OF SKIN CANCER PATIENTS

Melanoma in the elderly

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ABSTRACT

BACKGROUND: Among older patients, melanoma in general presents biological features related to a more aggressive biology, such as more locally advanced tumor. Management of melanoma in elderly may be difficult, mainly due to comorbidities. We report the experience of the Melanoma Unit of ASST Spedali Civili in Brescia, Italy.

METHODS: Study subjects were drawn from 3444 patients with histological confirmed melanoma. Data were extracted from electronic database of the Melanoma Unit of ASST Spedali Civili in Brescia, Italy. Patients who received diagnosis of cutaneous melanoma at age of 65 years or older were retrospectively evaluated. For each diagnosed melanoma, histological characteristics, treatment, and outcomes were evaluated.

RESULTS: Of the 805 patients described in this study, 444 were males and 361 females. Statistically significant differences were found between patients aged 65-80 years and those aged >80 years considering melanoma prognostic factors, such as Breslow thickness, number of mitoses/mm^2 and ulceration.

CONCLUSIONS: Older age is recognized as an independent poor prognostic factor in melanoma patients, and melanoma in older patients have a distinct natural history. It was found that management of cancer in old person represents a major challenge to medical practice. We believe that the choice of therapy should be individualized and based upon the individual's overall health and that, particularly in these cases, management often requires interdisciplinary cooperation between dermatologist, surgical specialist, oncologist and geriatrician.

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Key words: Melanoma - Aged - Skin neoplasms - Patient care management.

The incidence of cutaneous melanoma (CM) in elderly patients has increased in recent years with an unfavorable oncologic outcome. It is commonly reported that age is an important prognostic factor in CM. Elderly patients seem to have worse outcomes than younger patients with CM, regardless of the clinical and histological characteristics of the primary tumor.[1-4] The current Tumor, Node, and Metastasis (TNM) Staging System has significant predictive capabilities for patient prognosis and survival.[5] However, some studies suggest that patient age should be incorporated into staging systems as it represents an important predictor of CM outcomes.[2, 4, 6]

To date, universal accepted recommendations regarding the management of CM in older ages have not been made. Improving the cancer management of elderly people, defined as individuals aged over 65 years, has become a major public health issue as the worldwide population ages. Treatment decisions for older patients with CM are controversial, and there seems to be underutilization of sentinel lymph node (SLN) biopsy, lymph node dissection and use of systemic agents in the elderly population.[7] Moreover, CM management in the elderly may be difficult, mainly due to comorbidities.

We report here the experience of the Melanoma Unit of ASST Spedali Civili in Brescia, Italy. The character-

istics of CM in patients aged >65 years were analyzed, a literature review was carried out focusing on the main factors associated with CM in elderly age.

Materials and methods

Study subjects were drawn from 3444 patients with histological confirmed CM. Data were extracted from electronic database of the Melanoma Unit of ASST Spedali Civili in Brescia, Italy. We retrospectively evaluated patients who received diagnosis of CM at age of 65 years and older. Within this group, CM developed in 805 patients. The sample was then divided into two groups according to age: Group 1 (G1), patients aged 65-80 years old and Group 2 (G2), patients aged >80 years. For each diagnosed CM, histological characteristics, treatment and outcomes were evaluated.

Clinical variables included patients' age, gender and site of primary CM categorized into four anatomic locations: head and neck, trunk, upper extremities and lower extremities. Pathological features analyzed Breslow thickness, number of mitoses, ulceration, histologic subtype and SLN details when performed.

All data were elaborated using Student's *t*-test, χ^2 test and Fisher test. A significance level of 0.05 was considered for all analysis.

Regarding literature review, articles were gathered using "melanoma" as heading term and "elderly", "older ages", "geriatric" as subheading terms or checking the bibliographies of previously assembled reports.

Results

Among our database, that includes 3444 records, 805 patients aged 65 years and older received diagnosis of CM, 444 men (55%) and 361 women (45%). They represented 23% of all CM patients included in our database. The median age resulted to be 73.63 years (ds±5.97, range 66-95). Regarding anatomical site of CM, 125 patients (16%) presented the primary tumor on head and neck, 331 (41%) on the trunk, 142 (18%) on the upper extremities and 197 (24%) on the lower extremities. For the 10 patients left (1.2%), data about primary site of CM resulted non-available.

The median Breslow thickness resulted to be 1.28±2.11 mm (range 0-22), the median number of mitoses was 3.29±7.18/mm^2 (range 0-70) and 95 CM (12%) presented ulceration.

Female patients had thinner CM than male patients (1.16 mm in women *vs.* 1.38 mm in men) but this difference was not significant (P=0.14).

Regarding CM histological subtypes, we found 329 superficial spreading melanoma (SSM) (41%), 131 undetermined melanoma (UM) (16%), 113 (14%) *in-situ* CM, 112 lentigo maligna melanoma (LMM) (14%), 56 (7%) nodular melanoma (NM), 19 (2%) acral lentiginous melanoma (ALM), and 2 desmoplastic melanoma (DM) (0.2%). In 43 patients (5.3%), the histological subtype resulted to be unknown.

Among all patients, 157 (20%) underwent SLN biopsy and 32 (20%) of them resulted to be positive. Nine patients (1.1%), despite having a CM TNM stage that assumed SLN biopsy, did not perform the operation for comorbidities reasons, also considering their families' will.

We divided the sample of 805 patients into two groups: G1 included 684 patients aged 65-80 years (80%) and G2 counted 121 patients of 81 years and older (15% of total).

Between these two groups, we found some relevant statistical differences: patients of G1 presented a medium Breslow thickness of 1.12 mm, while Breslow thickness of G2 patients resulted to be 2.17 mm, and the difference between these values appeared to be significant (P<0.001) (Figure 1).

We found the same significance analyzing the pres-

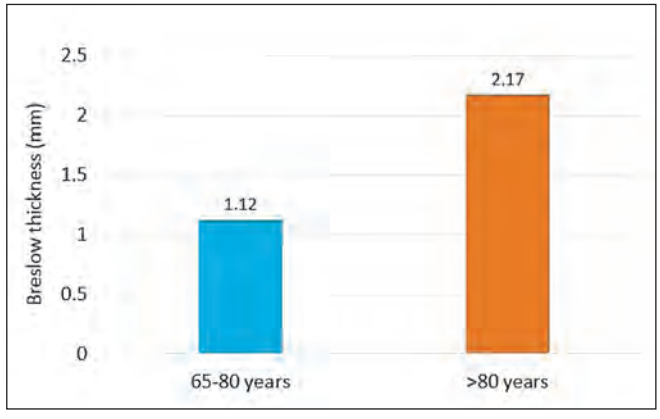

Figure 1.—Average CM Breslow thickness in G1 and G2.
CM: cutaneous melanoma; G1: Group 1 (patients aged 65-80 years); G2: Group 2 (patients aged >80 years).

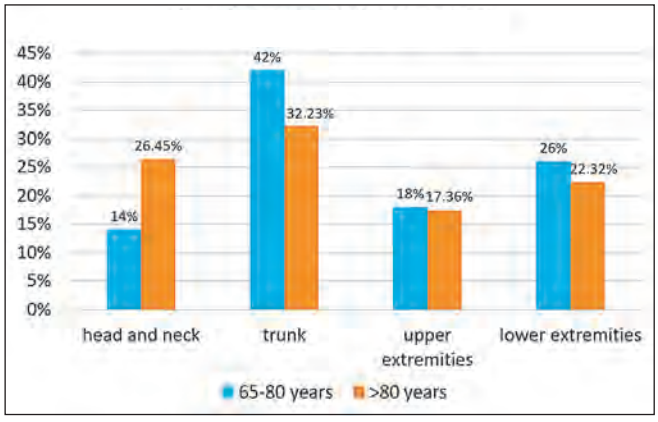

Figure 2.—Site of primary CM in G1 and G2.
CM: cutaneous melanoma; G1: Group 1 (patients aged 65-80 years); G2: Group 2 (patients aged >80 years).

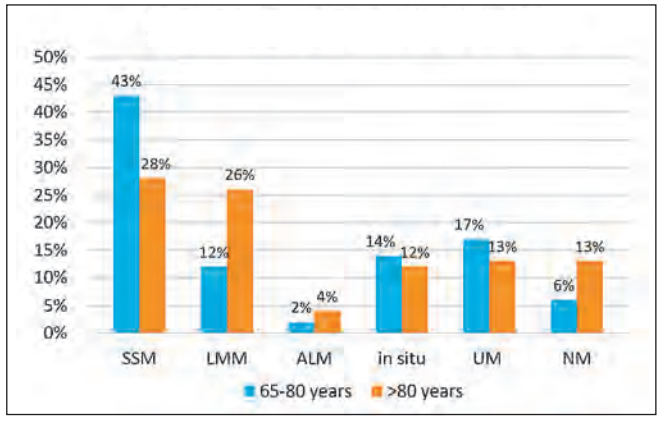

Figure 3.—CM histological subtype in G1 and G2.
CM: cutaneous melanoma; G1: Group 1 (patients aged 65-80 years); G2: Group 2 (patients aged >80 years); SSM: superficial spreading melanoma; LMM: lentigo maligna melanoma; ALM: acral lentiginous melanoma; UM: undetermined melanoma; NM: nodular melanoma).

ence of ulceration: this pathological feature involved 66 CM of G1 (9.6%) and 29 CM of G2 (29%). In addition, the number of mitoses was different: while G1 CM had an average number of mitoses/mm^2 of 2.82, patients included in G2 presented CM with an average number of 6.05 mitoses/mm^2 (P=0.0002).

A relevant difference resulted also considering the anatomical site of primary CM: in both groups the trunk was the most common site, but a significant percentage of G2 patients had primary CM localized on head and neck (26%) than G1 (14%) (Figure 2).

Regarding histological subtype, SSM resulted to be the most prevalent in both groups, although in G2 groups we found a significant occurrence of LMM (26%) and NM (13%) than G1, where we counted 12% of LLM and 6% of NM (Figure 3).

SNL biopsy revealed nodal metastasis in 20.3% of G1 patients and 21.1% of G2 patients. No significant differences were found between the two groups.

Discussion

Over the last two decades, there has been an expansion of the older population and especially of people over 80 — the so-called "oldest old."[8] Regarding CM, nearly half of these tumors are now diagnosed in elderly population and the majority of CM-related deaths is rising among patients aged 65 years and older.[9, 10] Older patients are more likely to present with locally advanced CM with higher rates of recurrence and distance metastases.[11-13] In particular, males aged 70-89 years have an incidence rate almost twice that of females [7] and the highest percentage of CM-related deaths occur in patients aged 75-84.[2, 4, 6] Furthermore, elderly patients have a higher incidence of DM, ALM and LMM, which are related to a poorer outcome.[3, 4] It is still unclear whether age differences in CM death and lymph node metastasis are due to the biology of the tumor itself or variations in hosts' immune response.[6, 12]

Thus, it seems apparent that CM at the extremes of age have a distinct natural history and that the variable behavior of CM in older patients might be due to differences in the aggressiveness of the tumor and/or altered host response to the disease, changes in lymphatic flow, comorbidities, or, more likely, to a combination of these factors.[4, 6, 7, 13, 14]

Results of our study seem to show that patients aged 81 and older present worse prognostic factors than patients aged 65-80. Moreover, we have to consider that the so called "oldest old" could suffer from more comorbidities and could be frailer than patients <80 years. The increasing number of medical comorbidities should be considered when planning treatment.[15]

Our study showed that most of our patients with CM aged >65 and older presented the primary tumor on the trunk. This result is not in line with previous reports in which the detection of CM in older patients involved mainly the extremities, as showed by Cavanaugh-

Hussey *et al.* in their study,[6] and head and neck, as demonstrated by Montero *et al.*[16] Furthermore, Montero's study also showed that patients aged 65 and older had a higher incidence of LMM and ALM, while almost half of our sample (41%) had a SSM as histological CM subtype.

Recent perspectives consider the role of occasional evaluation of the skin essential in early detection of CM, especially in elderly patients. Moreover, older people are often less observant to changes on their skin: this is probably due to low awareness of CM risk, fewer possibilities of undergoing medical specialists and elderly people's frailty.[17]

Previous reports that focused on demographic characteristics of CM in older patients showed a higher incidence rate of CM in males.[14-19, 20-22] Also, in our experience, we found a higher incidence of CM in males aged over 65, even if this difference resulted to be not significant. In addition, Khosrotehrani *et al.*[20] and Moreno-Ramirez *et al.*[23] evaluated patients in function of age and gender and both studies reported a significantly lower Breslow thickness in female population. In our sample, females presented thinner CM than males; however, this difference was not significant.

Surgery is still the most important treatment for CM.[18] Older patients are often less likely to receive adequate surgical excision margins resulting in higher rates of local recurrence, however none of our patients had a local recurrence. In addition, SLN biopsy for CM provides accurate staging information, can reduce regional recurrence and potentially improves survival for it can be critical for adjuvant therapy decision.[19] However, these therapies in the elderly are often poorly tolerated. Among our patients, 20% of them underwent a SLN biopsy and no difference was found between the two age groups. In our experience, it was found that the management of cancer in old people represents a major challenge to medical practice. Indeed, diagnostic and therapeutic treatment regarding older people should consider interventions tailored for each patient.[24, 25]

Conclusions

The increase of elderly population makes it necessary to investigate the features of CM, above all considering that CM incidence is constantly increasing. It would also be important to explore the benefit of secondary prevention in elderly age, focusing on the early detection of CM. In this regard, the occasional total skin evaluation during a routine medical care could have an important impact on the suspicion of CM in elderly patients, directed to early surgical excision.[17] Indeed, early recognition is the most important intervention to improve melanoma prognosis. In this regard, dermatologists play a crucial role in skin cancer prevention and represent an important check-point to start a multidisciplinary approach to CM.[26] Among the oldest patients, we believe that the choice of therapy should be individualized and based upon the individual's overall health and frailty. Particularly in these cases, management often requires interdisciplinary cooperation between dermatologist, surgical specialist, oncologist and geriatrician.

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REVIEW

PRACTICAL RECOMMENDATIONS FOR THE MANAGEMENT OF SKIN CANCER PATIENTS

Targeting subjects at high-risk of melanoma

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ABSTRACT

The current strategies for early diagnosis result at the moment insufficient to make further progresses in the fight against the melanoma**.** Reaching people not reached by screening activity yet is necessary for reducing mortality from this tumor. The well-known risk factors are still effective, but they have to be implemented by other variables at risk, like sex and age. People aged over 50 years old should be considered at risk, independently from other features. Renewed messages should be addressed to population and general practitioners. In this paper the old and new evidences on main risk factors will be analyzed and supported by the evidences of the literature.

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Key words: Risk factors - Melanoma - Secondary prevention - Early diagnosis.

The cutaneous melanoma incidence is rising deeply, most of all for the *in situ* and thin tumors (<1 mm of thickness according to Breslow), but the thick tumors and mortality are not decreasing.[1-4]

Evidently the current strategies for early diagnosis are been initially effective,[5] but the opportunistic screening, in the way which has been realized at the moment, results insufficient to make further progresses in the fight against the melanoma.[6]

Up to now, there are no evidences neither for or against screening population-based, because controlled randomized studies showing mortality reduction are missing. It remains fundamental to identify the subjects at greater risk, whom the secondary prevention activity should be addressed to.

The main risk factors for cutaneous melanoma are well established. They are both constitutional and environmental: high number of nevi and/or presence of atypical nevi, light phenotype and low skin type, familiarity for melanoma, previous melanoma, high sun-exposure, sunburns.

Nevertheless, for the previous considerations, it becomes necessary to wonder whether the identification of these features is sufficient to realize an effective activity of prevention or whether we have to identify even other factors.

Moles

High number nevi and/or the presence of atypical nevi is probably the most important constitutional risk factor. The meta-analysis by Gandini *et al.*[7] shows that a high number of nevi increases the risk of melanoma: a total number of common nevi of 16-40 units compared to 0-15 units brings a RR=1.47 (95% CI:1.36-1.59). A very high number nevi over the 100 units makes the risk almost seven times greater (pooled RR=6.89; 95% CI: 4.63-10.25). Also the count on the only arms can be a good predictor of risk for melanoma: 11-15 nevi *versus* no nevi, produces a risk almost five time greater (pooled RR=4.82; 95% CI: 3.05-7.62).

This variable has been confirmed more recently by

Argenziano *et al.*:[8] the presence of 20 nevi or more on the arms is an independent predictor of high total number nevi on whole body and of risk for melanoma. This aspect can be a rapid tool for general practitioners to identify some individuals at risk that must be addressed to dermatologists for a complete examination.

Nevertheless, if we want to obtain better results in early melanoma diagnosis, it is mandatory to consider some other data: in the paper of Williams *et al.*[9] the authors confirm that a high number of nevi represents a risk factor, but a considerable percentage of melanoma cases (42%) is found in patients with less than 20 nevi. Interestingly some recent studies [10, 11] pointed out that patients with a high number of nevi have melanomas with a lower thickness at diagnosis. This may be explained with a better awareness of the risk and a consequent more accurate surveillance (as skin self-examination or dermatological examination) in subjects carriers of numerous moles. Ribero [10] even found in the subgroup of patients with positive sentinel lymph node a better survival associated to high count of nevi. In this case, it could be assumed that the biological behavior of melanoma is different when associated to high number of moles.

Having many nevi is undoubtedly a risk factor for developing melanoma, nevertheless we always remember that the melanomas develop also in people with a few nevi and this condition is sometimes associated for various reasons to a worse prognosis.

For atypical nevi, the risk is even more elevated: even only one atypical nevus raises the risk (RR) to 1.60 (95% CI: 1.38-1.85,) up to 10.49 (95% CI: 5.05-21.76) if there are 5 atypical nevi.[7] In terms of risk, the weight of one atypical nevus in more is greater than one common nevus (RR 1.02 *versus* 1.51).

Regarding the topic "nevi", a condition especially at risk is the atypical mole syndrome (AMS),[12] characterized by a very high number of nevi and the presence of atypical nevi.

The incidence of AMS in the general population is not well-known,[13] but according to Greene *et al.*, the risk increases up to 500-fold when being carriers of this condition and when at least two family members are affected by melanoma.[14] The classic AMS [15] has 100 or more nevi, at least 1 nevus 8 mm or larger in diameter and at least 1 nevus clinically atypical. Other classifications are made by Newton *et al.*[16] (besides a high number of nevi moles are required in particular sites such as buttocks, scalp or iris) and by NIH [17] (here, an essential requirement is the familiarity for melanoma). According to Newton, the AMS is present in 15% of melanoma patients.[16]

Actually this condition can occur sporadically or in familial setting; according to Rigel *et al.*'s classification,[18] the risk increases from 6% if only AMS sporadic, up to 50% if there is also personal and familial melanoma.

It is very important to identify the subjects carriers of AMS, even if only a sporadic type, to monitor them very carefully, and to carry out an accurate anamnestic familial history. In these patients the practice of skin self-examination could be very difficult to perform, so it is probably prudent to submit them to dermatological examination about twice a year.

Sun exposure and indoor tanning

The IARC established that "*There is sufficient evidence in humans for the carcinogenicity of solar radiation. Solar radiation causes cutaneous malignant melanoma and non-melanocytic skin cancer*." [19] The intermittent sun exposure (RR=1.61) and sunburns (RR=2.03) are a proven environmental risk factor for melanoma.[20] A bias recall can weigh very much on the evaluation of this aspect, however, even in a recent Australian study, 63% of all melanoma and quite all keratinocyte cancers are attributed to high levels of sun exposure.[21]

Another very important environmental risk is the exposure to artificial ultraviolet (UV) light, as confirmed by IARC.[19] The exposure to tanning indoor devices strongly increases the risk of melanoma and non-melanoma skin cancer.[22, 23] In particular, if the first exposure is before the age of 30, the risk of melanoma increases of 75%.[24]

Indoor tanning is a risk factor for melanoma even without burns from indoor devices or outdoor sun exposure.[25] Melanoma risk is confirmed for users of tanning bed *versus* non-users, especially for more than 10 tanning sessions, and no statistical significance difference in the risk is found between before and after 2000, suggesting that more recent tanning instrumentals are not safer than older models.[26] Nilsen evaluated irradiance of indoor tanning devices and found an alarming emission of UVA higher than sun UV.[27]

Actinic damage

The role of UV radiation is confirmed even by the evidence that the subjects who are carriers of actinic

damage result at high risk for melanoma.[28] Actinic keratosis is caused by high chronic sun-exposure, and it is an important risk factor for basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), in particular it is a well-known precursor of the latter. It is very interesting to note that the carriers have an increased risk even for melanoma, and in particular it has been calculated that the risk increased from none to at least 10 AK for BCC, SCC and melanoma from 7.2% to 26.5%, from 1.2% to 13.6%, and from 0.7% to 1.9% respectively.[29]

A meta-analysis by Gandini [28] showed a strong positive association between melanoma and pre-malignant skin cancer lesions (RR 4.28; 95% CI: 2.80-6.55) and even between melanoma and other minor indicators of actinic damage, such as solar lentigo and elastosis (RR 2.02 95% CI: 1.41-3.29). The association between non-melanoma skin cancer and melanoma is confirmed in a more recent review and retrospective studies.[30, 31] These associations are a common evidence in our daily clinical practice, even if not quantified. So, the patients affected by BCC (a very frequent skin tumor) have to be followed because at risk for other BCC (frequently multiple over time) but also for the risk of melanoma.

It is also well-known, even by patients, and documented by many studies, that the subjects with light eye color, red or blond hair color, light skin color, low phototype, high density of freckles have a significant higher risk for melanoma.[28, 32]

Familiarity and multiple primary melanoma

About 8-12% of cutaneous melanomas occur in a familial setting and positive family history involves an increased risk (RR 1.74 95% CI: 1.41-2.14).[28] Only in about 10% of families with at least two cases the *CDKN2A* gene mutated is found. Even when there is only one relative of first or second degree, a yearly check of the cutaneous surface in dedicated surgery is very important. In families with *CDKN2A* mutation, it can be suitable to perform a skin check every six months. For the same reason every melanoma patient should be reminded the great importance of cutaneous examination for their relatives.

Multiple primary melanoma (MPM) is not a rare occurrence. The patients affected by melanoma develop a second melanoma in 1.3-8.6% of cases, according to the different surveys. The real incidence of MPM is not well known because changes by populations, but above all, many tumor registries record only the first tumor. The incidence of a second melanoma is estimated to be about 25-fold than that attended in the general population for melanoma. According to McMeniman,[33] 37% of MPM has familiarity for melanoma, 17% family history for MPM, and 42% presence of atypical nevi. This shows the strong influence of genotype in this group of patients. Obviously melanoma patients are already followed periodically for their disease, but it is necessary to underline the importance of a long follow-up even for the *in situ* or very thin melanoma. It is fundamental for early diagnosis, to suggest cutaneous check for the relatives. About 30% of MPM is synchronous (diagnosed at same moment or within three months), so it is of extreme importance to examine the overall skin surface, even when a patient is addressed to visit for a single suspicious pigmented lesion.

Gender, hormones, age

Cutaneous melanoma has a higher incidence rate in the male sex, and in both genders it is higher after 50 years of age. Nevertheless, there are some differences that have to be underlined.

Before the age of 50 the incidence results higher in females.[34, 35]

The increasing use in the last 30 years of female hormones as oral contraceptives or infertility therapy focused investigations on the possible relationship between exogenous hormones and melanoma risk. Many works documented also receptors for estrogens on nevi and melanoma. Controversial data are provided by the literature: Koomen *et al.* found increased risk of melanoma for assumption of hormonal therapy,[36] whereas other authors found no relationship neither between exogenous hormones and melanoma, nor between pregnancy and melanoma prognosis.[37] A more recent meta-analysis including studies published in the last 30 years, did not find increased risk of melanoma in women assuming oral contraceptives, fertility drugs or hormonal replacement therapy.[38]

It can be assumed that the excess of melanoma in females in younger age is attributable to more intensive use of tanning devices, as suggested from some epidemiological data.

On the other hand, it is interesting to note, even if this

does not concern strictly the secondary prevention, that women affected by cutaneous melanoma have a better prognosis than men, even under the same demographic, clinical and pathological variables. So the female sex seems to be protective on mortality from melanoma, even if the explanation is still unknown.[39, 40]

Cutaneous melanoma becomes more frequent in people aged over 50, and more and more with increasing age. In this group of age, males are affected more frequently than females. Moreover males develop melanomas with worse prognosis and consequently they have a higher mortality.[35, 41] For this reason the literature data are in agreement to consider males aged over 50 as subjects at greater risk of deadly melanoma.[42-44]

Particular people groups

Some groups of people, albeit a small part of the population, seem to be at greater risk of melanoma: recipients transplant, HIV/AIDS positive patients, and those affected by Parkinson's disease.

Because of the immunosuppressive therapy, the transplant recipients have an increased risk to develop tumors. Among the skin tumors, NMSCs are particularly frequent, but even the occurrence of melanoma is more elevated than in the general population (2.4 fold).[45] Moreover the behavior of disease seems to be worse, characterized by a lower survival.[46]

Patients affected by HIV/AIDS have a significant higher risk of NMSC [47] and melanoma. This latter risk seems unchanged by introduction of antiretroviral therapy.[48]

Melanoma incidence results higher in patients affected by Parkinson's disease.[49] This association has been investigated for a long time and at first it has been attributed to LEVODOPA therapy. More recently this link has been denied even if it is not clear what is shared by the two pathological conditions. The recent work carried out by Hu *et al.*[50] showed a possible explanation in a germline PARK2 mutation (PARK2 is a tumor suppressor gene) found in PD, in melanoma patients and in cell line melanoma.

Conclusions

In absence of an organized screening program, we need to improve the secondary prevention strategy for melanoma. So, we must reach the subjects carriers of classical and well-known risk factors, consider the risk derived from gender and age, and especially consider all people not sensitized on melanoma problem at risk for thick melanoma with bad prognosis. People not aware or who underestimate this type of risk, do not perform self-skin examination and/or dedicated clinical examination. As such, they should be considered at risk of deadly melanoma. All subjects over 50 years should periodically undergo clinical examination at least performed by a general practitioner, and addressed to a specialist in case of suspicious lesions or important risk factors.

As Weinstock said: "*A successful attack on the melanoma mortality problem must address this very high-risk population, but cannot be restricted to it because of the majority of melanomas that will arise outside of that high-risk group*".[51]

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REVIEW

PRACTICAL RECOMMENDATIONS FOR THE MANAGEMENT OF SKIN CANCER PATIENTS

Melanoma: clinical and dermoscopic diagnosis

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ABSTRACT

Missing the diagnosis of a melanoma is the worst dermatologist nightmare, especially when melanomas have a non-alarming clinical appearance and imitate a completely benign lesion. The use of dermoscopy has provided an effective tool to facilitate the differential diagnosis and to increasingly allow an early diagnosis of melanoma. The aim of this article was to summarize the most recent and important clinical and dermoscopic pearls to recognize melanoma at the earliest stages of its development.

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Key words: Melanoma - Dermoscopy - Diagnosis.

Today the real goal of a clinician is the diagnosis of *in situ*/thin melanomas. Dermoscopy allows recognizing patterns and specific criteria at the earliest stages of their development. In this article we provide an updated review of clinical and dermoscopic appearance of the different melanoma subtypes.

Superficial spreading melanoma (melanoma of trunk and extremities)

Clinical presentation

Superficial spreading melanoma (SSM) originates from melanocytes at the dermo-epidermal junction and is initially restricted within the epidermis (melanoma *in situ*). The radial growth phase is characterized by the invasion of melanocytes at all levels of the epidermis (pagetoid spread).[1]

Melanoma usually arises *de novo*, with only 20-30% of melanomas being associated with a nevus.[2, 3] At the earliest stages it presents as a light-to-dark brown or black flat macule often difficult to distinguish from a morphologically atypical melanocytic nevus. Characteristic features of SSM are the irregular shape and border and the presence of multiple colors. The mnemonic ABCDE rule and the Glasgow 7-point checklist are easy tools for the clinical recognition of suspicion lesions and are well known also among general practitioners.[4]

After a period that ranges between few months and many years, melanoma invades the dermis and acquires a significant metastatic potential. Clinically the evolution from radial to vertical growth occurs with the appearance of a papular/nodular component within the lesion. Bleeding and ulceration are also expressions of an advanced stage.[5]

The differential diagnosis of SSM includes other pigmented skin tumors, mainly nevi, seborrheic keratosis and pigmented basal cell carcinoma (BCC). The clinical discrimination between nevi and melanoma is basically

based on the natural symmetry of nevi, in contrast to the irregular morphology that typifies melanoma. Also nevi increase in size, especially in young individuals, but usually they enlarge symmetrically in all directions, remaining uniform in color and shape. Certainly, morphologically atypical nevi do exist, especially in the context of patients with the so-called "atypical mole syndrome". In such cases, an accurate diagnosis is impossible without coupling clinical examination with dermoscopy.[6]

Seborrheic keratoses may mimic SSM because of their intense pigmentation and asymmetric shapes. On the contrary the characteristic "stuck-on" appearance and the sharp demarcation allows an easy diagnosis. In the case of very tricky lesions (absence of specific clinical criteria, asymmetry and presence of multiple color) dermoscopy is mandatory.[7] Pigmented BCC might also be very difficult to discriminate from melanoma. An elevated border, a translucent hue, and tendency to early ulceration are in favor of pigmented BCC, but it often is impossible to differentiate these two tumors on clinical ground only.[8]

Dermoscopy

When evaluating a pigmented flat macule localized to trunk or extremities the first step is the classification into a melanocytic lesion or not. The presence of a reticular, globular, starburst or homogeneous pattern is suggestive of a melanocytic lesion while blue-gray blotches, arborizing vessels, milia-like cysts, comedo-like openings, red-blue lacunae and central white patches are local criteria specific for BCC, seborrheic keratosis and dermatofibroma, respectively.

Noteworthy when there is no evidence of a specific pattern/criteria we have to consider that lesion as a melanocytic one.[9, 10]

The second step consists in differential diagnosis between benign nevus and melanoma. The dermoscopic methods available are divided into qualitative (pattern analysis) and semi-quantitative analysis (ABCD rule, 7-point checklist, Menzies' method, CASH-color, architecture, symmetry and homogeneity-rule, and chaos and clues method).[11-15]

The pattern analysis has been described as the "simultaneous assessment of the diagnostic value of all dermoscopy features (global and local) shown by the lesion" [16] and it is the diagnostic process more often used by dermoscopy experts. Indeed, experts tend to review a dermoscopy image and reach a diagnosis without the use of structured algorithms. The latter were developed to simplify melanoma detection for novices in dermoscopy and are structured as scoring systems of easy interpretation and application. Some of them focus on the overall organization of the lesion colors and structures while others principally analyze the regularity or irregularity of specific dermoscopic features. Taken together the global and local criteria for melanoma diagnosis present in the different algorithms are resumed in Table I.[17]

The diagnostic importance of these criteria has been recently revalidated; moreover two new identified dermoscopic patterns significantly associated with melanoma resulted to be negative network and white shiny structures. At the same time, all the dermoscopic algorithms available were reviewed and compared in order to analyze their diagnostic accuracy and overall performance in melanoma of trunk and extremities detection. Surprisingly they show similar but modest levels of diagnostic accuracy.[17] Noteworthy in this study, the 7-point checklist was applied with a cut point ≥3, while according to the revised version of the algorithm, the presence of one of the specific SSM criteria is already mandatory for excision (Table II).[18] This approach considerably raises the sensitivity of this scoring system, but not specificity.

TABLE I.—*Global and local dermoscopic criteria of melanoma present in the various algorithms: ABCD rule, 7-point checklist, Menzies' method, CASH-color, architecture, symmetry and homogeneity- rule, and chaos and clues method (Modified from Carrera* et al.*).*[17]

Global criteria	Symmetry in colors or structures
	Border sharpness
	Quantity of specified colors
	Quantity of specified structures
	Architectural disorder
Local criteria	Blue-white veil
	Any blue or white color
	Atypical dots or globules
	Regression
	Streaks
	Atypical network
	Atypical vessels
	Irregular blotch
	Negative network
	White shiny structures

TABLE II.—*Seven-point checklist.*

Atypical pigmented network	Black, brown, or gray, thickened and branched line segments distributed irregularly throughout the lesion and sharply interrupted at the periphery
Blue-white veil	Irregular, confluent, gray-blue to whitish-blue diffuse pigmentation usually present in the elevated part of the lesion
Atypical vascular pattern	Linear-irregular or dotted vessels and/or milky-red areas
Irregular dots/globules	Black or brown round to oval structures, different in size and shape and unevenly distributed throughout the lesion
Irregular streaks	Brown to black finger-like projections asymmetrically distributed at the periphery of the lesion
Irregular blotches	Diffuse brown-black pigmentation that obscure the recognition of other dermoscopic features
Regression structures	White scar-like areas and/or blue areas (gray-blue areas, peppering, multiple blue-gray dots)

Pattern analysis remains the method with highest specificity and sensitivity but is still not well reproducible being influenced by the dermoscopy experience of each clinician. Many of the most important criteria associated with melanoma such as atypical network (Figure 1), irregular blotch, regression, streaks, pseudopods, atypical dots or globules, atypical vessels, any blue or white color, and blue-white veil, result to still have fair levels of inter observer agreement.[17]

In this light, further efforts to create a unique, valid, reliable and didactic algorithm are needed.

Lentigo maligna (facial melanoma)

Clinical presentation

Facial melanomas usually occur in severely sun-damaged skin of the elderly. The natural history of lentigo maligna (LM) is characterized by a long period of growth within the epidermis (LM is actually a melanoma *in situ*) followed after many years by the development of nodules as expression of vertical growth (LM melanoma).

Clinically it presents as a tan macule slowly expanding peripherally and changing in color unevenly. Regression, seen as whitish areas within the lesion might also occur. When LM becomes invasive, it may acquire all the characteristics of invasive melanoma (asymmetry, irregularity of shape and border, development of papular/nodular part, ulceration, bleeding).[18]

As opposed to melanoma of the trunk that has to be differentiated mainly from nevi, the differential diagnosis of facial melanoma includes non-melanocytic tumors, namely pigmented actinic keratosis (PAK) and solar lentigo/seborrheic keratosis (SL/SK).[19] The discrimination among these lesions and facial melanoma represents one of the most challenging clinical scenarios, even after dermoscopic examination.[20-23]

Dermoscopy

Facial melanoma is almost always of the LM subtype and displays different dermoscopic characteris-

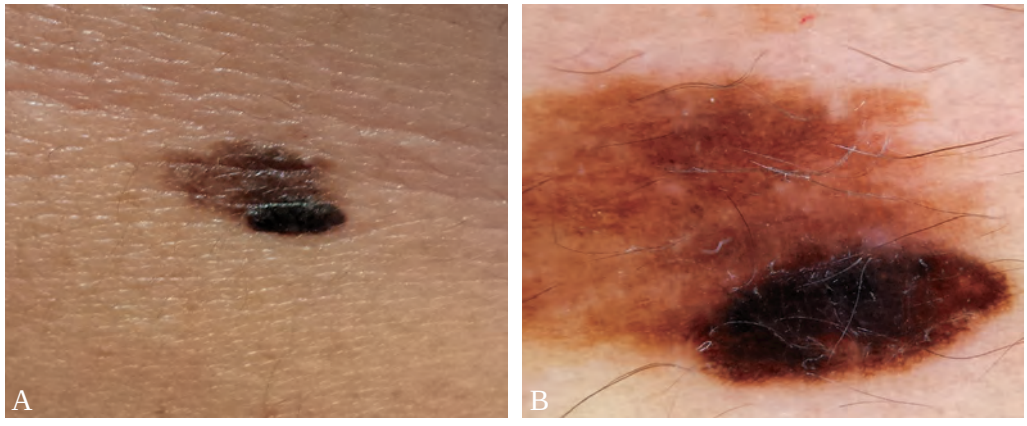

Figure 1.—A) Clinical aspect of a superficial spreading melanoma; B) dermoscopy reveals an atypical pigmented network and an eccentric brown-black pigmentation that obscure the other dermoscopic features.

tics, as compared to the conventional melanoma of the trunk.[20] Facial skin is characterized by numerous folliculo-sebaceous units and an effaced epidermis, therefore dermoscopic features of melanocytic proliferations on the face (both benign and malignant) do not reproduce the classical reticular network. Junctional melanocytic proliferations on the face are dermoscopically seen as structureless diffuse brown pigmentation, interrupted by numerous, variably broad hypo-pigmented holes, which correspond to hair follicles and sweat gland openings. The dermoscopic pattern of the latter, known with the term "pseudonetwork", is highly unspecific, since it can be seen in nevi, melanoma and non-melanocytic tumors.[24]

Several dermoscopic criteria have been suggested to characterize LM. A dermoscopic progression model has been described several years ago, introducing 4 main criteria of LM that appear sequentially as the tumor progresses: gray dots, gray globules, asymmetric follicular openings and rhomboidal structures.[20, 24] At a later stage, the pigmentation obliterates the follicular openings, while blue color and atypical vessels can be seen in advanced tumors. More recently, the detection of gray circles surrounding the follicular openings has been suggested as a specific clue of LM, while the presence of gray color (irrespectively of the corresponding structure) has been assessed as the most frequent dermoscopic criterion of LM (Figure 2).[25, 26] Vascular criteria have also been described.[27] Table III summarizes the numerous features that have been reported to characterize LM.

TABLE III.—*Dermoscopic criteria of LM.*

Pseudo-network	Structureless diffuse brown pigmentation intermingled by non-pigmented follicular openings due to the dermo-epidermal junction chronically damaged by the sun.
Gray circles	Asymmetric pigmented follicular openings.
Circles in the circle	Gray dots in the follicular openings.
Granular-annular pattern	Fine gray dots, globules and streaks around the follicles.
Rhomboidal structures	Thick pigmented lines around appendageal openings due to melanocytes that surround or completely obliterate the follicular openings.
Black/gray blotches	Diffuse brown-black pigmentation that obscure the recognition of other dermoscopic features.

Facial melanoma has to be differentiated mainly from non-melanocytic lesions rather than nevi. This represents one of the most challenging differential diagnoses in clinical practice and includes melanoma, pigmented actinic keratosis (PAK) and SL/early SK. With the addition of dermoscopy, the discrimination between LM and SL/SK is usually feasible, mainly based on the characteristic features of the latter.[24, 28] In contrast, PAK may display virtually all the criteria of LM, rendering the differential diagnosis between the two entities highly problematic.[23, 29] However, it has to be underlined that very often a definite diagnosis is impossible without a histopathologic confirmation.

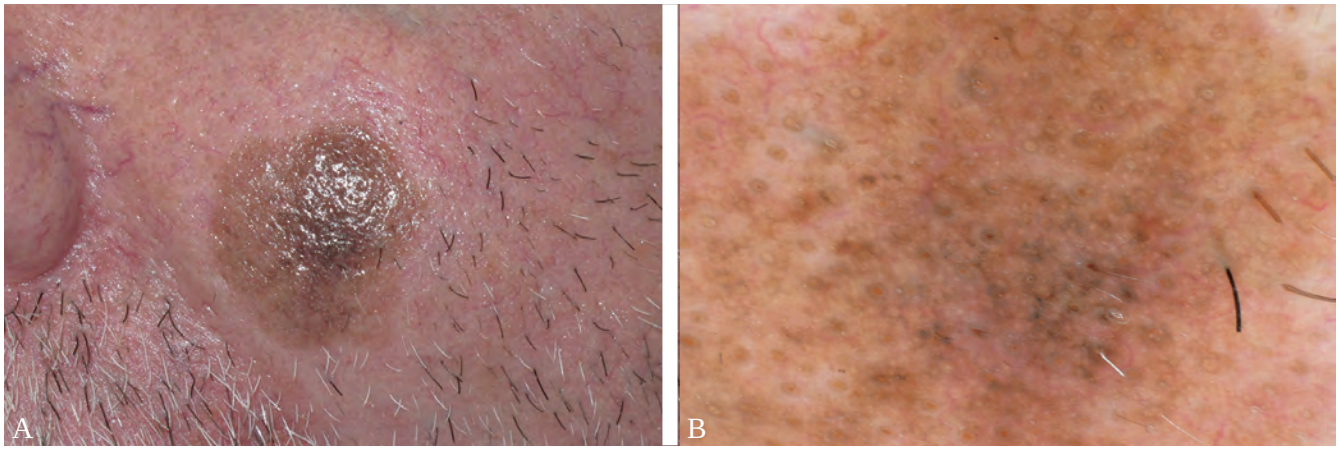

Figure 2.—A) Clinical aspect of a facial melanoma; B) dermoscopy is characterized by a structureless diffuse brown pigmentation, multiple brown and gray circles, isolated gray dots and rhomboidal structures.

Nodular melanoma

Clinical appearance

Nodular melanoma (NM) arises without an apparent precursor radial growth phase and is characterized by a high metastatic potential, even at its early stage.[30]

The most frequent anatomic sites of NM development are the head/neck area, the trunk and the extremities. It presents as a symmetrical, firm nodule with a rapid growth rate; the color is often quite homogeneous compared with SSM and may range from a black-brown to a pink-reddish (amelanotic NM).[31] It is often ulcerated at the time of diagnosis which is delayed due to the quite regular clinical aspect of this tumor. Effectively, the clinical ABCD rule is completely inefficient for the detection of NM. Clinically it may mimic every type of both benign and malignant lesion such as dermal and blue nevus, BCC, hemangioma, pyogenic granuloma, angiokeratoma, seborrheic keratosis and other neoplasms.[32, 33]

Dermoscopy

Even the dermoscopic appearance of NM is misleading as many of the "classic" dermoscopic features of SSM are observed less commonly. This is because classic features correspond to pigment deposition at the level of the dermo-epidermal junction, while the neoplastic cells in NM are located within the dermis.

Sometimes a little flat area of tumor is present, showing some of the criteria for SSM rendering the diagnosis easier.

Three dermoscopic criteria have been associated with NM and the detection of any of them in a nodular lesion should warrant immediate excision:[31-33]

1. the simultaneous presence of blue and black color within the same lesion (blue-black rule), provided that the black color does not correspond to clear-cut comedy-like openings or vascular lacunas (Figure 3);
2. an atypical vascular pattern, consisting either of linear irregular vessels or of more than two morphologic types of vessels;
3. a pink (milky red) dermoscopic color, even in the absence of any recognizable structure.

However, in clinical practice, the diagnosis of NM cannot be based exclusively on the detection of one of these 3 criteria. The only safe strategy to minimize the possibility of missing NM is excise any lesion that cannot be confidently diagnosed as a benign tumor.

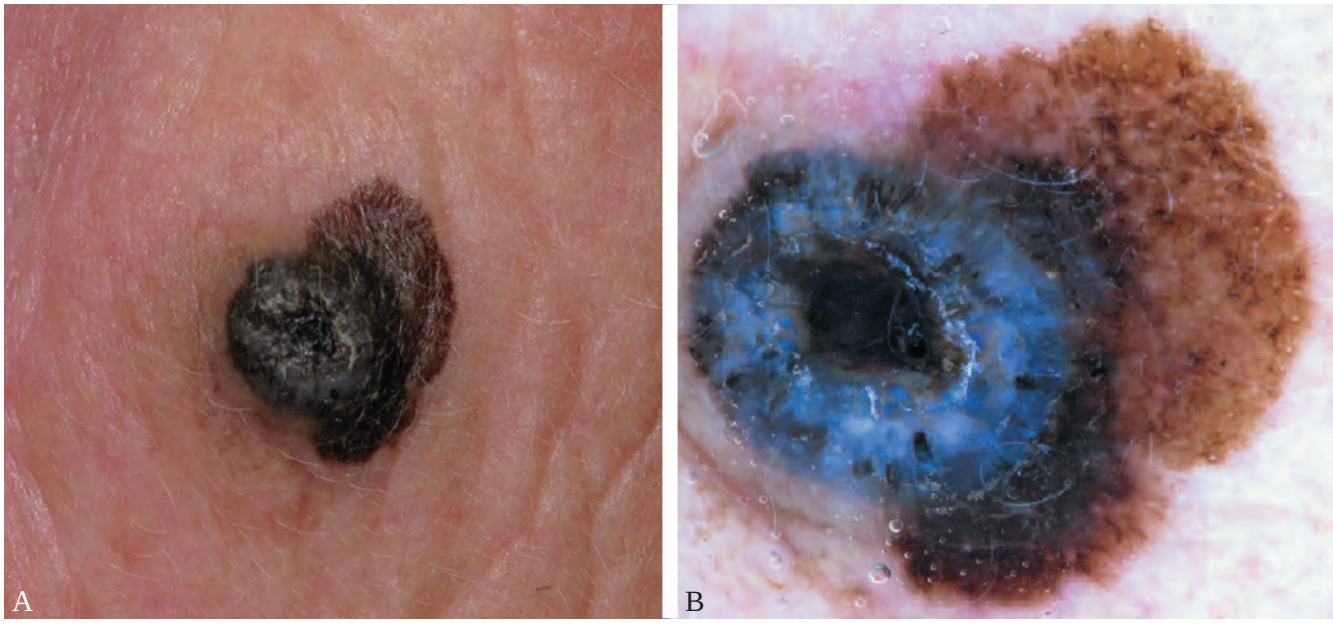

Figure 3.—A) Clinical appearance of a nodular pigmented melanoma; B) dermoscopy shows a predominance of brown-black colour in the flat part and blue-black colour in the nodular one.

Amelanotic melanoma

Clinical presentation

Melanoma partially or completely lacking melanin pigmentation is defined as amelanotic/hypopigmented melanoma. In some cases the tumor itself produces little or no melanin, while in others the absence of pigmentation is due to regression. Whatever the cause, this peculiar clinical presentation is often responsible for a delayed diagnosis, resulting in a poorer overall survival if compared to pigmented melanoma.[34]

Amelanotic melanomas (AM) can be found among all histologic subtypes, including superficial spreading, nodular, LM, and acral lentiginous melanoma. A common characteristic of all these subtypes is the whitish to pinkish or flesh-colored hue.[35, 36]

When flat, a possible clue for the recognition of AM is its shiny surface and the differential diagnosis includes superficial BCC, *in situ* squamous cell carcinoma, and regressed nevus and lichen planus-like keratosis (regressed SL or SK).[37]

Nodular AM develops as a rapidly growing pink or red nodule (Figure 4). It has to be differentiated from other non-pigmented nodular tumors including, BCC, squamous cell carcinoma, Merkel cell carcinoma, vascular tumors and other less frequent neoplasms.[35]

Dermoscopy

A completely AM is rare and through dermoscopy it is possible to recognize little areas of pigmentation, often present at the periphery of the lesion as blue or white-blue to grayish color. Sometimes a residual pigmented network or globules may also be identified.

Negative network and white shiny structures are newly defined dermoscopic patterns strongly associated with melanoma, being correlated to fibrosis in case of extensive regression.[18]

When there is a complete lack of pigmentation, evaluation of vascular pattern is the only clue available.[33] Specifically, the detection of dotted vessels is highly suggestive of a melanocytic tumor (nevus or melanoma), while BCC exhibits linear vessels, and actinic keratosis a diffuse perifollicular erythema (strawberry pattern). Intraepidermal carcinoma (Bowen's disease) also displays dotted vessels, but they are usually larger in diameter and coiled (glomerular vessels).[38]

Different types of vessels (atypical vascular pattern) together with a pink (milky-red) background color often coexist in nodular AM. However these features are highly aspecific, since they can be found in several other tumors including poorly differentiated SCC, Merkel cell carcinoma, atypical fibroxathoma and many others.[39]

The absence of pathognomonic dermoscopic criteria for AM detection is critical for clinicians. In this view, when evaluating a pink macule or nodule it is essential to look for any dermoscipic structure typical of benign (nevus) or non-melanocytic lesion (BCC, Bowen, AK). If a specific diagnosis is not feasible, the possibility of AM has to be taken into account and histopathology is mandatory.

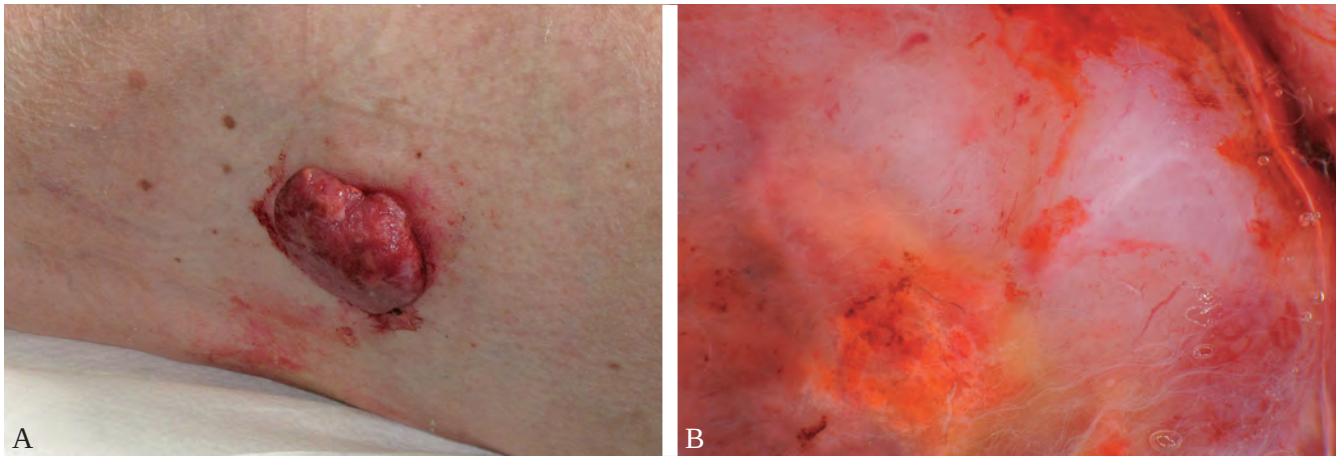

Figure 4.—A) Clinical appearance of an amelanotic melanoma; B) dermoscopy shows an atypical vascular pattern and milky red structures.

Acral melanoma

Clinical presentation

Acral melanoma is a relatively rare tumor in Caucasian population, while is the most common subtype in Asians, due the relatively rare frequency of other subtypes of melanoma.[40] It is most commonly seen on the sole, especially on the heel.

Clinical differentiation of early melanoma from benign melanocytic nevus on acral sites is sometimes difficult because both are seen as a brownish-black macule.

A well-established algorithm for the clinical discrimination between acral nevi and melanoma suggests excluding congenital melanocytic nevus and some other specific disorders (hematoma, viral warts or diabetic ulcers), as first step. Then, the diameter of the lesion has to be taken into account. Usually nevi do not exceed 7 mm in diameter, thus larger lesions should be considered suspicious and histopathologically examined.[41]

However, if a smaller lesion shows marked irregularity in shape and/or color or atypical dermoscopic pattern, a biopsy is recommended.

Dermoscopy

The peculiar anatomy of the acral skin, characterized by marked orthokeratosis and the presence of skin markings (dermoglyphics), modifies the dermoscopic appearance of melanocytic lesions from the "classical" aspect of nevi and melanomas of the trunk and extremities.

Indeed, melanocytic proliferations on the acral skin do not dermoscopically display a pigment network, but an accentuation of the pigmentation along the parallel furrows or ridges (pattern of parallel lines).[42, 43]

Dermoscopy might be very useful for the discrimination between melanoma and acral nevi, since in the former the pigmentation is distributed on the dermal ridges (parallel ridge pattern), whereas in nevi it is accentuated along the epidermal furrows (parallel furrow pattern). Notably, plantar nevi may exhibit several variations of this pattern (fibrillar pattern, lattice-like pattern, double-line pattern), depending on the precise localization of the nevus on the sole.[43-46]

The discrimination between ridges and furrows is usually feasible since ridges are much broader than furrows. In heavily pigmented tumors it might be difficult to assess whether the pigmentation follows the ridges or the furrows, but this is often clarified by focusing on the peripheral parts of the lesion.

Although parallel furrow pattern represents a highly specific finding, several melanoma slack this criterion. In order not to miss these melanomas, a more global morphologic assessment should be applied, considering several additional criteria that have been summarized in a recently introduced algorithm named the BRAAFF checklist (Table IV).[47]

TABLE IV.—*BRAAFF checklist for the diagnosis of acral melanoma.*

Acronym	Criterion	Points
B	Irregular blotches	+1
R	Parallel ridge pattern	+3
A	Asymmetry of structures	+1
A	Asymmetry of colours	+1
F	Parallel furrow pattern	-1
F	Fibrillar pattern	-1

A total score of ≥1 is needed for a diagnosis of melanoma.

Nail melanoma

Clinical presentation

Subungual melanoma usually originates from melanocytes of the nail matrix. Clinically it presents with a brown to black pigmented band that extends from the proximal nail fold to the distal end of the nail plate (Figure 5) (longitudinal melanonychia).

At this early stage diagnosis may be challenging, as longitudinal melanonychia is also typical of benign melanocytic proliferation (melanocytic nevi and benign melanocytic hyperplasia).

Nevi typically develop earlier in life and are characterized by a single pigmented band uniform in color that do not show enlargement over time. A special case is congenital nevus, since it may cover all the surface of the nail bed and also expand to the surrounding skin. However, in this case the patient will refer the congenital nature of the lesion. On the contrary, in melanoma the band increases in thickness and loses the uniform hue, developing sequential lines of different shades of brown or black color.[48]

At advanced stages, pigmentation of the whole nail

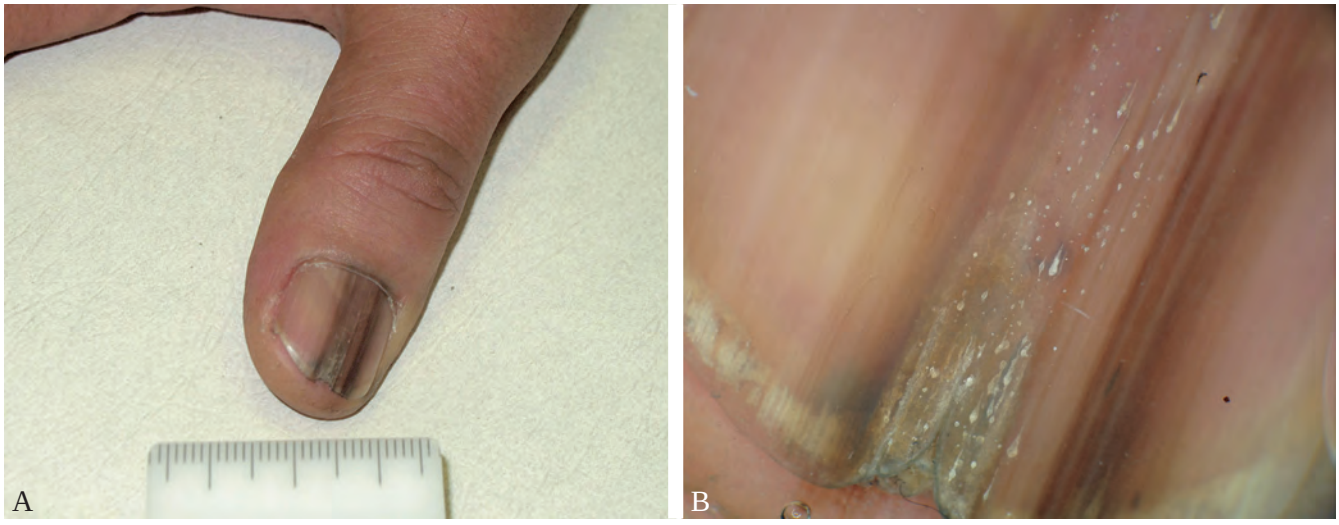

Figure 5.—A) Clinical aspect of a nail apparatus melanoma; B) dermoscopy reveals a pigmented band composed of parallel lines variable in the degree of pigmentation and thickness combined with isolated small brown granules.

plate is observed and erosion of the nail plate together with pigmentation of periungual skin may appear.

Clinical criteria for melanoma suspicion are the monodactyl involvement, the onset during adulthood, the enlargement of the band noticed by the patient over time, the triangular shape that the band may acquire (a thicker basis on the proximal fold and a thinner top on the distal end reflecting the rapid growth at the matrix) and the pigmentation of the periungual skin (Hutchison's sign, not to be confused with the pseudo Hutchinson's sign which corresponds to the visibility of the pigmentation through a translucent cuticle).[49]

Among non-melanocytic pigmentation, the differential diagnosis of nail melanoma includes subungual hemorrhage (or hematoma) and reactive pigmentation (*e.g.* drug-induced pigmentation or systemic diseases). The former is clinically characterized by a regressive evolution toward the distal edge of the nail plate while the latter usually involves more than one finger and toenails.[50] Noteworthy fungal infection may be a cause of melanonychia, thus also this alteration has to be included in the differential diagnosis.[51]

In rare cases nail melanoma is unpigmented and presents just with an erosion of the nail plate or a bleeding proliferative pseudo-vascular tumor. We always recommend a biopsy in those nail apparatus changes of difficult diagnosis.

Dermoscopy

According to the 2-step algorithm, a non-melanocytic origin of nail pigmentation (trauma, fungal infection, drug-induced pigmentation) shows a non-longitudinal pattern, whereas a longitudinal pattern is typical of melanocytic origin.[48]

Dermoscopy of pigmented nail band allows a better characterization of pigment distribution and borders. In subungueal hematoma usually the pigment is characterized by a homogeneous distribution and a sharp demarcation, while in the case of pigmentation due to fungal infection a spike and longitudinal striae patterns together with the coexistence of yellow and multicolor pattern reflect fungal invasion of the nail plate.[51] Finally in both cases dermoscopy often reveals that the pigmentation of the nail plate is interrupted before the proximal nail fold.

Once a melanocytic origin of the pigmentation has been proven, the challenge is to differentiate melanoma from benign melanocytic proliferation. Over the past decades many dermoscopic criteria has been proposed to this extent. The most relevant features include the presence of a black/multicolor pattern (in contrast with homogeneous brown of nevi) often associated with a brownish pigmentation of the background, and multiple bands of different thickness and irregularly spaced (Fig-

ure 5) (in contrast with the single band typical of nevi). Through dermoscopy, it is possible to identify micro Hutchison's sign (not visible with the naked eye) and discriminate between Hutchison's and pseudo Hutchison's signs.

In a recent study [53] evaluating the dermoscopic features of nail melanoma and their predictive value, it has been confirmed that nail apparatus melanomas involved more than 2/3 of the nail plate while most of the benign lesion show less than 1/3 of nail involvement. A strong association between the presence of gray or black color, together with irregular brown pigmentation, and melanoma has been demonstrated. Moreover, also the presence of granular pigmentation results a predictor of a malignant lesion. Finally, the presence of nail dystrophy increases three times the risk of detecting a nail apparatus melanoma.

Mucosal melanoma

Clinical presentation

Mucosal melanoma is an exceedingly rare variant of melanoma that, due to its rarity, is poorly described in literature. Primary sites of origin include the oral mucosa (55%), the anorectum (24%) and the vulvovaginal region (18%).[52, 53] Unlike cutaneous melanoma, these body areas are not exposed to UV light, thus UVs cannot be considered as a risk factor. Although some studies have suggested some correlations between mucosal melanoma and various predisposing risk factors, there is no definitive evidence that common carcinogens such as tobacco and formaldehyde, or exposure to carcinogenic viruses such as the human papilloma viruses, human herpes viruses or polyomavirus, have a role in its pathogenesis.[54]

Vulvar melanoma usually presents as a raised pigmented lesions with irregular borders. A minority of cases of amelanotic neoplasms does exist. Early lesions are completely asymptomatic while late symptoms include bleeding and pruritus. Indeed, most vulvar melanoma are diagnosed at locally advanced stages and it is not uncommon to find a regional nodal presentation of the disease.

The differential diagnosis of pigmented vulvar lesions includes vulvar nevi and vulvar melanosis (also referred to as vulvar lentiginosis or vulvar melanotic macule), particularly in younger individuals. Vulvar nevi tend to present clinically as evenly pigmented papules or macules with regular borders. Their colors range from red to dark brown-black and they typically measure less than 1 cm. Vulvar melanosis is characterized by single or multiple, irregularly pigmented, tan to black macules or patches with uneven borders.[55] Male genital melanomas share their clinical aspects with the female counterpart.

Dermoscopy

The dermoscopy of genital melanoma has been described in literature. The most reported patterns are the presence of blue, gray, or white colors with or without structureless areas and/or a whitish veil. These findings are in contrast with the described dermoscopic features of vulvar melanosis, which include a ring-like pattern, a homogeneous or structureless pattern, a reticular pattern, and a globular pattern.[56] Moreover, nevi and benign melanotic macule of the genital area typically show a light or slightly dark brown coloration. Dermoscopy is useful also to identify atypical areas for biopsy in doubtful lesions.

Management rules not to miss melanoma

Most melanomas are easy to be diagnosed clinically and dermoscopically. The question remains open concerning the correct strategies to detect those melanomas that look morphologically inconspicuous from a clinical and/or dermoscopic point of view. In our estimation, when morphology is not enough to recognize melanoma, one has to use specific management strategies. Herein we summarize the following 7 simple and practical rules that outline the need for a more general approach integrating clinical information with dermoscopic examination: 1) look basically at all lesions; 2) undress high-risk patients; 3) use the 10 seconds rule in single lesions; 4) compare and monitor lesions in patients with multiple moles; 5) excise doubtful nodular lesions; 6) combine clinical and dermoscopic criteria; 7) combine clinical and histopathologic criteria.

A few of these rules merit further explanation:

Rule 3: use the 10 seconds rule in single lesions. With experience, dermoscopic diagnosis of benign and malignant skin tumors requires usually only a few seconds. This is because the vast majority of skin lesions exhibit repetitive morphologic characteristics which, if

seen enough times previously, are easily recalled and recognized. However, a small proportion of lesions exhibit a dermoscopic pattern not typical enough to allow a definite diagnosis of a benign or malignant tumor with certainty. This results in a diagnostic dilemma, which is expressed by the pro-longed time of dermoscopic examination. These lesions should therefore be considered suspicious and excised or closely monitored;

Rule 6: combine clinical and dermoscopic criteria. Usually benign lesions are characterized by a certain harmony between clinical characteristics and dermoscopic features, with dermoscopic examination revealing, more or less, expected findings. Lesions lacking this kind of correlation should be carefully managed, and when the clinical scenario is strongly suspicious, the lesion should be eventually excised even in the absence of clear-cut melanoma-specific dermoscopic criteria;

Rule 7: combine clinical and histopathologic criteria. A clinically and dermoscopically difficult lesion is very likely to be equivocal also histopathologically, especially if the pathologist is not provided with relevant clinical information. As a rule, histopathologic reports should be interpreted in the context of the clinical information, and the diagnosis of lesions lacking a satisfactory clinico-histopathologic correlation should be managed with caution.[57]

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REVIEW

PRACTICAL RECOMMENDATIONS FOR THE MANAGEMENT OF SKIN CANCER PATIENTS

Histological reporting in patient management: frequently asked questions

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ABSTRACT

The recognition of a melanocytic tumor as a melanoma is not based upon the search of single, objective and easily reproducible morphological diagnostic features but, instead, it stems from a constellation of diagnostic criteria whose implementation, meaning and relative weight vary considerably from one case to another. We have herein tried to summarize the most reliable criteria. In conclusion, the pathologist should provide the surgeon with a report containing sufficient information to allow an evidence-based patient management planning, and to permit an accurate indication of prognosis to be determined.

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Key words: Patient care management - Melanoma - Skin neoplasms - Diagnosis - Prognosis.

The histological diagnosis of melanocytic lesions is one of the greatest challenges for pathologists. The pathologist's role in the management of cutaneous melanoma is crucial, not only in determining the diagnosis but also in determining the margins of excision and in providing prognostic indicators. Although most benign and malignant melanocytic lesions can be diagnosed with certainty, significant numbers of cases are diagnostically difficult and may lead to varied diagnoses even among expert dermatopathologists. This difficulty in diagnosis is compounded by the fact that smaller and less classical lesions are being removed because of increasing awareness among clinicians and patients about the need for vigilance concerning melanocytic lesions. Histopathologic analysis remains the gold standard for the diagnosis of melanoma. Despite this, the literature is replete with examples of subjectivity in the histologic interpretation of melanoma and other melanocytic tumors. The recognition of a melanocytic tumor as a melanoma is not based upon the search of single, objective and easily-reproducible morphological diagnostic features but, instead, it stems from a constellation of diagnostic criteria whose implementation, meaning and relative weight may vary considerably from one case to another.[1]

For these reasons, the histopathological diagnosis, being based upon the simultaneous evaluation of several criteria, is no more than an assessment of probability and, as such, is often matter of a sizable disagreement and inter-observer variability, still waiting for clear-cut information from molecular techniques. In the pathological reporting of a primary malignant melanoma, desirable features include: characteristics of the melanoma, including subtype; whether the lesion has been completely excised; and an evaluation of prognostic indicators. This will allow critical decisions regarding

extent of surgery and possible use of potentially morbid adjuvant therapy to be made, as well as reducing the chances of local recurrence and removing melanoma cells that could serve as a source of metastases.[1, 2]

There is no single histological criterion that definitely separates benign and malignant melanocytic lesions. Every characteristic that has been described in melanoma has also been found in benign nevi. There are several different types of benign melanocytic nevi, including banal junctional, compound, and dermal melanocytic nevi, as well as congenital, dysplastic (atypical), blue, cellular blue, deep penetrating, Spitz, ancient, desmoplastic, balloon cell, and halo nevi. Each type of benign nevus may simulate malignant melanoma and vice versa. A further confounding factor in the diagnosis of melanoma is that melanomas are frequently adjacent to or admixed with benign nevi including banal and dysplastic types. The histological features must be evaluated in conjunction with clinical and macroscopic data. Pathologists should make their histological assessment initially without reference to the clinical context. However, the histological features have to be further assessed in the clinical context. This stresses the importance of the clinician providing all relevant data in the pathology request form including age, sex, site, pregnancy, history of previous melanomas or nevi at the site of the current lesion or elsewhere, and recent change in the lesion. The clinician should also provide their provisional and differential diagnoses to the pathologist. Accurate diagnosis of melanocytic lesions may require a consultation between the clinician and the pathologist.[3, 4]

However up to now the goal of a standardized and reproducible histopathological diagnosis and reporting of melanoma is far from being achieved. The historical classification into lentigo maligna melanoma, superficial spreading melanoma, nodular melanoma, and acral (and mucosal) lentiginous melanoma is still an acceptable startpoint.[5, 6] The WHO 2006 classification [2] recognizes additional subtypes of melanoma but is still incomplete:

— superficial spreading melanoma;
— nodular melanoma;
— lentigo maligna;
— acral-lentiginous melanoma;
— desmoplastic and desmoplastic neurotropic melanoma;
— melanoma arising from blue nevus;
— melanoma arising in a giant congenital nevus;
— melanoma of childhood;
— nevoid melanoma;
— persistent melanoma.

However, the clinicopathologic and prognostic implications of such a classification are not so sharp as originally thought.[7]

The histological diagnosis of melanocytic lesions requires assessment of architectural and cytological features. Histological features that are more often present in melanoma than benign nevi include asymmetry, ulceration, cytological atypia, pagetoid involvement of the epidermis, lack of maturation, and dermal mitoses including deep and atypical types.[4-7]

Symmetry is assessed by looking at the silhouette of the lesion, and the distribution of its characteristics around a central vertical plane. Benign nevi are usually symmetrical whereas melanomas are often asymmetrical. This reflects the disordered growth of melanomas. Features contributing to symmetry and asymmetry include the extent of lateral spread, the pattern of dermal infiltration, the presence of pigmentation and the host response. Benign lesions are usually uniform and monotonous, whereas melanomas are varied and disordered. However, many melanomas, especially the small ones, may be symmetrical and uniform.

The presence of non-traumatic ulceration is an indication that the lesion is probably malignant. In addition, ulceration is a poor prognostic feature in melanomas and the greater the extent of ulceration the worse the prognosis. There is great variety in the cells that may be present in melanoma. The cells may vary from very small to very large in size. Their shape may be round, oval, polygonal, spindled, dendritic, or irregular.

The cytological features of melanoma are protean, and melanoma may mimic other neoplasms. Cytological atypia is usually present and is assessed mainly by looking at nuclear features. The nuclei often have irregular membranes, coarse chromatin, and hyperchromasia. Nuclear pseudoinclusions may be present. There is also great variety in the cytoplasmic features of melanoma. The cytoplasm may be eosinophilic, basophilic, amphophilic, granular, ground glass, pale, and/or vacuolated. While the cytological features of melanoma are usually atypical, in some cases the melanoma cells can be very bland and resemble a banal nevus, the so-called nevoid melanoma. On the other hand, some benign nevi

such as Spitz nevi exhibit cytological atypia that may lead to confusion in diagnosis.

Melanin may or may not be present in the cytoplasm of melanoma cells. If no pigment is present, the pathologist needs to seek confirmation that the lesion is melanocytic in origin. This confirmation can include the presence of a junctional component or the use of immunohistochemical stains such as S100 protein or Melan-A. One useful feature that may help in the diagnosis of melanomas is the presence or absence of pigment in the deepest component of the lesion. Nevus cells usually — but not always — lose their pigment with descent into the dermis, whereas melanomas often exhibit melanin in the deep dermal cells. The presence of atypical melanocytes singly and in small groups of two or three cells above the basal layer is a clue to the diagnosis of melanoma, particularly if the melanocytes are present up to and including the granular layer. This is termed pagetoid epidermal invasion, because of its similarity to Paget's disease of the nipple. The differential diagnosis of atypical cells in the epidermis includes Bowen's disease, in situ melanoma and Paget's disease. Pagetoid spread may also be seen at times in benign melanocytic lesions including recurrent, Spitz and acral nevi. As the melanocytes of benign nevi descend into the deeper dermis they usually become smaller both in cell and nuclear size compared with the melanocytes present in the superficial zone of the lesion. This is termed maturation. In melanomas, there is often poor or no maturation, or there may be apparent maturation in which the cells become smaller but still possess significant atypia. It is also important not to mistakenly label as maturation the presence of an associated banal nevus at the base of a melanoma.

Mitoses are generally not present in benign nevi. Exceptions include Spitz nevi, traumatized nevi, halo nevi, and nevi in pregnant women. The presence of mitoses in a melanocytic lesion, especially if numerous and particularly if they are deep and/or atypical, should arise suspicion of malignancy. Each lesion must be judged in its entirety together with the clinical details, including age, site and history of recent change.[4, 7] Additional special techniques may also be used. Optimal evaluation of any melanocytic lesion requires complete excision that incorporates the full thickness of the involved lesion removed intact. "Shave" procedures that do not include the intact base of the lesion should be avoided. Similarly, "punch" procedures suffer from limitations due to the "sampling" of the lesions and must be therefore restricted to the preoperative differential diagnosis between melanocytic and non-melanocytic lesions, whose *in-toto* excision would lead to cosmetic and/or functional impairment.[7]

Because of the lack of objective and reproducible diagnostic criteria, ancillary techniques have been increasingly implemented in routine practice. Immunohistochemistry is the most widely used for the demonstration of a melanocytic histogenesis for undifferentiated (anaplastic) malignancies in their either primary cutaneous or metastatic site, for the recognition of nodal melanocytic deposits in sentinel node biopsy, for the identification of prognostic factors (comprising Breslow's thickness) in melanoma and for the differential diagnosis among benign and malignant melanocytic tumors in the skin.[8, 9]

The first three goals can be achieved with the use of "panmelanocytic markers":[10-12] the best approach is to use S100, which is the most sensitive and less expensive marker, plus one more specific lineage-specific marker (Melan-A/MART1, tyrosinase, MITF1, p75/NGFr, or SOX10). The most efficient and less expensive couple of reagents are S100 and Melan-A/MART1, but with two main caveats: 1) desmoplastic melanoma is negative to MART1, tyrosinase, and MITF1 and can be identified solely with the anti p73/nerve growth factor receptor antibody, or with the anti SOX10 antibody; 2) on sun-damaged skin, a nuclear marker (MITF1 or SOX10) should replace Melan-A/MART1, which can give a melanoma-like staining pattern by labelling either hyperplastic dendrites or "pseudomelanocytic nests" of keratinocytes involved in a lichenoid tissue reaction.

Lineage-specific markers can be also used to refine the measurement of Breslow's thickness of melanoma. Cases of melanoma with halo-reaction and/or regression can show 'blurred' deep margins: therefore, immunohistochemistry can highlight deeply entrapped melanocytes thereby avoiding under-microstaging. Mitotic rate is a strong prognostic indicator in melanoma. In addition, the 2010 American Joint Committee on Cancer (AJCC) Staging System has replaced Clark's level IV with mitotic rate $\geq$1 mitosis/mm^2 to define pT1b melanoma and to select patients for sentinel node biopsy.[13]

The differential diagnosis between benign and malignant melanocytic tumors is the most ambitious task for

immunohistochemistry.[10-13] An acceptable compromise between cost, increase in technical routine workload, and diagnostic impact is the adoption of an antibody panel composed by the anti-cell cycle-related protein Ki-67, the anti-human melanoma black (HMB-) -45 and the anti p16 protein. Ki-67 staining can be evaluated either with a systematic count of neoplastic cells (<5% of neoplastic cells labelled in common nevi; 5-13% of neoplastic cells labelled in "dysplastic" nevi and Spitz nevi; >13% of neoplastic cells labelled in melanoma) or with an evaluation of the staining pattern (tidy in nevi, untidy with clusters of proliferating cells in melanoma). HMB-45 expression recalls the "maturation" of nevi (progressive loss of reactivity from the surface inwards) and the architectural disorder of melanoma ("patchy" reactivity, with isolated or clustered cells being labelled throughout the dermis). The anti-p16 antibody stains nevi in an either strong and diffuse or a tidy ("checkerboard") pattern; instead, melanomas typically show confluent foci of complete loss of reactivity.[9]

Unfortunately, the above illustrated rules have relevant exceptions and limitations. It must be therefore emphasized that immunohistochemistry must be always evaluated within the morphological context; that not any single immunostain is able to give clear-cut information for the differential diagnosis between nevus and melanoma. Immunohistochemistry may be needed to confirm diagnosis, however, there are yet no reliable markers that are both highly sensitive and specific for melanoma diagnosis. The ideal biomarker, defined as any measurable molecular change (DNA/chromosomal, epigenetic, mRNA, or protein) in a cancer cell, should be sensitive, specific, reliable, rapidly analyzable, cost effective, and should "add value," prognostically or therapeutically, to our current set of assessment tools. Several molecular and chromosomal events that influence the development and progression of melanoma show promise in improving differentiation of melanomas from benign melanocytic proliferations.[14, 15]

Molecular techniques are being increasingly proposed with the aim of looking for specific pathways toward melanomagenesis. When matched with morphologically obvious melanocytic tumors, all these techniques show a greater specificity than sensitivity, thereby allowing to 'rule in' and not to 'rule out' melanoma. Biomarkers that move beyond the current clinical pathological and radiological parameters, helping to identify those patients with early disease at high risk of relapse and guiding therapy choices for patients with metastatic disease, are still needed. A number of potential candidate biomarkers, including immunological markers warrant further evaluation in melanoma. Circulating or tumor-resident immune cells, including those associated with immunosuppressive forces in melanoma such as Treg MDSC, $IgG4^+$ B cells, and also cytokines, chemokines, checkpoint molecules, and antibodies may point to yet unexplored biomarker signatures associated with particular clinical outcomes. Despite the considerable progress made in immune monitoring technologies, it has been challenging to draw accurate correlations between immunological parameters and clinical outcomes or patient responses to therapeutic agents. The reasons might include complex interactions between immune and tumor cells and the variable patient immune responses, making it difficult to account for all the interactions required for adequate prognostic readouts. Even when associations with melanoma are demonstrated, there is significant variability among patients, possibly reflecting the heterogeneity of individual tumors and of individual patient immune responses. Therefore, the histopathology remains the major source of the most reproducible tool for diagnosis and prognosis in melanoma patients.

In general, the histopathological report must include all the pertinent clinical information and a thorough macroscopic description comprising the sampling protocol adopted.[1, 2] A microscopic description of the tumor, as well as the implementation and the results of the ancillary techniques are optional if the final diagnosis is clear-cut.

Mandatory histopathologic parameters include:

1. ulceration (present *vs*. absent): this is the sloughing of dead tissue. This can sometimes occur in the center of a melanoma lesion. Ulceration is thought to reflect rapid tumor growth, which leads to the death of cells in the center of the melanoma. It is defined as a full-thickness epidermal defect above dermal melanoma growth, with reactive tissue changes (fibrin, neutrophils) and atrophy or hypertrophy of the surrounding epidermis, with no history of trauma or surgery;

2. mitotic rate: this term describes the frequency of cell division within the melanoma. Higher mitotic rates are associated with more rapidly dividing cells and therefore larger lesions, with greater potential for

metastasis and poorer prognosis. Mitotic rate is thought to be the second most important factor (after Breslow thickness) in determining prognosis, with a higher rate being predictive of a poorer prognosis. This value is used to stage very thin melanomas (<1 mm). To measure the mitotic rate, the pathologist identifies the area of the tumor sample with the most mitoses (referred to as the hot spot) and counts the number of mitoses in a square millimeter surrounding this area. It is reported as a value per mm^2 or may be given as a range (*e.g.*, 1-4 mitoses/mm^2). The mitotic rate should be given as an integer number; if no mitotic figure is found in the invasive component of the tumor, the mitotic rate must be given as 0 mitoses/mm^2;

3. regression (present *vs*. absent): if it is present, the extent of regression is identified. Regression describes an area where it appears there had been melanoma cells, but these have been destroyed by the immune system and replaced with inflammation or scar tissue. When regression is present, the total size of the melanoma is hard to characterize because it is difficult to tell how extensive it was before the regression occurred. It can be focal (involving a portion of invasive tumor), partial (involving the entire invasive tumor), or complete (involving the entire tumor). Since complete regression and regression involving more than 75% of the lesion has been reported to carry adverse prognostic importance in invasive melanoma, it is recommended to assess regression;

4. lymphovascular invasion: blood vessel or angio invasion as well as lymphatic invasion is described as being present or absent. If present, it means that the melanoma cells have invaded the blood or lymphatic system;

5. perineurial invasion (present *vs*. absent): since melanoma is typically S100-positive just like nerves, an immunostain for the perineural sheath with the anti-epithelial membrane antigen (EMA) can be used to individuate the nerve fibers;

6. Breslow's thickness: maximum tumor thickness is measured with a calibrated ocular micrometer at a right angle to the adjacent normal skin. The upper point of reference is the granular layer of the epidermis of the overlying skin or, if the lesion is ulcerated, the base of the ulcer. The lower reference point is the deepest point of tumor invasion (*i.e.*, the leading edge of a single mass or an isolated group of cells deep to the main mass). If the tumor is transected by the deep margin of the specimen, the depth may be indicated as "at least X mm" with a comment explaining the limitation of thickness assessment;

7. satellitosis: satellite lesions (also called micro satellites) are areas of tumor/melanoma located more than 0.05 mm, but less than 2 cm, from the primary lesion. Satellites are described as being present or absent. These are also reflected in the staging. It is also recommended to include microsatellites into Breslow's thickness itself;

8. status of the surgical margins: microscopically measured distances between tumor and labeled lateral or deep margins are appropriately recorded for melanoma excision specimens because these neoplasms may demonstrate clinical "satellitosis." The report will describe the location of the tumor to the margins, or edges of the biopsy or tissue sample. "Negative margins" mean a small amount of normal tissue around the entire tumor was also removed and is free of cancer cells; this ensures the entire melanoma is removed. "Positive margins," on the other hand, indicate that melanoma extends all the way to the edge of the tissue removed, and that it is possible some melanoma may not have been removed. The report may also state how close the tumor cells were to the margins (edges) of the sample. Positive or close margins may require further surgery to achieve clean or negative margins.

Optional parameters of the histopathological report are the following:[1, 7]

1. histological subtype: this can be considered as an optional parameter because the current classification of melanoma evaluates a sum of criteria which are neither purely histopathologic nor exclusively tumor-related; therefore, features of different subtypes can be present in a given tumor, and, conversely, different subtypes of tumor can have similar histopathologic features. Even more important, the prognostic and therapeutic value of the current classification is probably minimal;

2. cell type: this parameter is partially incorporated into the WHO 2006 Classification. There are some data suggesting that thick (>5 mm) melanomas with a Spitzoid or spindle cell morphology have a slight survival advantage compared with other cytotypes, but evidence is too weak;

3. amount of pigmentation (none, mild, moderate, heavy): this is a very subjective assessment which lacks

any prognostic significance even on univariate analysis;[53]

4. Clark level,[5] which is defined as follows:
   I. intraepidermal tumor only;
   II. tumor present in but does not fill and expand papillary dermis;
   III. tumor fills and expands papillary dermis;
   IV. tumor invades into reticular dermis,
   V. tumor invades subcutis.

Although, as stated above, anatomic level has been replaced by mitotic rate in the AJCC 7th edition tables for the subclassification of pT1 lesions as T1a or T1b, in the text and in a table of the AJCC chapter, Clark levels IV or V is referred to as a tertiary criterion for T1b in cases with no ulceration and "if mitotic rate cannot be determined." Clark level should therefore be reported whenever it would form the basis for upstaging T1 lesions;[13]

5. tumor growth phase (radial/horizontal *vs.* vertical): in the radial (horizontal) growth phase, the tumor demonstrates a uniform cytological appearance and is generally wider than it is deep; a commonly applied criterion is presence of melanoma in situ three or more rete ridges beyond the invasive component. Vertical growth pattern in superficial spreading melanoma is defined as the presence of one or more dermal clusters larger than the largest epidermal cluster and/or the presence of any mitotic activity in the dermis. Nodular melanomas are by definition vertical growth phase tumors;

6. tumor-infiltrating lymphocytes (TILs): they describe the patient's immune response to the melanoma. When the pathologist examines the melanoma under the microscope, he/she looks for the number of lymphocytes (white blood cells) within the lesion. This response, or TILs, is usually described as "brisk," "non-brisk," or "absent," although it may occasionally be described as "mild" or "moderate." TILs indicate the immune system's ability to recognize the melanoma cells as abnormal;

7. associated nevus (present): this is considered a favorable prognostic factor, probably because nevus-associated melanoma is clinically detected in an earlier phase, with a lower mean value of Breslow's thickness;

8. the pT parameter of the pathologic stage according to the AJCC, 7th edition: this is optional, provided that all the microscopic criteria to retrieve it ar given in the report;

9. the morphology code of the International Classification of Diseases for Oncology (ICD-O) 3rd edition and the Systematized Nomenclature of Medicine Clinical terms (SNOMED CT). The code is /3 for invasive melanoma and /2 for *in-situ* melanoma.

Conclusions

Usually the clinical information provided to the pathologist in the final report is limited to the essential demographic data (age, gender, and body site of the lesion). However, there is growing evidence that providing clinical and dermoscopic images to the pathologist have the potential to improve his/her diagnostic confidence. Several previous studies have indeed demonstrated the benefit of integrating clinical with pathologic information, not only in the field of inflammatory skin disease but also in the context of skin melanocytic tumors. After the excision of a skin lesion, the clinician fills the referral sheet with demographics, site of the lesion and reports his/her clinical diagnosis. When the histopathologic report is rendered, the clinician reviews the case considering the clinical-dermoscopic pictures. Cases for which a good clinical-pathologic correlation is missing are jointly reviewed by the referral clinicians and the referral dermatopathologist combining all relevant clinical and histologic data including clinical-dermoscopic images and a picture selection of histopathologic slides.

A final consensus diagnosis is then reached in light of the case discussion. Fundamental first steps in multidisciplinary care require close cooperation between surgical oncologists and pathologists. The most important aspect of this cooperation is clear and free exchange of information between them. The surgeon should provide the pathologist not only with an adequate tissue sample for examination, but also with clinical details that will assist in establishing a diagnosis. The location and orientation of specimens, and areas of particular concern, should always be indicated. Operative digital photographs may assist this process.

The pathologist, in return, should provide the surgeon with a report containing sufficient information to allow an evidence-based management plan to be made for the patient, and to permit an accurate indication of prognosis to be determined.

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REVIEW

PRACTICAL RECOMMENDATIONS FOR THE MANAGEMENT OF SKIN CANCER PATIENTS

Staging and follow-up of cutaneous melanoma patients

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ABSTRACT

Melanoma is responsible for the greatest number of deaths caused by skin malignancies. The purpose of monitoring patients diagnosed with melanoma is to allow early detection of recurrence and any subsequent primary tumors. Several dermatological and oncological societies developed their own set of guidelines for the surveillance and management of melanoma patients depending on the stage of the disease. The object of this article is to provide a comprehensive, systematic overview that summarizes and interprets previous studies, to characterize current practices regarding progression of melanoma, division into stages of development, and subsequent surveillance. We have performed a systematic review search to December 2016 using the MEDLINE database and performed a manual search of selected references. We examined the staging system and the different surveillance programs for melanoma patients. Consistent recommendations with proven evidence are available for staging melanoma patients. Conversely, recommendations are more controversial for follow-up procedures. Given the inadequate number of randomized controlled trials, consensus on the best, universally-applicable follow-up procedure has not been reached and interpretation of the roles of imaging and laboratory tests, as well as of the appropriate frequency and duration of physical examinations, vary widely. Based on a universally-accepted staging system different surveillance procedures have been developed, which may be mainly classified in two groups: low- and high-intensity strategies.

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Key words: Melanoma - Neoplasm staging - Follow-up studies.

Cutaneous melanoma is the most deadly cutaneous neoplasm, representing 3-5% of overall skin cancers, but accounting for up to 65% of deaths. Its incidence varies from 3-5/100,000/year in Mediterranean countries to 12-20/100,000/year in Nordic countries, and is still rising.[1] In addition, *in situ* and superficial melanomas are not always included in the statistics, since they are often treated in outpatient clinics, without being reported. The increase in exposure to ultraviolet light seems to play an important role in the on-going increase in incidence. The lifetime risk of developing melanoma is estimated to be 1 in 50, with each affected individual losing an average of 15 years of potential life. This ranks the disease second only to adult leukemia in this respect.[2] Although incidence is still increasing, signs of stabilization of mortality have been identified over recent decades, except in elderly males.[3] For this reason, melanoma represents a major health concern for dermatologists, oncologists, and surgeons, who are constantly seeking to improve prevention, early diagnosis, treatment and early detection of local, regional and distant metastases, as well as any subsequent primary tumors.

Establishing an optimal follow-up strategy for melanoma patients is imperative. This strategy protocol should be related to the staging system, given that survival rates vary according to the specific stage of the disease. There is currently a universally-accepted TNM

staging system, published in 2009 in the seventh edition of the American Joint Committee on Cancer (AJCC) Melanoma Staging and Classification (Tables I, II).[4] However, at present, there is no common surveillance program, largely because insufficient randomized controlled trials have been carried out.[5] The purpose of this review is to present the current staging system with associated recommendations on the follow-up care of patients with cutaneous melanoma at different stages of development.

Diagnosis and staging

The stage at melanoma diagnosis has a significant impact on the course of the disease. Most melanoma patients (82-85%) present with localized disease, followed by regional involvement (10-13%), and distant metastatic disease (2-5%).[6] Staging is the main prognostic factor of melanoma, determining management, treatment and surveillance.

Biopsy and histopathological examination

A confirmed diagnosis of melanoma requires a full-thickness excisional biopsy with a minimal side margin of 1 to 2 mm.[7] Enlarging examination criteria to increase the number of sentinel node examinations performed reinforces that wider excisional biopsy side-margins must be avoided. An incisional biopsy may be acceptable for larger lesions and a deep saucerization biopsy may be satisfactory when the lesion is flat and the suspicion of melanoma is not high.[8] Biopsies should be analyzed by an experienced pathologist since diagnosis and initial local staging of the TNM system depend on them. The pathologists should include not only the maximum thickness of the tumor in millimeters (Breslow thickness), but they should also report all of the criteria that are deemed necessary in the AJCC staging system; thus information should be provided on ulceration, regression, lympho-vascular tumor infiltration and clearance of the surgical margins, as well as the mitotic rate only in cases where tumor thickness is less than 1 mm. From these histological features the T-staging grading can be defined. Other features such as anatomical site, degree of sun damage and melanoma type, as superficial spreading melanoma, *lentigo maligna* melanoma, acral lentiginous melanoma and nodular melanoma should be also present in the histological report.[9] These histological subtypes are not actually considered independent prognostic factors.[10, 11] However, the growth pattern conclusively determines the prognosis.[12] The radial growth phase is characterized by melanocytic proliferation in the epidermis and superficial dermis without producing proliferative nodules while the vertical growth phase represents the tumor ability to metastasize.

Breslow thickness is the most important prognostic factor and must be measured from the granular layer, or from the end of the ulceration if present. Depending on Breslow thickness, invasive melanomas are mainly clas-

TABLE I.—*TNM (tumor thickness, nodes and metastasis) staging categories for cutaneous melanoma. Adapted from the final version of 2009 AJCC melanoma staging and classification.*[4]

T-classification	Thickness (mm)	Ulceration status/mitoses
Tis (*in situ*)	Not applicable	Not applicable
T1	≤1.00	a: Without ulceration and mitosis <1/mm²
		b: With ulceration or mitosis ≥1/mm²
T2	1.01-2.00	a: Without ulceration
		b: With ulceration
T3	2.01-4.00	a: Without ulceration
		b: With ulceration
T4	>4.00	a: Without ulceration
		b: With ulceration
N-classification	**N. metastasis nodes**	**Nodal metastasis burden**
N0	0	Not applicable
N1	1	a: Micrometastasis*
		b: Macrometastasis**
N2	2-3	a: Micrometastasis*
		b: Macrometastasis**
		c: In transit metastases/satelites without metastatic nodes
N3	4 +	
	- Metastatic nodes, or	
	- Matted nodes, or	
	- In transit metastases/satellites with metastatic nodes	
M-classification	**Site**	**Serum LDH (lactate dehydrogenase)**
M0	No distant metastases	Not applicable
M1a	Distant skin, subcutaneous, or nodal metastases	Normal
M1b	Lung metastases	Normal
M1c	All other visceral metastasis	Normal
	Any distant metastasis	Elevated

*Micrometastasis are diagnosed after sentinel lymph node biopsy; **macrometastasis are defined as clinically detectable nodal metastases confirmed pathologically.

TABLE II.—*Stage groupings for cutaneous melanoma. Adapted from the final version of 2009 AJCC melanoma staging and classification.*[4]

	Clinical staging *				Pathologic staging **		
	T	N	M		T	N	M
0	Tis	N0	M0	0	Tis	N0	M0
IA	T1a	N0	M0	IA	T1a	N0	M0
IB	T1b	N0	M0	IB	T1b	N0	M0
	T2a	N0	M0		T2a	N0	M0
IIA	T2b	N0	M0	IIA	T2b	N0	M0
	T3a	N0	M0		T3a	N0	M0
IIB	T3b	N0	M0	IIB	T3b	N0	M0
	T4a	N0	M0		T4a	N0	M0
IIC	T4b	N0	M0	IIC	T4b	N0	M0
III	Any T	N>N0	M0	IIIA	T1-4a	N1a	M0
					T1-4a	N2a	M0
				IIIB	T1-4b	N1a	M0
					T1-4b	N2a	M0
					T1-4a	N1b	M0
					T1-4a	N2b	M0
					T1-4a	N2c	M0
				IIIC	T1-4b	N1b	M0
					T1-4b	N2b	M0
					T1-4b	N2c	M0
					Any T	N3	M0
IV	Any T	Any N	M1	IV	Any T	Any N	M1

*Includes microstaging of the primary melanoma and clinical/radiologic evaluation of metastases; **includes microstaging of the primary tumor and pathological information about the regional lymph nodes after sentinel node biopsy of complete lymphadenectomy.

sified into four groups (T1-T4), which are significantly correlated with survival rates. The subgroups (a and b) are based on the presence or absence of ulceration, and, only in T1, on mitotic rate (≥1 mm^2). The 10-year survival rate for melanoma ≤1 mm with no ulceration and mitosis (T1a) is 95%, but drops to 85-88% if the mitotic rate is ≥1 mitosis/mm^2 and/or ulceration is present (T1b).[3] The inevitable margin of error with Breslow thickness measurement is an important concept that clinicians should keep in mind when making management decisions. Ge *et al.*[13] demonstrated a statistically significant difference between the measures of 125 invasive melanomas, with original recorded Breslow thickness of 0.9-1.1 mm, by two different pathologists. This group has identified two possible reasons for this. The first is failure to follow standardized thickness measurement guidelines (including use of an ocular micrometer) and the second, the phenomenon of terminal digit bias, not previously identified as a problem in this field.

Clark level of invasion has an additional prognostic value only in melanomas with Breslow thickness <1 mm,[14] however in the last AJCC edition it has been replaced by mitotic rate. The number of mitosis per square millimeter has been one of the new covariates added in the seventh AJCC edition since it has been demonstrated to be an independent prognostic factor in primary melanoma with Breslow thickness ≤1 mm. This measure should be made in the invasive area of the tumor with the highest mitotic rate, known as the 'hot spot'. If this is not clear, the fosfoistone-H3 antibody can help to identify it. Clark level of invasion is still considered in defining T1 melanomas only in the rare circumstances when mitotic rate cannot be determined. Ulceration is another important prognostic factor that helps to determine the metastatic potential of melanoma. Although not included in the last AJCC staging system, ulceration size may be an additional prognostic factor.[15]

Regression is an immunologic phenomenon that may be categorized into three stages: early regression when lymphocytic infiltrate disrupts the tumor mass and intermediate-late regression when dermal fibrosis is already found. The prognostic significance of tumor regression is controversial, however it has been recently consolidated that tumor-infiltrating lymphocytes grade is a powerful

independent predictor of sentinel lymph node status, melanoma-specific survival, and recurrence-free survival in patients with clinically localized primary cutaneous melanoma ≥0.75 mm in thickness. Those with a pronounced lymphocytic infiltration have an excellent prognosis.[16] Intermediate and late regression are still under discussion. It has been demonstrated that the presence of dermal fibrosis in radial growing melanoma has a negative prognostic value,[17] although it has not been confirmed in further studies.[18, 19] Currently, there is no conclusive statement mainly because of lack of standardized histological criteria. If extensive regression is present (≥75%, in agreement with the College of American Pathologists - CAP - 2009),[20] it should be reported in.

Intralymphatic metastases (*i.e.*, satellites and in transit metastases) belong to the N-staging grouping and are another important histological criteria. The microscopic satellites are defined as any discontinuous nest of metastatic cells larger than 0.05 mm in diameter, that are well separated by normal dermis from the main invasive component of melanoma by a distance of at least 0.3 mm. In transit metastasis is a similar concept but they are found further (>2 cm) from the main invasive tumor component. According to AJCC staging Committee, these early lymphatic metastases are retained in the category of N2c melanoma, that corresponds to IIIB substage. It has been demonstrated that survival outcome of these patients is comparable to that of patients with clinically detectable satellite metastases.[21, 22] The presence of intralymphatic metastases has a considerable impact on prognosis, increasing the probability of cutaneous relapse and local lymph node involvement.

Mutational analysis

Mutational genetic analysis of tumor melanocytes has been recently introduced in clinical practice since advances in the understanding of the molecular basis of melanoma led to the introduction of target therapies. Mutations of different genes have been described in melanoma, mainly in molecules of the RAS/RAF/MEK/ERK mitogen activated protein kinase (MAPK) pathway, responsible for cell proliferation and differentiation. The most frequent involved gene is *BRAF*, a proto-oncogene that codify for a serine/threonine-protein kinase, mutated in approximately 50% of melanomas. Its most frequent mutation is V600E (nearly 80% of *BRAF* mutations).[23] Patients with V600E *BRAF* mutation are usually younger (fifties) with a high number of melanocytic nevi and develop melanoma on intermittent sun exposed areas.[24] Mutational testing is mandatory in patients with advanced disease (unresectable stage IIIC or stage IV) and highly recommended in high-risk resected disease stage IIC, and stage IIIB-IIIC. Testing for *NRAS* and *c-KIT* mutations should be performed when the tumor is *BRAF*-wild type. As *BRAF* and *NRAS* mutations are mutually exclusive, *NRAS* mutations are tested when *BRAF* mutations are not detected.[25] Although there is no available specific target therapy for *NRAS* mutant melanoma, good therapeutic results have been obtained when treating these patients with MEK inhibitors.[26] Mutations of proto-oncogene *c-KIT* are screened when *BRAF* and *NRAS* genes are wild-type; the frequency of *c-KIT* mutations is quite low (1-3% of melanomas) [27] but they are especially common in acral and mucosal melanoma, as well as in melanoma arising on chronic sun exposed areas.[28] When a *c-KIT* mutation is detected, an off-label treatment with *c-KIT* inhibitors can be initiated.[29, 30] Lastly, genetic analysis should be preferentially performed in the metastatic tissue, since discrepancies have been reported between mutations of target genes in the primary tumor and metastatic sites.[31]

Physical examination and instrumental investigations

An accurate physical examination is necessary to complete the initial staging with special attention to other suspicious pigmented lesions, tumor satellites, intransit metastases, regional lymph node and systemic metastases.

Further step is to define the instrumental investigations to complete staging, which are however not recommended in all stage groupings. There is no broadly accepted protocol, but version 2.2016 of the National Comprehensive Cancer network (NCCN) Guidelines is the actual reference (Figure 1).[14]

Lymph node basins are the most common site of melanoma metastasis and are often the first site involved by metastatic disease. It determines the N category, which is primarily defined by the number of metastatic nodes and tumor burden (microscopic vs macroscopic). The sentinel lymph node biopsy (SNLB) is the first clinical decision since lymph node metastases have a great impact on prognosis.[32] Its focus is to identify lymph me-

tastases that are not clinically detected, by analyzing the first single or selected few lymph nodes receiving drainage from the primary melanoma lesion. The presence or absence of nodal micrometastases is the most important prognostic factor in early-stage melanoma, particularly in intermediate thickness melanoma.[33] If lymphatic nodal micrometastasis are left untreated, they may develop into macrometastases and later to a distant disease. The earlier is the diagnosis and the better will be the control of regional disease. SNLB is recommended for patients with melanoma thickness >1 mm and clinically uninvolved regional lymph nodes and should be offered and discussed in selected patients with melanoma thickness <1 mm with high-risk features (T1b, mitotic rate ≥1 per mm^2, ulceration, young age).[22, 34, 35] The depth of invasion is directly proportional to lymph involvement, which reaches 60% in melanomas of more than 4 mm.[36, 37] The use of SLNB in patients with melanomas thicker than 4 mm was initially debated due to their high risk (30-40%) of developing distant metastasis,[38] but it is recommended because recent studies demonstrated its valuable prognostic factor.[39] In patients with intralymphatic metastasis, in transit or satellites (at least IIIB stage) the SLN status does have prognostic significance, and it can upstage a patient to stage IIIC.[40] Decision not to perform SLNB in all these patients may be based on significant patient comorbidities, patient preference, or other factors.

According to the latest AJCC staging system, sentinel lymph node is already considered to be positive with the presence of isolated tumor cells that are solely identified with inmunohistochemical stains such as HMB45 or Melan A/Mart 1. The number of SLNs analyzed and their positivity must be included in the histological report. In addition, the localization with respect to the lymph capsule (intra/extracapsular) of the metastatic cells should be reported.[41] A complete lymphadenectomy should be performed in all positive cases as well as in regional lymph metastatic nodes clinically detected and confirmed by cytology or biopsy sample.[42, 43] When a SLN contains micrometastases, approximately 20% of positive SLN cases will have additional nodes beyond the sentinel node, which also contain metastatic melanoma cells.[44]

Imaging indications are controversial. NCCN guidelines 2.2016 recommend using baseline imaging from stage III; in lower stages only specific signs or symptoms should be explored in the history and physical examination. The guidelines of the Italian Association of Medical Oncology (AIOM) 2015,[35] recommend to perform from stage IIC total body computed tomography (CT) scans (alternatively positron emission tomography [PET] + encephalic magnetic resonance imaging [MRI]), and from stage IB regional lymph nodes and abdominal ultrasounds (Figure 2). Due to the low sensitivity, chest X-ray is discouraged.[45]

The M category is defined by the site of distant metastases and an elevated serum lactate dehydrogenase level (LDH). Therefore, at the time stage IV disease is

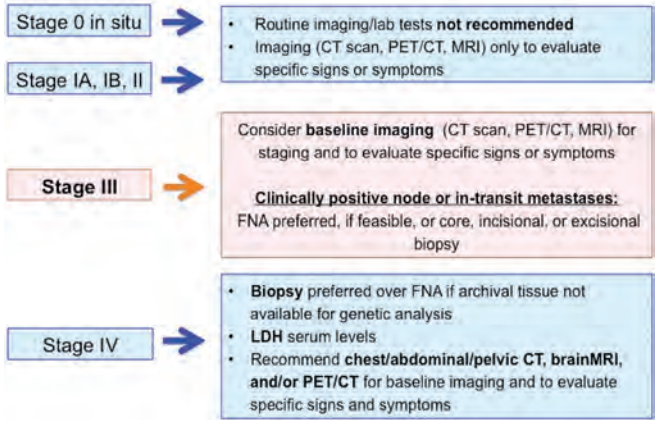

Figure 1.—Clinical stage and staging workup. Adapted from NCCN Clinical Practice Guidelines of Melanoma, version 2.2016.[14]

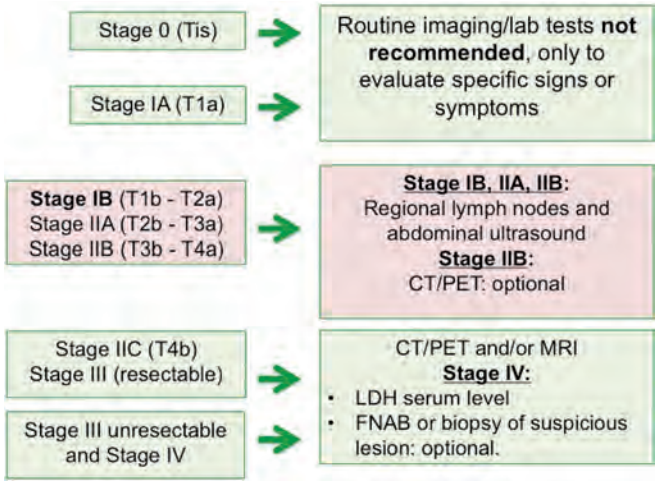

Figure 2.—Clinical stage and staging workup. Adapted from AIOM melanoma guidelines, 2015 edition.[35]

documented, LDH level should be measured, and if elevated, the stage would be M1c regardless of the site of distant metastasis. The updated AJCC melanoma staging database has demonstrated a significant decrease in survival rates in patients with distant metastases and increase in serum LDH (32%, 1-year-overall survival) compared with normal serum LDH (65%, 1 year-overall survival).[4] Furthermore, LDH level was among the most predictive independent factors of shorter survival in all published studies when it was analyzed in a multivariate analysis, even if taking in account the site or number of metastases.[46, 47] The mechanisms or sources of elevated LDH isoenzymes are unclear.

Patient education and follow-up

Patient education

Education of the patient diagnosed with melanoma plays an important role, since more than half of disease recurrences are detected by patients themselves or their partners.[48] This rate may be improved by a better training; patients must be counseled on how to perform monthly self-examination of their skin and peripheral lymph nodes. Moreover, correct sun behavior has to be highlighted, providing information about sunscreens and protective cloths. Finally, information about the increased risk of melanoma in family members is also mandatory.

Follow-up and surveillance

The goal of any cancer follow-up regimen is early detection of local, regional and distant metastases, as well as early recognition of eventual subsequent primary tumors. Thereby an impact on long-term outcome may be possible with an early therapeutic approach. However, this potential benefit has not been proven yet in melanoma, therefore follow-up remains a controversial issue, in the attempt to balance clinical benefits and costs of intensive follow-up.[34]

There are considerable data showing that most recurrences will develop during the first 5 years.[49] Eight percent of all melanoma patients develop a second melanoma within 2 years after the initial diagnosis [50] and 35% of patients with *lentigo maligna* melanoma develop another cutaneous malignancy within 5 years.[51]

Recommendations for follow-up, frequency and instrumental investigations, are strictly dependent on disease staging, given that survival rates vary according to the specific stage. The different dermatological and oncological societies published evidence-based follow-up strategies that try to balance medical needs of the patient with improved survival and economic costs for health care system. Due to lack of randomized controlled trials, wide variations in guidelines and recommendations exist for patient follow-up and surveillance. Currently, surveillance strategies can be broadly classified into low intensity strategies (USA, UK, Australia and New Zealand) [6, 52-55] and high intensity strategies (rest of Europe other than UK).[35, 56-61]

All strategies agree with the importance of medical history and physical examination, but there is no consensus on the optimal frequency and duration after a primary melanoma diagnosis. Overall, they are recommended to be lifelong follow-ups and at least once a year, mostly adjusted by personal risk factors such as fair skin, multiple nevi and family or personal history of melanoma. Once a year would be enough for patients with melanoma *in situ*, but frequency may become narrower for higher stages, particularly during the first five years after diagnosis.

Imaging is a topic of debate and challenge. Low intensity strategies recommend imaging mostly based on symptoms, with this determination being argued by the little evidence of increasing patient outcome with more exams and by the economic burden. It also considers the anxiety that patients can experience with frequent imaging procedures, especially in cases of false positive findings.

The American Academy of Dermatology (AAD) Guidelines [52] advise clinicians not to perform imaging exams in any stage unless specific symptoms are present, and although they can be considered in patients with high risk for recurrence, they are not recommended after five years.

The NCCN [14] recommends imaging from stage IIB to IV. Chest X-ray, CT and/or PET scans are indicated every 3 to 12 months, and MRI scans of the brain once a year. Routine follow-up radiologic imaging is not recommended for stage IA to IIA melanomas.

Several researchers have questioned the benefit of chest X-ray for screening of pulmonary metastasis in asymptomatic patients; it has a high rate of false positive and negative findings and does not improve surviv-

al.[62-64] Another imaging modality limited by the number of false positive rates is CT scans. It has been recently demonstrated to be able to detect distant recurrence in 18.5% of stage II and 33% of stage III disease.[65] CT scans should be recommended only in patients with high risk of developing distant metastasis.

The Guidelines for the management of melanoma in Australia and New Zealand [54] recommend only ultrasound as imaging modality in patients with advanced disease, but only if performed by an experienced ultrasonographer.

Ultrasonography of regional lymph nodes has been reported to improve survival rates and is clearly superior to palpation for the detection of lymph node metastases.[66] It has been considered the most accurate imaging procedure in surveillance of patients with stage III and IV melanomas.[65-67] Ultrasound penetration is limited, therefore intestinal metastasis cannot be early detected. Furthermore, imaging by ultrasound is modified by abdominal air, therefore abdominal sonography has a low sensitivity for small metastases detection.

The European Society of Medical Oncology [57] and The British Association of dermatologists [55] do not provide specific guidelines for imaging exams, but CT and/or PET scans are suggested in high risk (*i.e.*, those with thick primary tumors or recent tumor resection) and in stage IIIB and IV patients.

The Swiss Melanoma Guidelines [58, 59] offer specific recommendations depending on TNM staging. Locoregional lymph node sonography, abdominal sonography and chest X-ray are recommended from stage I (T2N0) to III, and whole body imaging by CT, PET or MRI from stage IIC to III. In patients with stage IV, all physical, laboratory, and imaging evaluations are considered on an individual basis. These guidelines are very similar to the Italian recommendations proposed in 2007,[61] and to those of AIOM,[35] that propose locoregional lymph node and liver sonography from stage IIB and CT/PET scans from stage III.

The German Cancer Society and German Dermatologic Society [60] advise locoregional lymph node sonography for patients stage IB to IV and abdominal sonography only if symptoms are present. CT, MRI or PET scans are suggested in patients with stage IIC to IV.

Use of blood tests to monitor disease recurrence and progression is also controversial in follow-up programs. The ESMO, German and Swiss guidelines recommend testing serum S100 protein levels. A meta-analysis performed by Mocellin *et al.*[68] suggested that S100 may play a role in surveillance of patients with stage I to III

TABLE III.—*Follow-up scheme proposed by an Italian group of expert clinicians. Adapted from Moscarella* et al.[5]

Stage	Clinical and dermoscopic examination Interval	Imaging exams		
		Lymph node ultrasound	Abdominal ultrasound	Total body CT scans
0 Melanoma *in situ*	- 12 months - 6 months if multiple nevi and/or personal or family history of melanoma	None	None	None
IA Melanoma ≤1 mm with <1 mitosis/mm²	- 6 months for 5 years then - 12 months	Every 12 months for 5 years or based on clinical exam	None	None
IB - Melanoma ≤1 mm with mitoses or ulceration or - Melanoma 1.01-2 mm with no ulceration	- 4 months for 5 years then - 6 months for 5 more years than - 12 months	Every 6/12 months for 5 years or based on clinical exam	None	None
II - Melanoma 1.01-2 mm with ulceration or - Melanoma >2 mm	- 4 months for 5 years then - 6 months for 5 more years than - 12 months	Every 12 months for 5 years	Every 12 months for 5 years	Once per year for 5 years At 6 months interval with ultrasound
III - Any T with lymph node metastasis and/or - In transit or satellite metastasis	- 4 months for 5 years then - 6 months for 5 more years than - 12 months	Twice per year for 5 years then once per year for more 5 years (visit I and III in the year)	Twice per year for 5 years then once per year for more 5 years (visit I and III in the year)	Once per year for 5 years Alternating with ultrasound (visit II in the year)

disease, but it should not be implemented routinely as a prognostic marker in all melanoma patients. LDH is not recommended during follow-up by any guidelines, but only in the initial workup of stage IV disease by the NCCN and AAD.

Finally, a simplified schedule for routine follow-up of melanoma patients was developed by an Italian expert group of clinicians (Table III).[5] The aim of this group was to standardize the follow-up recommendations based on the currently used 2009 AJCC staging system. As the other strategies, clinical examination remains the most solid and useful tool and is intended to be lifelong. Type and frequency of clinical and imaging examinations (regional lymph nodes and abdominal sonography, total body CT scans) depend on stage of disease. Due to the low positive predictive value of blood tests (LDH, S100) they are not included in the surveillance process.[68] These recommendations should be flexibly understood, since history and physical examination of every patient tailor the examinations required.

Conclusions

Currently, a universally accepted staging system is available in order to classify melanoma patients. However, there is not yet an optimal follow-up program, since surveillance imaging and blood tests have not yet demonstrated a significant improvement of life expectancy. Because of this, the different monitoring strategies vary in the frequency of history and physical examination but largely in the utility of imaging and blood exams. It will certainly change in the near future with the increasing use of the new therapeutic agents, that greatly improve survival of melanoma patients with advanced disease.

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REVIEW

PRACTICAL RECOMMENDATIONS FOR THE MANAGEMENT OF SKIN CANCER PATIENTS

Treatment of metastatic melanoma: a multidisciplinary approach

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ABSTRACT

The prognosis of stage IV metastatic melanoma is poor. An overall 1-year survival of 25.5% and a median survival of 6.2 months were reported without any significant improvement during the last 30 years before the introduction of new drugs (immune checkpoint inhibitors and targeted therapies) which completely modified the therapeutic approach and induced an overwhelming improvement on the survival rates of these patients. This review will analyze the therapeutic tools available for the treatment of patients with metastatic melanoma, including adjuvant interferon and locoregional therapies (surgery, radiotherapy and electrochemotherapy) and will mainly focus on the presentation of results obtained by the new treatments (checkpoint inhibitors and targeted therapies).

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Key words: Melanoma - Neoplasm metastasis - Molecular targeted therapy - Electrochemotherapy - Radiotherapy.

The prognosis of advanced metastatic melanoma is poor. The unsatisfactory results obtained by (bio) chemotherapy were clearly summarized in a review study by Korn *et al.*,[1] which analyzed the clinical data of more than 2000 patients enrolled onto 42 phase II trials since 1975. An overall 1-year survival of 25.5% and a median survival of 6.2 months were achieved, without any significant improvement during the last 30 years.

It is well known that T-cell responses are regulated through a complex balance of inhibitory and activating signals and that the tumor itself can dysregulate these pathways, leading therefore to an impairment of the immune system activities. The relevant new concept that was developed following the failure of cytokine-based immunotherapy and the increasing evidence of the clinical activity of different targets therapies in several cancer types was constituted by the potential of targeting these inhibitory and activating immunological synapses as a new tool to promote the immune response.[2] Until now, two main types of immune modulating drug antibodies have been developed and used in the treatment of advanced metastatic melanoma, the first targeting the CTLA-4 antigens, the other the PD-1/PD-1L pathway. Both compounds interact with immunological checkpoints physiologically

TABLE I.—*Decision-making factors in advanced metastatic melanoma: pre-treatment parameters to be evaluated.*

Parameter		Significance	Marker
Mutation pattern	–	Possibility to prescribe a molecular target therapy	BRAF, NRAS, cKIT
Performance status (PS)	–	Candidate for active therapy or only palliation	ECOG Performance Status
	–	Need to obtain a quick response	
High/low tumor load	–	Responsiveness to systemic treatment	LDH Blood chemistry CT/MRI images PDL-1 expression
Brain metastases	–	Risk of CNS symptoms	CT/MRI images
Progression pattern (low/fast)	–	Responsiveness to systemic treatment	LDH
	–	Need to obtain a quick response	PET scan CT/MRI images
Clinical trials	–	Availability of clinical trials	N/A

CNS: central nervous system; LDH: lactate dehydrogenase; CT: computed tomography; MRI: magnetic resonance imaging; PET: positron-emission tomography; N/A: not available.

leading to inhibitory signals for the immune system; the blockade of these pathways by the drugs allows to release the break to the immune system thus fostering, maintaining and stimulating the T-cell responses.

The other key stone achievement in melanoma is the identification of "driver" mutations in specific genes involved in the pathways of growth and differentiation of melanoma cells. The most frequent is represented by the BRAF mutation, which is harbored by approximately 50% of melanomas, more frequently those arising on skin without chronic sun-induced damage (Table I).[3]

The introduction of new therapeutic drugs, both the immune checkpoint inhibitors and the target therapies, has determined a striking effect on our possibilities to manage the metastatic disease, giving rise to an overwhelming improvement on the survival rates of these patients.[4]

This review will analyze the therapeutic tools available for the treatment of patients with metastatic melanoma, including adjuvant interferon and loco-regional therapies (surgery, radiotherapy and electrochemotherapy) and will mainly focus on the presentation of results obtained by the new treatments (checkpoint inhibitors and targeted therapies) (Figures 1-3).

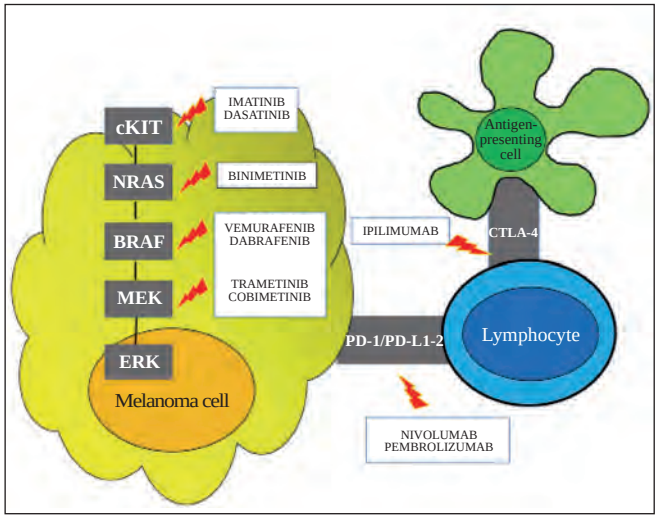

Figure 1.—The scenario of new treatments: targeted therapies and immune checkpoint inhibitors.

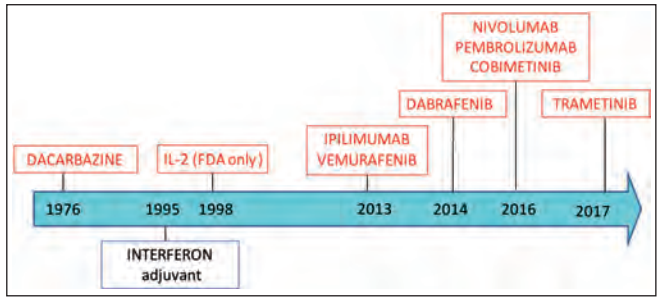

Figure 2.—Time point of approval for systemic treatments in metastatic melanoma.

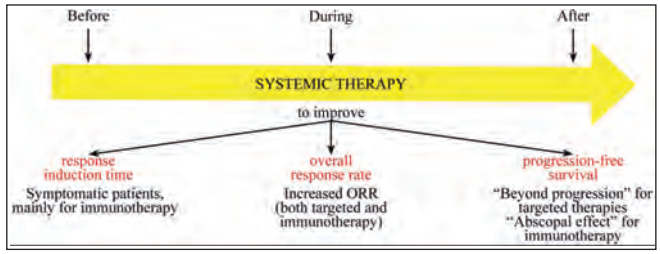

Figure 3.—Association of local and systemic therapies: why, when and in which patients.

Locoregional therapies

Surgery

Surgery can be considered to treat a localized disease or a symptomatic lesion in a patient with widespread disease and still constitutes a relevant therapeutic tool in the treatment of stage IV metastatic melanoma.

The most important rational for performing surgical resection as first option in stage IV melanoma is mainly based on the evidence that, despite new medical treatment options, complete responses occur only in a minority of patients following these approaches whilst surgery can allow the complete removal of a lesion with acceptable morbidity and mortality. However, surgery alone is seldom curative in a metastatic setting as the majority of patients develop disease progression in other sides due to the widespread diffusion of micrometastatic disease clinically silent and not detectable by imaging at the time of surgical treatment.

It is therefore relevant to select very carefully the patients amenable of surgical treatment, which is possible as first choice of therapy in about 25% of stage IV patients according to literature data.[5] The factors to be considered as prognostic parameters for surgery have been identified:[6] *i.e.*, limited disease extension with the possibility to complete resection, prolonged disease-free survival before and a tumor-volume doubling time potentially of >60 days.[7, 8]

No randomized clinical trials are available comparing surgery *vs.* systemic therapies in this patient setting, however, a series of single arm or comparative studies reported very favorable survival rates in selected series of patients. The Southwest Oncology Group trial 9430 reported the results of 64 patients treated by surgery with complete excision and prospectically included. The median relapse-free survival was 5 months, whilst the 1- and 2-year percent survival rates were 75% and 47%, respectively.[9] Data of patients included in the MSLT-I trial were analyzed as to the survival after stage IV diagnosis according to the treatment (surgery or standard medical treatment or both): the results clearly demonstrated a survival advantage for patients treated by surgery with or without standard medical treatment, with a median survival of 15.8 *versus* 6.9 months for patients treated by standard medical treatment alone, and 4-year survival 20.8% *versus* 7.0%.[10] It should be taken in consideration that the majority of the studies have been performed in an era when target or immunotherapy were not available yet.

The introduction in the clinical practice of new drugs at the beginning was seen as the end of the role of surgery in the treatment of stage IV melanoma. With the increasing evidences about the possibilities and the pitfalls of the new drugs, clinicians have realized that we will not assist to a decrease in the relevance of surgery, but to an increasing need of understanding the possible integration with the different treatment approaches in the management of patients. For example, surgery can be positively associated with immunotherapy in an attempt to reduce the disease burden and thus increase the effectiveness of the immune response and the clinical activity of the treatment. In this regard, it has been reported that a smaller disease burden is associated to a higher response rate and to longer survival.[11] Thus, surgical accessible metastasis should be potentially excised or treated with electrochemotherapy.[12] The main issue still under consideration is the timing, because a surgical approach can delay the start of a potentially life-treating treatment. Data on timing of surgery in potentially immunotherapy-enrollable stage IV melanoma patients are inconsistent.

Furthermore, surgery can play a role at the end of immunotherapy to remove the partial responded metastasis. Metastases after immunotherapy can regress leaving scar tissue or necrotic area potentially impacting on the quality of life. In this case, surgery can be performed.

So far, immunotherapy moreover shows a response rate around 11% for anti CTLA-4 treatment and around 40% for antiPD-1. Thus at least around the 50% of the patients will not respond to immunotherapy, and if not BRAF mutated, the surgical option can be considered as well as radiotherapy or other loco-regional approaches among the available palliative treatments to increase the quality of life of patients with symptomatic metastasis.

Similarly, surgery can be favorably associated to target therapy, to increase the percentage of complete responses (usually not so high) and thus render the patient NED or to manage the local progression and maintain the systemic treatment ("beyond progression"). At the same time, surgery could be in some cases performed at diagnosis when it is demonstrated that smaller metastatic volumes usually are associated to longer response to treatment.

Also for target therapy, the integration with surgery is

a matter of discussion in terms of timing. The medical treatment can be used in a neoadjuvant or adjuvant setting or in case of a not radical excision.

It should not be forgotten that surgery is always a potential life treating option in case of severe complication for immunotherapy and targeted therapy (bowel perforation or obstruction). The role of surgery for side effects (such as squamous cell carcinoma) should also be considered in stage 4 melanoma patients under target therapy.[13, 14]

Electrochemotherapy

Electrochemotherapy is a recently introduced but already well-recognized therapeutic tool for the local treatment of cutaneous and subcutaneous metastases of different tumor origin which foresees the delivery of electric pulses directly onto the skin lesions in association with the administration by local or systemic injections, of a low dosage of chemotherapy (bleomycin or cisplatin). A large series of data have demonstrated its clinical activity and tolerability in patients with cutaneous melanoma metastases, with response rates ranging up to 90% of treated lesions.[15-17] Recently factors influencing treatment efficacy were identified: *i.e*. coverage of deep margins, absence of visceral metastases, presence of lymphedema, treatment of non-irradiated areas and tumor size <3 cm.[18] The main indication for this treatment is in patients with loco-regional metastases, *i.e*. with stage III melanoma, whilst in stage IV the frequent contemporary presence or the high risk of developing in a short period visceral metastases in association with the skin localizations imply that apart from selected cases, it is recommended to treat these patients with a systemic approach.

Recently, the safety and effectiveness of electrochemotherapy on recurrent melanoma has also been retrospectively evaluated after interferon alpha (IFN-α) adjuvant therapy of melanoma patients.[19] Taking into account all metastases treated from all patients together there was an 85% complete response rate probably due to immune system activation by electrochemotherapy, which was previously modulated by IFN-α.

Electrochemotherapy however is favorably associated both with target therapy and with immunotherapy to increase the local response in the skin or to induce responses in sites non-responding to treatment.

The association between electrochemotherapy and BRAF-inhibitors was first reported in a case regarding a patient undergoing dabrafenib therapy. Electrochemotherapy during dabrafenib proved to be a safe and valuable option in a challenging patient who developed tumor resistance exclusively on superficial metastases.[20]

After this first experience a study was conducted with the aim to explore *in vitro* the effectiveness of electrochemotherapy during the treatment of melanoma patients with BRAF inhibitors.[21] The study demonstrated that electrochemotherapy with bleomycin is as effective, or even more effective on BRAF mutated, compared to non-mutated melanoma cells (*BRAF*^{V600E} mutated cells required 2 times lower concentration of bleomycin compared to non-mutated cells), although the exact biological mechanism still needs to be explored. Furthermore, an interaction of electrochemotherapy and vemurafenib treatment was observed in BRAF mutated melanoma cells, indicating on more than additive or synergistic effectiveness. The enhanced effectiveness supports the possibility to use electrochemotherapy concomitantly during the treatment with BRAF inhibitors even if further clinical studies with larger number of patients are needed.

There is also a robust rationale for the association of electrochemotherapy and immunotherapy. Recent findings suggest indeed that electrochemotherapy may exert a role in boosting anti-tumor immunity by promoting Langerhans cell migration from the tumor to draining lymph nodes and dendritic cells recruitment at the site of the lesion thus inducing a sort of *in-situ* vaccination.[22] An enhanced exposure of tumor-associated antigens and better accessibility of immune cells to tumor antigens may explain the development of an antitumor immune response. Dendritic cells can efficiently prime melanoma-specific $CD8^+$ lymphocytes, stimulating their migration to the inflamed skin. The inflammatory infiltrate shows, after an initial Treg cell decrease, a substantial presence of $CD8^+$ lymphocytes and NK that surrounds the tumor cells from the earliest phases until 2 months after electrochemotherapy.[23, 24]

On the basis of these findings, a series of clinical cases have been reported highlighting the synergistic activity of electrochemotherapy and anti-checkpoint inhibitors.[25, 26]

A retrospective analysis of the combination of an approved anti-CTL4 immuno-oncology agent (ipilimumab) with electrochemotherapy in patients with advanced

melanoma allowed to obtain a disease control rate of 60% and a stable disease rate on 44% after 3 months.[27] Recently, the retrospective evaluation of electrochemotherapy combined with ipilimumab or PD-1 inhibition in 33 patients with unresectable or metastatic melanoma showed local overall response rate (ORR) of 66.7%. The systemic ORR was 19.2% and 40.0% in the ipilimumab and PD-1 cohort, respectively.[28] In both series the local response was lower than reported for ECT only.[15-17] This can be attributed to the micro-environmental biological changes caused by previous local or systemic treatments administered to patients. In a third retrospective series of 45 melanoma patients, the addition of a local treatment (radiotherapy or electrochemotherapy) to systemic immunotherapy significantly prolonged overall survival.[12]

In conclusion, the combined treatment of electrochemotherapy with immune checkpoint blockade (ipilimumab, pembrolizumab, nivolumab) as with BRAF and MEK inhibitors proved to be feasible, tolerable and showed a high systemic response rate. However prospective trials are needed to consolidate the role of this local procedure together with the new treatment strategies in order to improve the outcome of melanoma patient.

Radiotherapy

Melanoma was generally considered a radio-resistant tumor, thus radiation therapy (RT) for long time represented a purely palliative option. RT was usually reserved to patients with painful lesions mainly located to the bones, nodes or skin, or in the presence of bleeding lesions not amenable with other treatments; patients with brain metastases were also considered as candidate for RT, mainly to manage the symptoms related to edema, however with discouraging results. Whole-brain radiotherapy (WBRT) represented the standard approach, with the most common dose/fractionation schedule being 30 Gy in 10 fractions over two weeks. Although studies have shown a potential clinical benefit, WBRT has no significant impact on overall survival and may be associated to detrimental neuro-cognitive outcomes.[29, 30] Retrospective observational studies showed a median survival time of only 14 weeks after WBRT alone.[31] Many attempts were made in order to improve the results of WBRT on brain metastases, through radiation dose escalation, radio sensitization or the addition of chemotherapy, without significant therapeutic gains.[32] The striking results obtained so far by the use of targeted agents and immunotherapy for metastatic melanoma, in different sequences, opened a new window for the multidisciplinary management of patients with brain metastases, both at diagnosis or at the time of intracranial relapse/progression.[33] At the same time, magnetic resonance imaging (MRI) and radiosurgery (SRS)/fractionated stereotactic RT (FSRT) became standard procedures, rapidly replacing WBI in most situations. Patients with <3 metastases treated with surgery or SRS had better survival in comparison with those with diffuse disease receiving WBI.[34] Recent studies also showed promising results for SRS for patients with more than 3 lesions. Yamamoto *et al.*[35] evaluated the role of SRS in a cohort of 1194 patients with 4 or more metastases from various histologic examinations, showing an equivalent median survival in patients with 5 to 10 brain metastases compared to those with 2 to 4 lesions. Likewise, no differences in median survival time, neurological deterioration and toxicity emerged in another study from the same Authors evaluating the role of SRS alone in patients with 10 or more lesions compared to patients with 2 to 9 metastases.[36]

The timing of brain directed focal treatments in combination with new systemic therapies, especially immunotherapy response evaluation and potential additional toxicities are critical issues to be addressed in the future. Barker and Postow recently reviewed the clinical outcomes of the combination of various immunotherapy strategies and RT for melanoma:[37] investigators at Yale University reported on 77 patients with brain metastases treated with SRS, with patients who received ipilimumab having a median survival of 21.3 months *vs.* 4.9 months for those who did not. Survival was not significantly different whether the drug was given before or after SRS.[38] In a similar study, investigators from New York University reported on 58 patients treated with brain SRS. No difference in local tumor control, survival, or frequency of intracranial hemorrhage in patients who did or did not receive ipilimumab was reported.[39] Investigators at Memorial Sloan-Kettering Cancer Center (MSKCC) recently reported on 45 patients treated with ipilimumab and brain SRS. On multivariable analysis, prolonged survival was associated with the delivery of SRS during ipilimumab.[40] The potential compli-

cations of brain SRS and ipilimumab were studied in a small series of 3 patients: in patients receiving brain SRS to 20 Gy in 1 fraction, followed several months later by ipilimumab, radiation necrosis was observed histologically in 1 case and radiologically in 2.[41]

Parallelly to the increasing use of SRS for brain metastases, also extracranial stereotactic radiotherapy, namely stereotactic body radiotherapy (SBRT) or stereotactic ablative radiotherapy (SABR) rapidly expanded in recent years, for lung, lymph-nodal, liver, bone, adrenal gland metastases.[42]

Its application has been tested for advanced melanoma patients, especially those who are candidate for immunotherapy, due to the peculiar synergistic effects occasionally observed between the two treatments and the great potential for future possible combinations.[43-46] Experimental findings showed that RT is able to convert the tumor in an *in-situ* vaccine, altering the microenvironment towards the development of an "immunogenic hub:" in particular hypofractionated SBRT resulted to be able to promote both the priming and effector phases of anti-tumor immune response in animal models, with initial promising clinical results. Retrospective clinical reports showed that the combination of RT, often used as palliative treatment, and CTLA-4 inhibitors was able to trigger the abscopal effect in a proportion of melanoma patients, and that this effect might prolong survival.[44, 45, 47-50] In particular, Grimaldi *et al*. reported on 23 patients treated with RT on various metastatic sites after ipilimumab, reporting abscopal responses in 11/23 (52%). Most patients were treated for disease progression at a median time of 5 months after the last ipilimumab dose. Median OS for patients with abscopal response was significantly higher than for patients without abscopal response (22.4 months *vs.* 8.3 months).[50]

With regards to the association between RT and target therapies, particularly BRAF inhibitors, few clinical data are available, mostly on brain metastases.

Preliminary clinical reports evaluated the efficacy and safety of RT plus vemurafenib in patients with *BRAF*-^{V600E} mutated melanoma brain metastases;[51] 6 patients received SRS and 6 WBI, respectively; vemurafenib was administered prior to radiation therapy in 7 and concomitantly in 5. Median time to intracranial progression was 14.5 months. Ahmed *et al.*[52] also showed very good local control rates after vemurafenib and SRS with low toxicity. The 6-month intracranial control of about 45% appears comparable to first reports; a median time to intracranial progression of 12.9 weeks was then reported by Gaudy-Marqueste *et al.*[53] In the latter series, 53 lesions were treated with gamma knife SRS prior to or concurrent with vemurafenib or dabrafenib, in 30 patients; 20/30 received concomitant treatment without any increase of radiation induced toxicity (scalp radiation dermatitis). Median OS was 48.8 weeks, 6-month overall survival rate 78.8%, and 12.9-week median time to intracranial progression. Xu *et al*.[54] reported on a cohort of 65 patients treated with SRS and BRAF divided in 3 groups: Group A (BRAF-mutated untreated, N.=13); Group B (BRAF mutated treated with BRAF inhibitors, N.=17); and Group C (wild-type BRAF, N.=35). Median OS was superior for BRAF mutated patients treated with SRS in conjunction with BRAF inhibitors (Group B) *vs.* patients with wild-type BRAF (Group C, 23 *vs.* 8 months and 13 *vs.* 5 months, respectively; $P<0.01$).

The toxicity profile of the combination of SRS and BRAF/MEK inhibitors is uncertain. Ly *et al*.[55] found an increased risk of hemorrhage (61% *vs.* 23%, no statistical analysis) in their retrospective study on 17 patients, in which BRAF inhibitors were interrupted during SRS. In contrast, Wolf *et al*.[56] showed no significant difference in bleeding rate after combined treatment (16% after BRAF and SRS *vs.* 8% after SRS; not statistically significant). Special caution should be paid to the few cases where WBRT is indicated for patients on treatment with BRAF inhibitors, due to the potential occurrence of severe skin acute side effects. This phenomenon, which is more frequent with vemurafenib than dabrafenib, justifies the need to temporarily interrupt treatment before WBRT.[57]

Systemic therapies

Adjuvant interferon

Hypothetically, non-detected melanoma micrometastases might be the cause of future relapses and/or may induce tumor tolerance in the host. Adjuvant IFN-α therapies, which could induce TH1 anti-tumor responses, are based on these hypotheses and might be of benefit to some patients with possible micrometastases.[58]

Until now, IFN-α is the only drug approved in the EU for the adjuvant melanoma treatment (Table II). There

TABLE II.—*Summary of the most relevant studies analyzing interferon's clinical activity in the adjuvant treatment of melanoma.*

Clinical trial	Stage	N.	Study design/ IFN dose	DFS HR	DFS P value	OS HR	OS P value
NCCTG	I, II	262	20 MU/m^2 IM tiw ×3 mo	0,76	NS	0.90	NS
E1684	IIB-III	280	HDI *vs.* controls	0.67	0.0023	0.73	0.0237
AMCG	IIA-IIB	311	3 MU ×3 wk then tiw ×11 mo	0.61	0.02	NR	NR
FCGM	IIA	499	LDI ×18 mo	0.74	0.035	0.70	NS
E1690	IIB-III	608	HDI *vs.* LDI *vs.* controls	0.81	0.03	0.98	NS
SMG	IIB-C (>3 mm) III	95	LDI ×6 mo *vs.* controls	0.80	NS	0.86	NS
E1694	IIB-III	774	HDI *vs.* GM2	0.67	0.006	0.72	0.04
WHO 16	III	444	LDI *vs.* controls	0.88	NS	0.95	NS
E2696	IIB, III	107	Phase II HDI + GM2	0.59	<0.05	NR	NR
UKCCCR	IIB, III	674	Phase III, LDI ×2 yr *vs.* controls	0.91	NS	0.94	NS
EORTC 18871	IIB-C, III	800	IFN-2α 1 MU/d ×1 yr *vs.* IFNγ 0.2 mg/d ×1 yr	1.05	NS	0.98	NS
EORTC 18952	IIB-C, III	1000	10 MU/d ×1 mo then 10 MU tiw ×11 mo *vs.* 5 MU tiw ×23 mo *vs.* controls	0.88	NS	0.91	NS
DeCOG	III	444	LDI *vs.* dacarbazine + LDI *vs.* controls	0.69	0.0045	0.62	0.005
EORTC 18991	III	1256	Peg-IFN *vs.* OBS	0.84	NS	1.0	NS
HeCOG	III	353	induction HDI *vs.* standard HDI	NR	NS	NR	NS
DeCOG	II (>1.5 mm)	850	LDI 18 mo *vs.* 60 mo	1.05	NS	1.1	NS
EADO	II (>1.5 mm)	898	LDI *vs.* peg-interferon	0.91	NS	NR	NR
UK	IIB-C, IIIB-C	194	HDI with or without maintenance	0.89	NS	0.59	0.05
DeCOG	III	649	Intermittent HDI *vs.* standard HDI	1.21	NS	1.03	NS
DeCOG	IIB-C, III	909	Peg-interferon *vs.* LDI	1.09	NS	1.05	NS

IFN: interferon; LDI: low-dose interferon; HDI: high-dose interferon; tiw: three times a week; mo: months; wk: week; d: day; yr: year; NS: not significant; NR: not reported.

is a possible benefit of this treatment in patients who are at higher risk of disease recurrence and in the daily practice the treatment should be offered to stage II and III melanoma patients.

Low- (LDI), intermediate- (IDI) and high-dose (HDI) IFN-α regimens have been tested in randomized trials in an adjuvant setting, but the studies greatly differed also in terms of the therapy duration, route of administration and the type of IFN used. These are some of the reasons for the still-ongoing discussions about the use of IFN in the adjuvant melanoma setting in the scientific community. Despite several studies comparing IFN at different dosages we still did not reach a consensus about the optimal dose schedule and treatment duration.

Some randomized trials reported a reduction of melanoma recurrence, but a significant impact on overall survival (OS) was only shown with the high-dose IFN-α2b intravenous regimen (HDI) when compared to observation only (US Intergroup trials E1684: median OS 3.82 *vs.* 2.78 years, P=0.0237) and the GMK vaccine (E1694: OS HR=1.52; P=0.009).[59-62] The trial was composed of a 4-week induction phase with 20×10^6 IU/m^2/day for 5 days, followed by a maintenance phase with IFN-α2b subcutaneously at 10×10^6 IU/m^2/day three times/week for 48 weeks. The outcomes of the E1684 trial in 1995 led to the regulatory approval of IFN by the US Food and Drug Administration (FDA).[63] However, these results were not confirmed in the following E1690 trial that compared HDI *versus* LDI *versus* control, failing to demonstrate a significant benefit of the HDI, but bearing more adverse events.[60] Furthermore, different randomized trials reported other conflicting results, never offering the real hint to the therapeutic benefit of IFN. The randomized phase III DeCOG trial compared LDI *vs.* LDI plus dacarbazine *vs.* observation in stage III melanoma patients. The colleagues found a DFS and OS for the LDI regimen, and, interestingly, a worse therapeutic effect when dacarbazine was added.[64] Other studies investigated the impact of IFN treatment duration. A phase II study compared the HDI induction phase only to induction HDI phase plus maintenance treatment, and found that clinical outcomes were bet-

ter in patients in the longer regimen, supporting the hypothesis that treatment duration and not the dose could be related to therapeutic benefits.[65]

The EORTC 18952 adjuvant IFN trial was performed including 1388 resected stage IIB/III melanoma patients.[66] In the trial the researchers compared a 4-week induction phase using IFN at 10×10^6 IU/m^2/day for 5 days/week for 4 weeks, followed by a maintenance phase with 10×10^6 IU three times a week for 12 months, to 5×10^6 IU 3 days/week for 24 months, to observation alone. After the long median follow-up of 11 years, the only difference reported was the distant metastasis-free interval with an HR of 0.95 for the shorter maintenance group *versus* HR of 0.82 for the longer maintenance group (P=0.027).

A meta-analysis found that IFN-α slightly improved DFS (risk reduction: 18%) and OS (risk reduction: 11%) in high-risk cutaneous melanoma patients, however, there were no differences between low and high dosages.[67] Wheatley *et al.* reported a 5-year absolute benefit of about 3%, with greater efficacy in patients where the primary tumor was ulcerated.[68] Indeed, also a pooled analysis of the EORTC trials 18952 and 18991 found that the primary tumor ulceration and the higher number of lymph nodes involved, could be predictive of IFN efficacy.[69]

Clinical trials comparing adjuvant HDI to ipilimumab (NCT01274338, NCT01708941, NCT02506153) or to pembrolizumab (NCT02506153) are ongoing, and the results are still pending.

Pegylated IFN-α (peg-IFN), which should have a longer half-life through IFN's covalent binding to polyethylene glycol, was tested in the European Organization for Research and Treatment of Cancer (EORTC) trial 18991.[70] The trial tested an induction dosage of subcutaneous peg-IFN at 6 μg/kg/week for 8 weeks, followed by a maintenance dose of weekly subcutaneous injections at 3 μg/kg for up to 5 years. A rather slight improvement of the relapse free survival for the peg-IFN was reported (7-year RFS rate: 39.1% *versus* 34.6%) but the authors did not find any differences in OS and distant metastasis-free survival between the treatment and the sole observation group 13. Moreover, one study reported peg-IFN's association with higher rates of grade 3-4 AEs (47.3% *versus* 25.2%; P<0.0001) and treatment discontinuations (54.3% *versus* 30.4%) compared to IFN-α.[71]

How should we choose patients who might benefit of IFN-α2?[72-75]

Clinical criteria: patients who have primary tumors with an ulceration, and/or microscopic nodal metastasis might be those who could benefit from IFN-α treatment.[75]

Biological criteria: one study found that a higher pSTAT1/pSTAT3 ratio in the tumor was associated with a longer overall survival in stage IIIB patients.[15] Pro-inflammatory cytokines IL-1β, IL-1α, IL-6, TNF-α serum levels might also correlate with longer relapse free survival rates.[58, 72, 73] A better clinical response to HDI was found in patients whose PBMC showed a reduced pSTAT1 induction upon IFN-α stimulation.[74] Higher numbers of infiltrating CD4$^+$ lymphocytes in metastases of patients with systemic disease was correlated to an improved clinical benefit of IFN-α treatment, suggesting a possible use of CD4$^+$ infiltrating cells in regional metastases to select patients who might profit from an adjuvant IFN-α treatment.

Checkpoint inhibitors

Even if it is well-known that T-cell responses are regulated through a complex balance of inhibitory and activating signals, and that tumor can dysregulate these pathways, leading to an impairment of the immune system activities, the relevant new concept which was developed following the failure of cytokine-based immunotherapy and paralleling the increasing evidence of the clinical activity of different targets therapies in several cancer types was constituted by the potential of targeting these inhibitory and activating immunological synapses as a new tool to promote the immune response.[2, 76, 77] Until now, two main types of immune modulating drug antibodies have been developed and used in the treatment of advanced metastatic melanoma, the first targeting the CTLA-4 antigens, the other the PD-1/PD-1L pathway. It is of relevance that both compounds interact with immunological checkpoints physiologically leading to inhibitory signals for the immune system; the blockade of this pathways by the drugs allow to release the break to the immune system thus fostering, maintaining and stimulating the T-cell responses (Table III).[78-92]

Ipilimumab is a fully-humanized monoclonal antibody that binds to CTLA-4, a receptor expressed on the T-cell surface that interacts with CD80 (B7-1) and CD86 (B7-2) on antigen-presenting cells (APCs) and downregulates T-cell response. CTLA-4 blockade allows CD28 to bind to B7-1 receptors, leading to im-

TABLE III.—*Summary of the most relevant phase II/III randomized studies on new target therapies and check point inhibitors whose results have been published.*[78-92]

Study	Reference	Phase	Study drugs/arms	N.	ORR (%)	CR (%)	PFS median (mo)	OS median (mo)
CA184-002	Hodi *et al*. (2010) [78]	III	Ipilimumab + gp100	403	5.7%	0.2%	2.76	10
			Ipilimumab alone	137	10.9%	1.5%	2.86	10.1
			gp100 alone	136	1.5%	0%	2.76	6.4
CA184-024	Robert *et al*. (2011) [79]	II	Ipilimumab + dacarbazine	250	15.2%	1.6%	3	11.2
			Dacarbazine + placebo	252	10.3%	0.8%	3	9.1
CA209-066 CheckMate 066	Robert *et al*. (2015) [80]	III	Nivolumab	210	40%	7.6%	5.1 mo	NR
			Dacarbazine (Untreated BRAF w/t)	208	13.9%	1%	2.2 mo	10.2-12.4
CA209-037 CheckMate 037	Weber *et al*. (2015) [81]	III	Nivolumab	272	31.7%	3.3%	4.7	–
			Chemotherapy (ipilimumab-progressed)	133	10.6%	0	4.2	–
								–
KEYNOTE-006	Robert *et al*. (2015) [82]	III	Pembrolizumab Q2W	279	33.7%	5%	5.5	NR
			Pembrolizumab Q3W	277	32.9%	6.1%	4.1	NR
			Ipilimumab	278	11.9%	1.4%	2.8	NR
KEYNOTE-002	Ribas *et al*. (2015) [94]	II	Pembrolizumab 10 mg/kg	181	21%	2%	5.8	–
			Pembrolizumab 2 mg/kg	180	25%	3%	5.4	–
			Chemotherapy (ipilimumab-refractory)	179	4%	0%	3.6	–
CA209-067 CheckMate 067	Larkin *et al*. (2015) [83]	III	Nivolumab + ipilimumab	314	57.6%	11.5	11.5	–
			Ipilimumab	315	19%	2.2	2.9	–
			Nivolumab	316	43.7%	8.9	6.9	–
CA209-069 CheckMate 069	Postow *et al*. (2015) [84] Hodi *et al*. (2016) [85]	II	Nivolumab + ipilimumab	95	59%	22%	8.9	NR
			Ipilimumab	47	11%	0%	4.7	NR
BRIM-3	Chapman *et al*. (2011) [86] McArthur *et al*. (2014) [87]	III	Dacarbazine	274	5%	1%	1.6	10
			Vemurafenib	275	48%	6%	6.9	13.3
BREAK-3	Hauschild *et al*. (2012) [88]	III	Dacarbazine	63	6%	2%	2.7	–
			Dabrafenib	187	50%	3%	5.1	–
Combi-D	Long *et al*. (2015) [89]	III	Dabrafenib + trametinib	210	67%	16%	11	25.1
			Dabrafenib + placebo	210	51%	13%	8.8	18.7
Combi-V	Robert *et al*. (2015) [90]	III	Dabrafenib + trametinib	352	64%	13%	11.4	NR
			Vemurafenib + placebo	352	51%	8%	7.3	17.2
CoBRIM	Larkin *et al*. (2014) [91] Ascierto *et al*. (2016) [92]	III	Vemurafenib + cobimetinib	247	68%	16%	9.9	22.3
			Vemurafenib + placebo	248	45%	10%	6.2	17.4

ORR: overall response rate; CR: complete response; PFS: progression-free survival; OS: overall survival; mo: months; NR: not reached.

mune activation, IL-2 secretion, cytotoxic T-cells expansion and proliferation.[2, 77] The interaction between CTLA-4 and B7-1/2 takes place in an early phase of the immune response, involving naïve T lymphocytes and APCs. This mechanism of action explains the characteristics of the clinical activity as well as the common side effects of this drug, consisting of immune-mediated reactions (irAEs) developing more frequently in the skin, gastrointestinal tract (mainly diarrhea), liver and endocrinal glands (Table IV;[78, 106] Figure 4). The first randomized phase II study comparing different dose regimens in metastatic melanoma (0.3, 3 or 10 mg/kg IV every 3 weeks), showed that both 3 and 10 mg/kg induced optimal response, even if the latter dose was

TABLE IV.—*More frequent adverse events from immune check-point inhibitors (anti-CTLA4 and anti-PD1) alone or in combination and from targeted therapies with anti-BRAF inhibitors alone or in combination with MEK inhibitors.*

Drug/regimen	Most frequently reported adverse events				
Ipilimumab [78]	Dermatologic events (43%)	Fatigue (42%)	Diarrhea (32.8%)	Nausea, vomiting (23.7%)	Endocrine (7.6%)
Pembrolizumab (KEYNOTE-002)	Fatigue (21%)	Pruritus (21%)	Rash (12%)	Diarrhea (8%)	Arthralgia (7%)
Nivolumab + ipilimumab [106]	Diarrhea (45%)	Rash (41%)	Fatigue (39%)	Pruritus (35%)	Nausea, liver (22%)
Vemurafenib (BRIM-3)	Rash (49%)	SCC (14%) Papilloma (15%) Hyperkeratosis (19%)	Arthralgia (39%)	Fatigue (34%)	Photosensitivity (31%)
Dabrafenib (BREAK-2)	Hyperkeratosis (27%) Papilloma (15%) SCC (10%)	Arthralgia (33%)	Fever (24%%)	Fatigue (22%)	Headache (21%%)
Dabrafenib + trametinib (Combi-D)	Fever (51%)	Fatigue (35%)	Headache (30%)	Nausea (30%)	Chills (30%)
Vemurafenib + cobimetinib (CoBRIM)	Diarrhea (56%)	Nausea (40%)	Vomiting (21%)	Rash (38%)	Photosensitivity (28%)

coupled with an increase in irAEs.[93] In 2010, in a phase III trial, ipilimumab with or without glycoprotein 100 peptide (gp100) vaccine was compared with gp100 vaccine monotherapy in patients with unresectable stage III or stage IV melanoma. Ipilimumab monotherapy significantly improved median OS compared with gp100 vaccine monotherapy (10.1 *vs.* 6.4 months).[78] In another important randomized phase III trial, the combination of ipilimumab (10 mg/kg) and dacarbazine (850 mg/m^2) resulted in significantly superior OS compared to dacarbazine (850 mg/m^2) plus placebo (11.2 *vs.* 9.1 months).[79]

Noteworthy, ipilimumab produced a plateau in survival curves: a recent pooled analysis of OS data for 1.861 patients enrolled in 10 prospective and 2 retrospective trials, with up to 10-year follow-up, showed that the survival curve began to plateau around 3 years after treatment. Three-year OS rates were 22%, 26%, and 20% for all, treatment-naïve, and previously treated patients, respectively.[94] Moreover, the results of the ipilimumab expanded-access program (EAP) in Italy resulted consistent with these data, confirming the activity of the drug also in specific patient's subsets such as the elderly, the mucosal or uveal primaries, and in the presence of brain metastases.[95]

Interestingly, the peculiar mechanism of action described above allowed to identify four distinct response patterns to anti-CTLA4: 1) shrinkage in baseline lesions, without new lesions; 2) durable stable disease (in some patients followed by a slow, steady decline in total tumor burden); 3) response after an increase in total tumor burden; and 4) response in the presence of new lesions.[96] This implies the need to adopt specific immune-related criteria to correctly evaluate response, based on the concomitant assessment of both the primary lesions and the appearance of new lesions.[97]

In the era of anti-PD1/PD-L1 blockers, one of the potential new field of application for ipilimumab is represented by the adjuvant setting.

In a phase 3 trial (EORTC 18071), 951 patients with stage III cutaneous melanoma (excluding lymph node metastasis ≤1 mm or in-transit metastasis) were randomly assigned to receive intravenous infusions of 10 mg/kg ipilimumab or placebo every 3 weeks for four doses, then every 3 months for up to 3 years. At a median follow-up of 2.74 years, median recurrence-free survival was statistically significantly higher (26.1 months) in the ipilimumab *versus* the placebo group (17.1 months) (hazard ratio 0.75).

Due to the high ipilimumab dosage (10 mg/kg instead of 3 mg/kg) and the re-treatment every three months, toxicity was significant. The most common grade 3-4 immune-related adverse events in the ipilimumab group were gastrointestinal (16%), hepatic (11%) and endocrine (8%). Adverse events led to discontinuation of treatment in 245 (52%) of 471 patients who started ipilimumab. Five patients (1%) died due to drug-related adverse events (three patients died because of colitis, one because of myocarditis, and one because of multi-organ failure with Guillain-Barré Syndrome).[98]

At a median follow-up of 5.3 years, the differences between ipilimumab and placebo were confirmed also

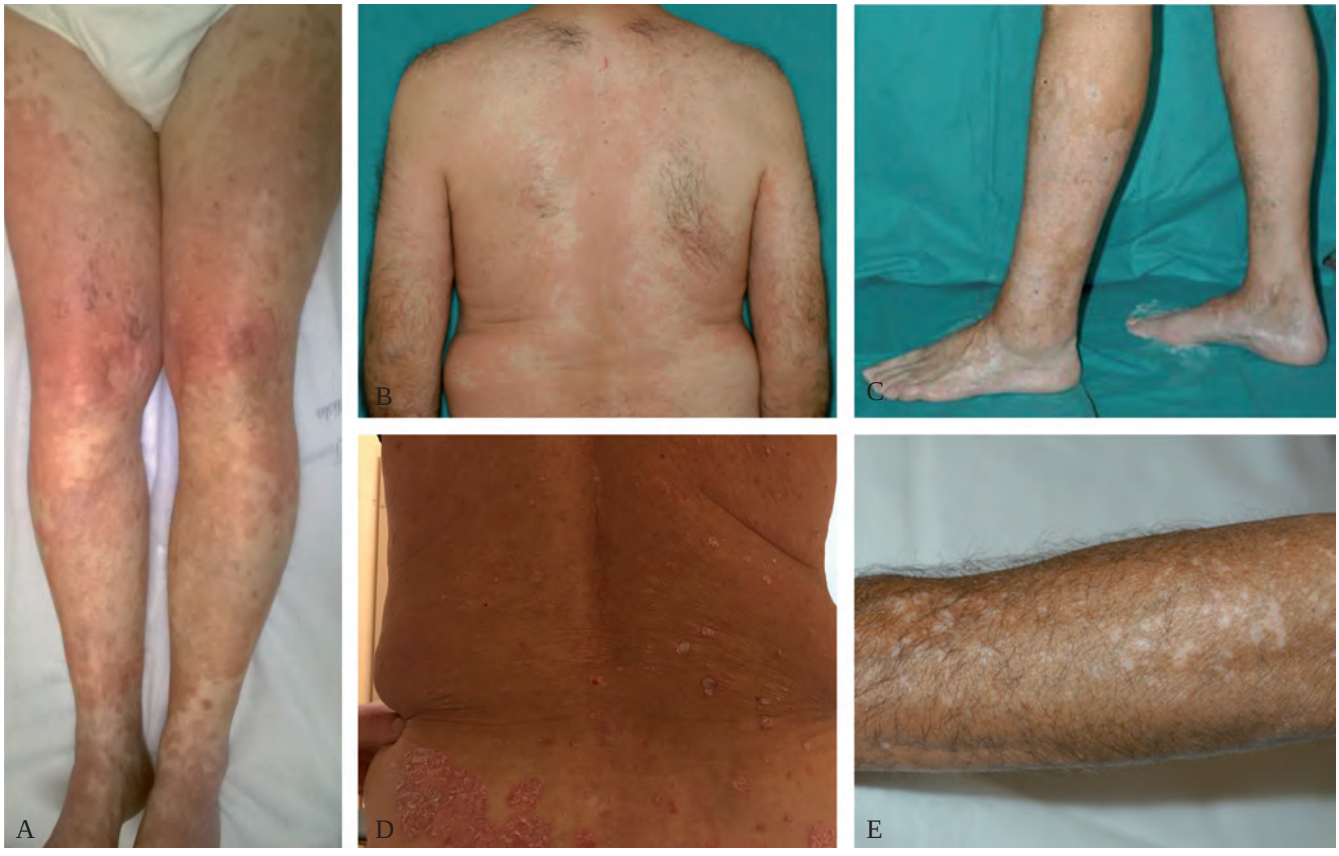

Figure 4.—Clinical pictures of skin toxicities from immune check point inhibitors: A) maculo-papular exanthema from anti-PD1; B) maculo-papular exanthema from anti-CTLA4; C) psoriasiform guttate eruption from anti-PD1; D) vitiligo lesions from anti-CTLA4; E) from anti-PD1.

for OS and DFS.[99] The rate of OS at 5 years was 65.4% in the ipilimumab group, as compared with 54.4% in the placebo group (hazard ratio for death, 0.72; P=0.001). Adjuvant ipilimumab were therefore shown to significantly improve recurrence-free, DFS and OS for patients with completely resected high-risk stage III melanoma even if the adverse event profile at higher incidences with that observed in advanced melanoma. At the moment, we are waiting for the results of other randomized trials which attempted to clarify the role of both target therapies and anti-PD1 (pembrolizumab and nivolumab) in stage III/IV disease-free metastatic melanoma patients and also, to compare ipilimumab *versus* high dose interferon.

PD-1 protein is a co-inhibitory receptor expressed on B and T cells, and has been shown to be involved in the negative regulation of T-cell activation.[100] PD-1 ligand (PD-L1) is expressed in different tumors and associated with a worse prognosis. The discovery that tumor cells were able to activate the PD-1/PD-L1 axis, leading to protection from cytotoxic T cells through exhaustion, leads to the development of specific anti-PD-1 inhibitors.[101] The anti-PD-1 monoclonal antibodies nivolumab (a fully-human anti-PD-1 IgG4) and pembrolizumab (a humanized anti-PD-1 IgG4) have shown to be highly effective for malignant melanoma, and in 2014 they both have been licensed in the USA and later in the EU for the treatment of advanced melanoma.[102] The toxicity profile of anti-PD-1 agents was reported to be similar to anti-CTLA-4, even if generally these drugs are better tolerated.[103] A randomized phase III study comparing nivolumab *vs.* dacarbazine in previously untreated melanoma without BRAF mutation demonstrated superior overall response rate (ORR 40% *vs.* 13.9%, respectively) and increased 1-year OS (72.9% *vs.* 42.1%, respectively). Moreover, nivolumab treatment-related

adverse events occurred in 11.7% of the patients receiving nivolumab and 17.6% of the patients receiving dacarbazine, respectively.[80] In CheckMate 037 phase III trial, patients were randomly assigned 2:1 to receive nivolumab 3 mg/kg every 2 weeks or investigators' choice chemotherapy (ICC) until progression or unacceptable toxic effects. Primary endpoints were the proportion of patients who had an objective response and OS. At first interim analysis on 120 and 47 randomized patients, confirmed objective responses were reported in 31.7% of patients in the nivolumab group *vs.* 10.6% of patients in the ICC group; no treatment-related deaths occurred.[81]

The activity of pembrolizumab for advanced melanoma was firstly shown in 2013 by a phase IB study achieving an ORR of 38% in both ipilimumab pretreated or not pre-treated patients.[104] Two different doses of pembrolizumab (2 mg/kg and 10 mg/kg) were then investigated and compared with ICC in the KEYNOTE-002 randomized phase II clinical trial. At enrolment, patients had progressive disease after ipilimumab or, if BRAF mutated, after BRAF or MEK inhibitors, or both. Results showed an improvement in progression-free survival (PFS) at 6 months as assessed by independent central review, with HR 0.57 for pembrolizumab 2 mg/kg and 0.50 for 10 mg/kg. Grade 3-4 treatment-related adverse events were more frequent and occurred earlier in patients receiving chemotherapy.[83]

In a large randomized phase III study, 834 patients with advanced melanoma were treated either with pembrolizumab at a dose of 10 mg/kg every 2 or every 3 weeks or with 4 doses of ipilimumab (3 mg/kg every 3 weeks). The estimated 6-month PFS rates were 47.3% for pembrolizumab every 2 weeks, 46.4% for pembrolizumab every 3 weeks, and 26.5% for ipilimumab, respectively. Estimated 1-year OS rates were 74.1%, 68.4%, and 58.2%, respectively. The response rate was improved when pembrolizumab was administered either every 2 or every 3 weeks, as compared with ipilimumab. The rate of treatment-related adverse events of grade 3-5 severity was lower in the pembrolizumab groups (13.3% and 10.1%).[82]

Preclinical models have shown that dual blockade of both CTLA4 and PDL1, as compared with inhibition of either pathway alone, synergistically improves antitumor responses.[105]

In phase II clinical trial (CheckMate 069) at a median follow-up of 24.5 months, 2-year overall survival was 63.8% for those patients assigned to nivolumab plus ipilimumab and 53.6% for those assigned to ipilimumab alone.[106]

Among patients with BRAF wild-type melanoma, the rate of confirmed objective response was 61% (44 of 72 patients) in the group that received both ipilimumab and nivolumab *versus* 11% (4 of 37 patients) in the group that received ipilimumab and placebo (P<0.001), with complete responses reported in 16 patients (22%) in the combination group and no patients in the ipilimumab-monotherapy group. Similar results for response rate and PFS were observed in 33 patients with BRAF mutation-positive tumors.[85]

In another clinical trial, Larkin *et al.* demonstrated that median PFS was 11.5 months with nivolumab plus ipilimumab, as compared with 2.9 months with ipilimumab alone, and 6.9 months with nivolumab alone. In patients with tumors positive for the PD-1 ligand (PD-L1), the median PFS was 14.0 months in the nivolumab-plus-ipilimumab group and in the nivolumab group, but in patients with PD-L1-negative tumors, PFS was longer with the combination therapy than with nivolumab alone (11.2 *vs.* 5.3 months).[84]

Targeted therapies

Anti-BRAF and anti-MEK inhibitors

The pharmacological inhibition of the mitogen-activated protein kinases (MAPK) pathway by targeting the mutant v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) is a milestone in the management of metastatic melanoma. At the time of writing, two BRAF-inhibitors are available for clinical use in the daily clinical practice, vemurafenib and dabrafenib. Vemurafenib and dabrafenib are approved for the treatment of patients with unresectable or metastatic melanoma with a *BRAF*^{V600E} mutation, as detected by an FDA-approved test. The recommended dosages of vemurafenib and dabrafenib are 960 mg and 150 mg, respectively, both taken orally twice daily. Similarly, two anti-MEK inhibitors have been already approved for clinical use, namely cobimetinib (in association with vemurafenib) and trametinib (in association with dabrafenib).

Vemurafenib was the first BRAF inhibitor (BRAFi) to be approved. Following phase 1 and 2 clinical trials which showed response rates of more than 50% in pa-

tients with metastatic melanoma, a randomized phase 3 trial was conducted (BRIM-3) comparing vemurafenib with dacarbazine in 675 patients with previously untreated, metastatic melanoma with the $BRAF^{V600E}$ mutation.[86] For the first time in the history of medical treatments for metastatic melanoma, a drug was proven to be superior to dacarbazine. Indeed, response rates were 48% for vemurafenib and 5% for dacarbazine and PFS was 5.6% *vs.* 1.3%, respectively. In an extended follow-up analysis of the total population and in the $BRAF^{V600E}$ and $BRAF^{V600K}$ mutation subgroups, the median overall survival was confirmed to be significantly longer in the vemurafenib group than in the dacarbazine group (13.6 *vs.* 9.7 months, without differences between V600E and V600K.[87] Similar results were obtained when comparing dacarbazine with the other BRAFi dabrafenib. In the BREAK-3 trial,[88] 250 were randomly assigned to receive either dabrafenib (N.=187) or dacarbazine (N.=63). Median progression-free survival was 5.1 months for dabrafenib and 2.7 months for dacarbazine, with a confirmed striking advantage for dabrafenib also in terms of response rates (50% *vs.* 6%). The significant clinical activity of dabrafenib was confirmed also in the phase-2 study BREAK-MB which included only patients with brain metastases showing a similar activity of the BRAFi when performed before or after a local treatment for brain metastases such as surgery or radiotherapy (28% *vs.* 20% response rate).[107] MEK inhibitors (MEKi), such as cobimetinib and trametinib have also been associated with improved progression-free and overall survival in BRAF mutant melanoma and neuroblastoma rat sarcoma viral oncogene homolog (NRAS) mutant melanoma. Despite these advances in melanoma treatment, disease progression occurs in approximately 50% of patients within 6 to 7 months from the starting of therapy with a BRAFi. This is due to several mechanisms of resistance, most of which seem to rely on reactivation of the MAPK pathway.[108-112] Therefore, in order to avoid or delay resistance to a single drug, combination therapies with BRAFi and MEKi have been explored. In phase 1 and 2 studies, combination regimens showed improved progression-free survival over single inhibitor therapy. Following these evidences, large phase 3 trials were conducted and the higher clinical activity of the combinations regimens coupled also with a reduction of side effects and toxicities, was confirmed by three randomized studies (Table III), clearly stating that the combo regimens (BRAFi and MEKi) constitute the standard for targeted therapies.

In the Combi-D study, 423 BRAF mutant patients were randomly assigned to receive dabrafenib and trametinib (N.=211) or dabrafenib only (N.=212). Median OS was 25.1 months in the dabrafenib and trametinib group *versus* 18.7 months in the dabrafenib only group with a median progression-free survival of 11 and 8.8 months, respectively.[89] Similarly, in the Combi-V study, 704 patients with metastatic melanoma with a $BRAF^{V600}$ mutation were randomized to receive either a combination of dabrafenib (150 mg twice daily) and trametinib (2 mg once daily) or vemurafenib (960 mg twice daily).[90] Median PFS was 11.4 months in the combination-therapy group and 7.3 months in the vemurafenib group. The objective response rate was 64% in the combination-therapy group and 51% in the vemurafenib group. The hazard ratio for death in the combination-therapy group was 0.69.

Also the combination of vemurafenib and cobimetinib was proven to be superior to vemurafenib alone in a phase 3 randomized clinical trial including 495 patients with previously untreated unresectable locally advanced or metastatic $BRAF^{V600}$ mutation-positive melanoma. The combination PFS (median: 9.9 *vs.* 6.2 months) And OS (9-month OS: 81% *vs.* 73%).[91]

The updated results presented in recent meetings (ASCO and ESMO 2016) of these combinations confirmed the potential of inducing long lasting remissions and their robust impact on long-term survival.

The percentage of best confirmed response is 69% in the Combi-D trial, 64% in the Combi-V, and 69.6% in the co-BRIM trial. Median response duration is 12.9 months, 13.8 months, and 12.98 months, respectively (median PFS 11, 11.4, and 12.2 months). Median OS is 25.1 months for the Combi-D trial, 26.1 months for the Combi-V, and 22.5 for the coBRIM. Most importantly, the landmark analysis confirmed a long-term benefit on overall survival, with 3-year survival rates of 44% in the Combi-D, 45% in the Combi-V and 37.4% in the coBRIM.

The analysis of treatment outcomes according to prognostic variables and predictors clearly showed that long term benefit in *BRAF* mutant patients treated by combo anti-BRAF and anti-MEK targeted therapies is significantly associated with the extent of metastatic disease, LDH values and ECOG performance status at

the time of treatment beginning. The data reported by Long *et al.*[113] from a pooled analysis of 612 patients included in phase 1 and 2 dabrafenib + trametinib studies, in the Combi-D and Combi-V trials showed that patients with normal range LDH values have a 3-year survival rate of 57% *versus* only 7% in patients with higher values. If considering also the number of metastatic sites, patients with less than 3 sites and normal LDH values show a 3-year survival rate of 70%. This clearly shows that patients with limited disease tumor burden at the initial time point benefit more than those with widespread disease from the treatment.

One major issue for a proper management of targeted therapies is represented by the treatment beyond progression.

Retrospective data suggest that treatment with BRAF inhibitors beyond progression is associated with improved survival, even if prospective data are needed.

Patients included in the phase 1 vemurafenib trial were allowed to continue anti-BRAF treatment after progression in the presence of progressive disease amenable to local therapy (surgery or radiotherapy). In the long-term follow-up analysis, median overall survival was 26 months (range, 7.7-56.1) among 20 patients who continued vemurafenib after local therapy. Continuation of targeted therapies after PD might be beneficial in some patients because remaining disease might continue to respond to BRAF inhibition.[114]

In another retrospective single-center study, 35 patients that continued vemurafenib treatment beyond progression are compared with 35 patients who stopped BRAFi treatment at disease progression. Median OS beyond progression was 5.2 *versus* 1.4 months (P=0.002) in favour of BRAFi TBP. Moreover, in the multivariate survival analysis, stopping treatment at disease progression was significantly associated with shorter survival.[115]

In a further single center experience, among 95 patients who received BRAFi monotherapy within clinical trials and showed progressive disease after response, the prosecution of treatment beyond progression was shown to be a favorable prognostic factor in both univariate and multivariate analysis of survival.[116]

The mechanism of action of BRAFi not only is responsible for its clinical activity but is also the basis for the development of side effects and adverse events. Treatment with vemurafenib causes a multitude of cutaneous adverse events (Figure 5), such as exanthema, photosensitivity, palmar-plantar dysesthesia or hand-foot syndrome (HFS), alopecia, pruritus, keratosis pilaris-like eruptions (KP), actinic keratosis (AK), hyperkeratosis, skin papillomas, keratoacanthomas (KA) and cutaneous squamous-cell carcinomas (SCC).[117-120] The most frequent cutaneous adverse events of dabrafenib are hyperkeratosis, papilloma, alopecia, and palmar-plantar erythrodysesthesia syndrome. Trametinib is more frequently related with the development of acneiform dermatitis or alopecia.[121] Less is known about the cutaneous adverse events related to cobimetinib. The development of hyperkeratotic lesions and particularly AK, KA and potentially SCC during BRAFi therapy is caused by activation of the MAPK pathway in keratinocytes with pre-existing RAS mutations commonly found in chronically sun-damaged skin. Although BRAFi potently reduce *RAF* signaling in *BRAF* mutant cells, leading to apoptosis and tumor shrinkage, they cause increased CRAF signaling in wild-type cells, leading to the development of SCC.[122-125] Thus, the development of SCC is consequent to an abnormal and paradoxical activation of the MAPkinase pathway.[123, 124] The concomitant administration of a MEKi reduces this activation and therefore has preventive effects on the development of SCC and KA. Indeed, when BRAF and MEK inhibitor drugs are combined, the development of cutaneous adverse events specific for each drug appear to be reduced (Figure 6). Photosensitivity is a specific side effect related to vemurafenib not related to the drug itself but to other compounds of the tablet.[126] Also with respect to dabrafenib, vemurafenib shows a more pronounced liver toxicity, whilst dabrafenib is characterized by the frequent occurrence of fever which can develop frequently during the first weeks of treatment. Even if generally, the combo regimens show less side effects with respect to the monotherapy with BRAFi, the association of dabrafenib and trametinib give raise to a more frequent occurrence of fever. The two BRAFi available in the clinical practice are therefore characterized from one side by a clinical activity profile substantially similar, but by potentially relevant differences in the spectrum of adverse events, thus implying that the choice of one instead of the other should be done on the basis of the age and comorbidities of the patient to reduce the impact of toxicities. The side effects associated to target therapies and observed in the more relevant studies are summarized in Table III and

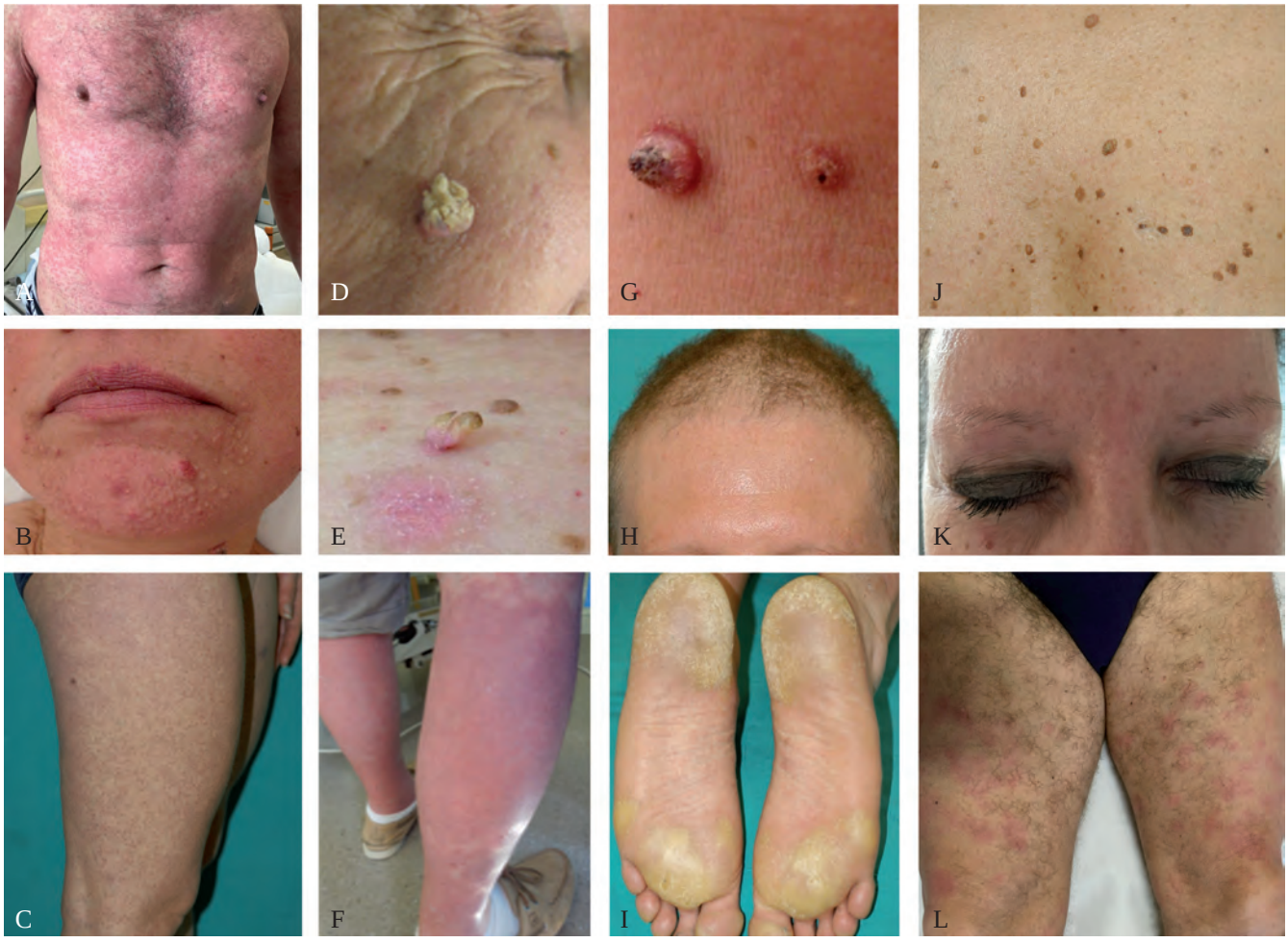

Figure 5.—Clinical pictures of skin toxicities from target therapies: A) macular exanthema on the trunk; B) pustular rash due to anti-MEK; C) maculo-papular eruption with follicular hyperkeratosis; D, E, G, J) verrucous and keratosis lesions; F) photosensitivity (from vemurafenib); H) hair alopecia; I) plantar keratoderma; K) eye-brow alopecia; L) erythema nodosum.

compared with dose of immune checkpoint inhibitors. It is important to remember that these drugs are globally well tolerated with side effects less severe than previous treatments such as chemotherapy and particularly bio-polychemotherapy, and a low percentage of severe toxicities.

cKIT INHIBITORS

Mutations and amplification of the KIT oncogene are more frequent in melanomas arising in the skin with chronic sun damage, acral sites or mucosal melanomas. A series of laboratory evidences and preclinical studies demonstrated that hot-spot mutations, most frequently constituted by substitutions at exons 11 and 13, induce a pathological activation of the *KIT* and thus an upregulation of the downstream signal transduction pathways, which are not only the MAPkinase but also the PI3K/AKT and JAK/STAT pathways. KIT gene expression has been correlated with activating mutations, which indicates the role of *KIT* in tumorigenesis in melanoma. Therefore, *KIT* has been suggested to be a potential therapeutic target for malignant melanoma.[127, 128]

Several trials have been conducted using KIT-targeted tyrosine kinase inhibitors in melanoma in both selected and non-selected patient populations. Trials of imatinib demonstrated responses if KIT was mutated but not if it was wild-type and amplified (Table V).[129-134] Other KIT

TABLE V.—*Summary of results of studies using cKIT inhibitors in stage IV metastatic melanoma.*[129-134]

	Guo *et al*. (2011) [132]	Carvajal *et al*. (2011) [131]	Hodi *et al*. (2008) [133]	Lebbe *et al*. (2014) [129]	Buchbinder (2015) [130]	Lee *et al*. (2015) [134]
N. patients	43	28	25	25	52	42
Drug	Imatinib	Imatinib	Imatinib	Nilotinib	Sunitinib	Nilotinib
RR (%)	23%	16%	29%	20%	7.7, 9.7%*	16.7%
TTP	3.5 mo	–	3.7 mo	47.5% (6 mo PFS)	–	34 weeks (median response duration)
Survival	54% (1-year OS)	11 mo (median)	12.5 mo (median)	67.2% (6-mo OS)	6.4 mo, 8.6 mo* (median)	–

RR: response rate; TTP: time to progression; PFS: progression-free survival; OS: overall survival; mo: months.
*With and without KIT mutation, respectively.

inhibitors such as dasatinib, sunitinib and nilotinib have also demonstrated responses in KIT-mutant melanomas. Taken together, however, these studies showed a percentage of responses around 20% and 30%, mostly of short duration without a significant impact on survival. Moreover, all these studies were performed on relatively small numbers of patients and no randomized trial is available. It can be therefore hypothesized that durable responses in c-Kit mutant melanoma may require combination therapies selectively inhibiting downstream pathways (mainly the PI3K cascade which is the dominant effector of cell proliferation following cKIT activation).

The limited clinical activity of targeting cKIT imply that cKIT mutant patients should be treated as first line with immune checkpoint inhibitors and only after the failure of these regimens, consider the potential of cKIT inhibitors.

NRAS MUTANT PATIENTS

NRAS mutations (codons 12, 13, and 61) can be detected in 15-20% of all melanomas. These alterations have been associated with aggressive clinical behavior and a poor prognosis.[135]

There is no drug available specifically targeting the NRAS mutant protein. Some studies have been reported analyzing the clinical activity of anti-MEK inhibitors in these patients.

In an open-label non-randomized phase 2 study aimed to assess the use of MEK162, a small-molecule MEK1/2 inhibitor, no patients had a complete response and 6 (20%) of 30 patients with NRAS-mutated melanoma had a partial response (three confirmed).[136]

On the basis of these data, a randomized phase III trial was designed, comparing binimetinib with dacarbazine. The results were recently presented at ASCO 2016. The study enrolled 269 patients in the binimetinib arm and 133 in the dacarbazine arm. Binimetinib significantly prolonged PFS and improved response rates with respect to the control arm even if the clinical benefit is slow, with median PFS of 2.8 months compared to 1.5. Furthermore, no differences in overall survival were achieved. An interesting point was that the benefit in terms of PFS appear to be higher in patients with a prior immunotherapy (median 5.5 months) even if this is a retrospective analysis and thus caution should be taken.

The therapeutic algorithm

The decision-making factors to define the best treatment approach in a patient with stage IV metastatic melanoma are represented by: mutation pattern, performance status, high/low tumor load, brain metastases, progression pattern (low/fast) and availability of clinical trials (Table I). On the basis of these parameters we can develop an algorithm for therapeutic interventions based on the clinical activity of the new drugs (Table VI, Figure 7).

Based on these parameters, in the presence of a BRAF-mutant patient, the presence of unfavorable Performance

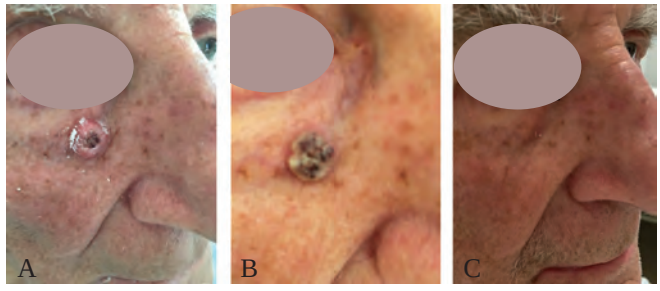

Figure 6.—Clinical regression of a keratoacanthoma on the cheek, arosen after the beginning of BRAF inhibitor treatment and cleared after the introduction of the anti-MEK: A) after 1 month (anti-BRAF); B) after 2 months (anti-BRAF + anti-MEK); C) after 4 months (anti-BRAF + anti-MEK).

Status, fast progression pattern, brain metastases and need to achieve an early response, represent specific factors leading to the decision of target therapies with anti-BRAF and anti-MEK. On the other hand, in the presence of a patient with low tumor burden (for example one or two localized metastases), low progression pattern and good PS, the decision could be either immunotherapy or BRAF inhibitors. One of the main differences in the clinical activity between target therapies and immunotherapy is the early response induction which characterizes the good positive results of the target therapies, whilst both regimens are significantly more active in the presence of a low tumor burden. Therefore, the low tumor burden by itself cannot be considered an indication for immunotherapy but only if associated with a good Performance Status and low progression pattern as well as no need to obtain a quick response.

Indeed, we do not have still available prospective clinical data which could clarify the best sequencing options (target therapies and then immunotherapy or viceversa) and even though this issue has been highly covered in the literature we are still waiting for the results of two main randomized clinical trials which could help in this challenging decision. However, in a recent metanalysis including 16 articles reporting randomised clinical trials involving 6662 patients treated by either target therapies or checkpoint inhibitors, there was no significant difference in OS between BRAF/MEK and PD-1, whilst a significant advantage of BRAF/MEK compared with all other treatment strategies was found for PFS. On the other hand, PD-1 were associated with lowest risk of serious adverse events.[137]

These results show overall a potentially similar clinical activity for the two approaches, underlying the need of a better knowledge of prognostic factors and biologic parameters leading to the identification of patients more likely to respond to each treatment approach. Also, these results suggest that our main issue could be more than the optimal sequencing, the treatment of patients with more aggressive disease who appear to achieve a limited benefit from both treatments. In these patients, the development of integrated target plus immunotherapies could represent a potentially favorable approach currently under investigation in several ongoing trials.

The values of LDH represent a very important marker: first of all, they represent a good indication for the turn-over of growth of metastatic cells but also as a direct consequence of a pathological activation of anaerobic glycolysis reflect a reduction of extra-cellular pH and thus may be associated to an impairment of lymphocyte functions. These evidences support the role played by LDH as unfavorable prognostic factor in patients treated by ipilimumab.

As for toxicity, both treatments (immune checkpoint inhibitors and molecular targeted therapies) show a good toxicity profile, with different features related to their mechanisms of actions. However, a common point for both classes of drugs is represented by the frequent occurrence of cutaneous side effects, which implies that the dermatologists should be aware of these manifestations, develop potential knowledge and experience to diagnose and treat them and moreover, they should be included in the multidisciplinary team for the management of these patients.

TABLE VI.—*Clinical activity and toxicities: comparison between the new drugs (data from major phase I-III studies included updated results presented at ASCO, ESMO, and SMR 2016).*

	Time to resp (mo)	% ORR	Resp duration (mo)	PFS (mo)	OS (mo)	2-yr OS	3-yr OS	%3-4 toxicity
Ipilimumab	3.18-3.32	5.7-15	11.5	2.76	10.1	29.8%	22%	17.4%-22.9%
Pembrolizumab	2.9	36%-37%	>22.8	5	>26	55%	-	11%-14%
Nivolumab	2.1-2.78	40%	23	6.9	20.3	48%	42%	16.3%
Combi-D	1.42	69%	12.9	11	25.1	52%	44%	32%
Combi-V	1.45	64%	13.8	11.4	26.1	53%	45%	48%
Cobrim	<1.8	69.6%	12.9	12.2	22.5	49.1%	37.4%	37%
IPI+NIVO	57.6%	>20.5	11.5	>27	64%	54%		

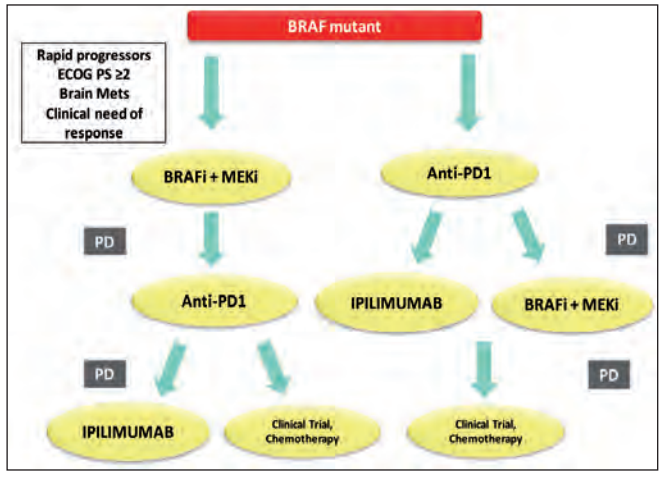

Figure 7.—Therapeutic algorithm of metastatic melanoma.

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REVIEW

PRACTICAL RECOMMENDATIONS FOR THE MANAGEMENT OF SKIN CANCER PATIENTS

Familial melanoma and multiple primary melanoma

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ABSTRACT

Cutaneous melanoma (CM) has the highest mortality rates among the most common skin cancers, and its incidence is rising worldwide, thus representing a significant health care burden. CM is considered the most lethal skin cancer if not detected and treated during its early stages. Susceptibility to CM is also associated with an increased presence of atypical nevi and the occurrence of multiple primary melanoma. Personal history of CM increases the risk of developing a second melanoma by 5-8%. A family history of melanoma has also been strongly associated with an increased risk of melanoma. Approximately 5-10% of melanoma cases occur in a familial context. The main genes involved are *CDKN2A*, *CDK4* and *MC1R*. The recent technological advances have allowed the identification of new genes involved in melanoma susceptibility: breast cancer 1 (*BRCA1*), *BRCA1*-associated protein 1 (*BAP1*), and telomerase reverse transcriptase (*TERT*).Tests on these genes allow to identify a larger number of high-risk individuals with a potential of developing familial melanoma and primary multiple melanomas. These patients also have a high risk of developing internal organ malignancies, especially pancreatic cancer. It is essential that these individuals receive adequate management along with frequent dermatological examinations, dermoscopic evaluation, genetic counselling and instrumental examinations aimed at the early identification of other tumors associated with CM.

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Key words: Cutaneous malignant melanoma - BRCA1 genes - p16 genes - Cyclin-dependent kinase 4 - Human TERT protein.

The incidence of cutaneous melanoma (CM) has increased over the past decades. In developed countries CM is the sixth most common cancer accounting for >47,000 deaths worldwide annually. The rise in incidence affects both young and older populations, while the global projected incidence of melanoma for the year 2025 is estimated to be 317,000 new cases compared to the 200,000 cases reported in 2008.

CM is considered the most lethal skin cancer if it is not detected and treated during its early stages.[1, 2]

According to the AIRTUM data on the frequency of tumors in Italy, CM is the third most common malignancy in the 20-44 age group both in males and females. Melanoma etiology is complex and heterogeneous as it involves environmental, phenotypic and genetic risk factors. The main environmental risk factor for CM is the exposure to ultraviolet radiations (UVR). UVR has been widely demonstrated to be implicated in nevogenesis and melanomagenesis. However, there are intrinsic risk factors that can predispose to melanoma: phenotypic factors, high number of nevi, personal and familial history of melanoma.[3]

The primary prevention campaigns have stressed risk factors such as solar sun burns, intense and intermittent sun exposures, especially during childhood and adolescence, the clear skin type, the number of moles, the presence of atypical moles, a family history of melanoma and finally the personal history of melanoma. The secondary prevention campaigns, however, have imposed rules as ABCDE, which surely contributed to

raise awareness regarding sun habits and the need to undergo periodic medical checks in patients.[4]

Susceptibility to CM is also associated with an increased presence of atypical nevi and the occurrence of multiple primary melanoma. Personal history of CM increases the risk of developing a second melanoma by 5-8%.[5] Finally, family history of melanoma has been widely associated with an increased melanoma risk. Approximately 5-10% of melanoma cases occur in a familial context. In individuals with a strong personal or family history of CM, the likelihood of finding a mutation associated with CM ranges from 30% to 40%. In contrast, the likelihood of finding a germline mutation in families with a single melanoma is ≤1%.[3]

General considerations on hereditary melanoma

The first documented case of familial melanoma was reported by Norris in 1820, his patient was a 59-year-old man with melanoma, a high total body nevus count and a family history of melanoma.[6] Over a century after Norris made his observation, Link and Krush described familial atypical multiple mole melanoma (FAMMM) syndrome, which comprised an association between pancreatic cancer, multiple nevi and melanoma.[7] In the very same period, Clark described a similar phenotype B-K mole syndrome, consisting of familial melanoma in the setting of numerous atypical nevi. In the early 1990's, several study groups reported germline mutations in the cell cycle gene p16 (now CDKN2A) among a subset of FAMM kindred.[8]

Nowadays, in light of all this knowledge, familial melanoma can be identified as an autonomous entity and with an autosomal dominant transmission model, which is responsible from a vertical transmission from affected individuals to 50% of their children (both male and female). Inbreeding does not affect transmission. According to the criteria provided by the Italian Society of Human Genetics (SIGU), a familial melanoma is diagnosed in the presence of at least two cases of melanoma in first-degree relatives or three or more cases in the same branch of the family. The presence of a relative affected by melanoma increases the risk of developing the cancer only moderately. The presence of three or more affected relatives increases the risk by 35-70 times. Members of some families with mutations in *CDKN2A* have up to 20% of risk of developing pancreatic cancer.[3, 5]

Familial melanoma recognizes high-penetrance genes *CDKN2A*, cyclin-dependent kinase 4 (*CDK4*) and low penetrance genes, among which the most important is MC1R.[16]

CDKN2A is located on chromosome 9p21 and its alterations are most commonly associated with the familial atypical multiple mole melanoma (FAMM) syndrome. *CDKN2A* is comprised of 4 exons that are used to encode for two proteins, p16 and p14 ARF. P16 inhibits *CDK4*.

CDK4 is located on chromosome 12q13, it is proto-oncogene and its recurrent mutations are ARG54-HIS-CYS. It encodes a protein that controls cell cycle progression trough the G1 phase. To date mutations in this gene have been described in 17 melanoma prone families. Mutations in *CDK4* are far rarer than those in *CDKN2A* and have been identified in approximately 20 melanoma families.[9]

CDKN2A and *CDK4* are high-penetrance genes. The penetrance is the ability of a mutated gene to induce the malignancy. High penetrance does not mean that the person with the mutation definitely develop cancer during his lifetime, but it certainly has a very high risk threshold.

CDKN2A mutations penetrance (or the likelihood of developing melanoma over time) also varies by geography. The estimated rates are 30% to 91%, 50% to 70%, and 13% to 58% in patients aged 50 to 80 in Australia, the United States and Europe, respectively.[10, 11]

BAP1 germline mutation have been associated with a cancer syndrome characterized by the presence of broad tumor typed: cutaneous melanoma, uveal melanoma, mesothelioma, renal cell carcinoma, atypical Spitz tumors and multiple basal cell carcinomas. The frequency of *CDKN2A* wild type melanoma-prone families with mutations in *BAP1* is not well established but beyond CM families bearing *BAP1* mutations seem to be enriched by uveal melanoma, mesothelioma, other cutaneous tumors.[12] The most recent findings in melanoma susceptibility involve genes that play a role in telomere maintenance. Telomeres consist of tandem nucleotide repeats (TTAGGG) and are located at the ends of chromosomes. Horn and colleagues identified a germ line mutation in the promoter of telomerase reverse transcriptase in a melanoma prone family.[13]

Other studies include 510 melanoma prone families without mutations in the known melanoma susceptibility genes to date.[11]

Overall, the germ line mutations in genes that play a role in telomere maintenance may explain around 1% of familial melanoma cases showing the relevance of telomere maintenance is melanoma susceptibility.

Melanocortin 1 receptor (MC1R) is considered a moderate-risk gene and its role in melanoma susceptibility has been widely studied. *MC1R*, located in 16q24, is one of the master regulator genes in human pigmentation and encodes the alpha melanocyte-stimulating hormone (alpha-MSH) receptor 1. *MC1R* is a highly polymorphic gene in the Caucasian population. *MC1R* variants are associated with skin and hair pigmentation and independently of their phenotypic effect, *MC1R* variants are associated with an increased risk of developing melanoma studies assessing the modulator effect of *MC1R* variants in *CDKN2A* carriers demonstrate that the presence of *MC1R* variant increase the melanoma penetrance in *CDKN2A* carriers.[14]

Management

In light of the above, it is important that families at greater risk of developing CM are submitted to frequent clinical and dermoscopic check-ups and receive genetic counseling. Currently, there are no specific guidelines for genetic testing for hereditary melanoma.[1] Recently, genetic counselling and testing criteria for *CDKN2A* mutations have been developed. In Italy, *CDKN2A* mutation testing is indicated in individuals affected by primary multiple melanoma, and in individuals who have one first-degree or three second-degree relatives diagnosed with melanoma or pancreatic cancer. Patients with known *CDKN2A* mutation are candidates for annual pancreatic cancer screening through ultrasound or magnetic resonance.[10] The National Comprehensive Cancer Network (NCCN) guidelines recommend that individuals with a personal history of CM, or *CDKN2A* carriers regardless of their CM history, undergo a yearly skin examination and recommends that patients are educated on skin cancer prevention and self-examination. The NCCN also notes that those at higher risk because of personal history (*i.e.*, with multiple primaries or early onset), multiple atypical moles or dysplastic nevi, increased UV exposure or other environmental risk factors, may require more frequent examinations.[15]

If a mutation is not identified in a patient with family history for melanoma, however, the patient has to undergo frequent dermatological checks cause in about 50% of cases do not yet know the genes that cause susceptibility in CM. As already mentioned, a *BAP1* mutation can cause a cancer syndrome with combination of more tumors including melanoma and atypical Spitz nevi.[15, 16] All families with a history of multiple cancers including renal cell carcinoma, ovarian carcinoma, breast and melanoma or atypical Spitz nevus must be submitted to genetic testing for the detection of mutations in *BRCA1*, *BAP1*, *CDKN2A*/*CDK4* and *MC1R*.

Conclusions

The recent advances in technology have led to the identification of new genes involved in melanoma susceptibility. This has allowed to explain less than 30% of the genetic susceptibility in melanoma-prone families.[17] It is very important that new predictive biomarkers and prognostic factor in CM are identified. This can be achieved through the correlation among genotype, phenotype and environmental risk factors. It is also crucial that all patients with familial and multiple melanoma receive genetic counseling for the identification of high-risk subjects who need targeted follow-up.

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REVIEW

PRACTICAL RECOMMENDATIONS FOR THE MANAGEMENT OF SKIN CANCER PATIENTS

MELTUMP: how to manage these lesions in the clinical routine

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ABSTRACT

Although most melanocytic lesions can be diagnosed by histopathologists as benign or malignant with high confidence, a subset of morphologically ambiguous lesions does exist and still represents a significant problem for pathologists. These lesions have been defined as MELTUMP, *i.e.* melanocytic tumors of uncertain malignant potential. MELTUMP could be considered as a large cauldron in which melanocytic lesions with equivocal morphologic features fall into, including most benign lesions and a minority of melanomas, unfortunately recognizable only *a posteriori* for their unfavorable outcome. As a consequence of the lack of uniformity in the biologic behavior of melanocytic lesions belonging to the heterogeneous subset of MELTUMP, confusion and lack of agreement in the management of these difficult lesions is increasingly growing up. As most MELTUMP have a favorable prognosis we recommend a conservative approach, avoiding over treatment for this group of lesions.

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Key words: Epithelioid and spindle cell nevus - Pigmented nevus - Skin neoplasms.

Although most melanocytic lesions can be diagnosed by histopathologists as benign or malignant with high confidence, a subset of morphologically ambiguous lesions does exist and represents a significant interpretative problem for pathologists.

Several terms have been employed to describe these equivocal tumors, such as "borderline melanoma," "minimal deviation melanoma," "dermal-based melanocytic lesion," atypical Spitz tumor (AST), and melanocytoma. All these terms can be grouped under the same terminology umbrella of MELTUMP, *i.e.* melanocytic tumors of uncertain malignant potential [1-7] (Figure 1).

Abraham *et al.*[8] defined MELTUMP as a provisional diagnosis made by a pathologist who is not able to make a definitive diagnosis on the basis of histopathologic morphology, suggesting that only long term (perhaps life-long) follow-up will reveal the biologic behavior of each tumor.

The current state of art in the diagnosis of MELTUMP

The concept of MELTUMP itself encloses a lack of consensus for the diagnosis of this entity, meaning that a tumor diagnosed as MELTUMP by a pathologist could

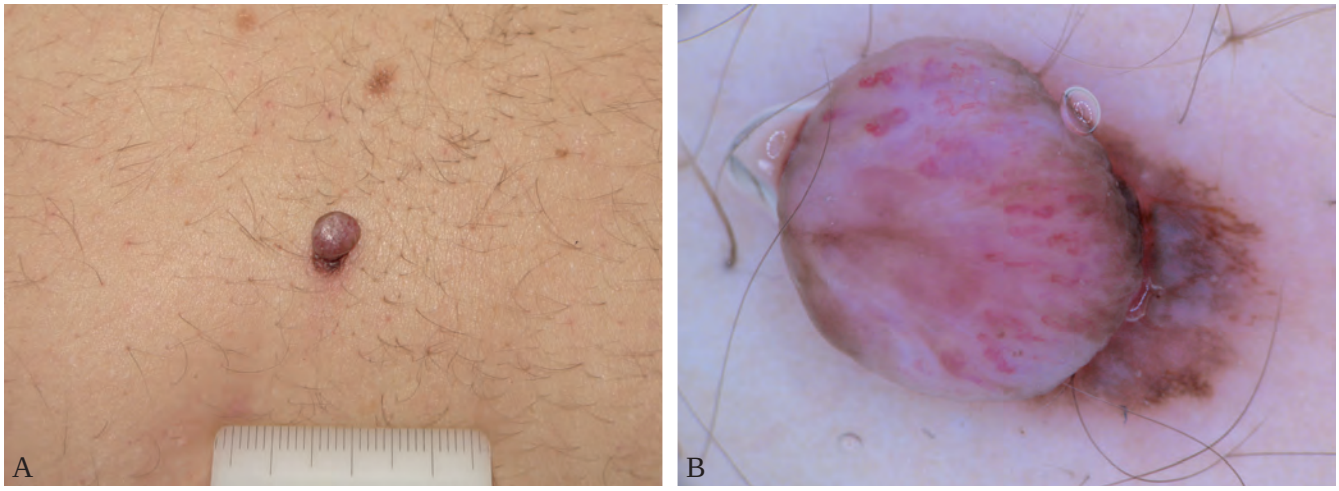

Figure 1.—Clinical (A) and dermoscopic (B) features of a lesion diagnosed as MELTUMP in a young patient. Clinical and dermoscopic features can often be undistinguishable from melanoma.

be diagnosed as either benign or malignant by another pathologist. Cerroni *et al.*[7] confirmed the difficulty of having a precise diagnosis in 57 ambiguous cases evaluated by a panel of internationally recognized experts.[7] The authors found a very low diagnostic consensus, as only 15.8% of the cases were either classified by the majority of panelist as uncertain or the diagnoses were split equally between benign and malignant, and the inherent difficulties in matching the histopathologic diagnosis with the biologic behavior of the tumors.[7]

From a speculative point of view, we can assert that the diagnosis of MELTUMP is operator dependent and the rate of lesions diagnosed as MELTUMP depends on the confidence of the pathologist towards difficult and doubtful melanocytic lesions. On the basis of these statements, it is obvious that diagnostic criteria, although always advisable and useful in clinical practice, are not so useful for MELTUMP. On the other hand, Cerroni *et al.*[7] hypothesized that the difficulties in classifying the tumors examined in their study as benign or malignant may be due to their particular biologic behavior rather than to the capability of the dermatopathologist assessing the histopathologic features of a given tumor.

Definitely, MELTUMP could be considered as a large cauldron in which all the melanocytic lesions with equivocal morphologic features fall into, including most benign lesions and a minority of melanomas, unfortunately recognizable only *a posteriori* because of their unfavorable outcome.

The prognosis of MELTUMP

To date, in the wide spectrum of melanocytic lesions, the dichotomy benign/malignant is progressively imposing as the most relevant classification both for the dermatologists and the patients, who need to have precise prognostic information after the excision and subsequent histopathological examination. Unfortunately, by using a Dantesque allegory, between heaven (benign) and hell (malignant) a purgatory (MELTUMP) does exist, in which lesions stand until the correct diagnosis is evident.

As a consequence of the lack of uniformity in biologic behavior of melanocytic lesions belonging to the heterogeneous subset of MELTUMP, confusion and lack of agreement in the management of these difficult lesions is increasingly growing up. A common behavior of clinicians when they are not sure of the biologic nature of a tumor, is to manage it "aggressively," as if it was malignant. The question is whether patients really benefit from an aggressive treatment in MELTUMP or not.

The concern derives from data on sentinel lymph node biopsy (SLNB) performed in equivocal cases. For example, it became clear that up to 40% of AST cases were associated with SLNB positivity despite a very low mortality rate.[9] Lallas *et al.*[10] performed a systematic review of published reports to assess the role of SLNB as a prognostic method in the management of atypical Spitz tumors. The results of their analysis

did not show any prognostic benefit of SLNB; having a positive sentinel lymph node does not seem to predict a poorer outcome for patients with atypical Spitz tumors.

For MELTUMP data are overlapping those of AST with a variability in SNLB positivity ranging from about 16% to 50%.[11-13] Nevertheless, in patients with SNLB positivity who underwent complete lymph node dissection, the detection of an invasion of further nodes was an exceptional event.[14] Moreover, most MELTUMP with nodal involvement did not usually develop systemic disease, although there was an occasional report of death.[2, 8, 11] It appears obvious that in patients with "metastatic MELTUMP" there should be a temptation to revise the diagnosis to melanoma.[9]

As most MELTUMP have a favorable prognosis, future perspective should aim at detecting histopathologic markers for the recognition of true melanoma, accounting for about 5% of all MELTUMP.[14] Recently, Abraham *et al.* found that the presence of lymphatic invasion detected by dual immunohistochemistry in MELTUMP is associated with poorer prognosis. This technique may, therefore, serve as a useful prognostic factor for risk stratifying patients with these diagnostically challenging lesions.[8]

Management: an approach proposal

Once the diagnosis of MELTUMP (or similar equivocal term) is made, the first thing to do is to discuss with the pathologist to understand why the diagnosis is ambiguous (Figure 2). The clinicopathological reappraisal may be sometimes helpful in the reconsideration of the diagnosis, by integrating the histopathologic morphology with the history, the age of the patients and the clinical and dermoscopic features. This approach could permit to throw out a given number of lesions from the group of MELTUMP and to diagnose them as benign or malignant.

Once the diagnosis of MELTUMP is eventually confirmed, a wide excision is recommended. The most widely adopted and applied margins are 1 cm. Although some national guidelines [15] suggest proceeding with SLNB in MELTUMP, data on the prognosis of this group of lesions do not support this procedure. We do not recommend SLNB in MELTUMP because evidence suggests that it is useless in terms of predicting survival, while important comorbidities are related to the complete lymphadenectomy that would follow the positive result of SLNB in about 40% of cases.

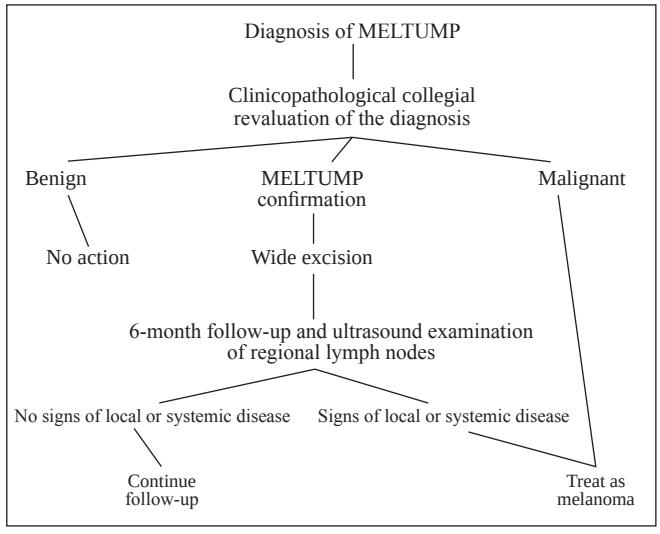

Figure 2.—Flow chart summarizing our proposal of management for patients diagnosed with MELTUMP.

A clinical 6-month follow-up of the patient with ultrasound examination of the regional lymph nodes is recommended. If clinical signs of local recurrence or systemic disease appear, the patient should undergo the same treatment as for conventional melanoma patients.

Conclusions

Despite the progress made in the field of diagnosing melanocytic lesions, MELTUMP is a provisional diagnosis still necessary to characterize doubtful tumors for which morphologic features are not sufficient to make a precise diagnosis. As most MELTUMP have a favorable prognosis, we recommend a conservative approach, namely wide excision, while SLNB is not justified by the existing evidence. Future research might help to better characterize this group of equivocal lesions and minimize the number of tumors diagnosed as MELTUMP.

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REVIEW

PRACTICAL RECOMMENDATIONS FOR THE MANAGEMENT OF SKIN CANCER PATIENTS

Management and treatment of mucosal melanoma of the genital tract

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ABSTRACT

The melanoma of the genital mucosa is a rare melanocytic neoplasm that affects both sexes. The diagnosis is often delayed; videodermatoscopy may represent a useful diagnostic tool. The treatment is complex and multidisciplinary. We report the main diagnostic features and therapeutic approaches for mucosal melanoma of the genital tract.

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Key words: Melanoma - Diagnosis - Disease management - Therapeutics.

Melanoma of the genital mucosa is a rare melanocytic neoplasm that affects both sexes. In women, vulvar melanoma (VM) occurs mainly in postmenopausal age, usually after 60 years of age, and represents the second vulvar cancer in order of frequency (5% of cases) after squamous cell carcinoma.

VM characteristics

There are about 500 cases of VM reported in the literature, most of all in Caucasian women. VM can present as an acral lentiginous melanoma or as a nodular melanoma (Figure 1A). About 27% of cases of VM are amelanotic. The anatomical features of this site, which is rich in lymphatic vessels, permits a rapid vertical infiltration.[1] The diagnosis is often delayed; therefore, the mean Breslow thickness of VM at diagnosis is equal to 3.08 mm (the mean Breslow thickness of cutaneous melanoma (CM) being equal to 0.9 mm at diagnosis), with a 5-year survival ranging from 20% to 50% and a higher rate of recurrence compared to CM. The patient with VM often refers to a physician when

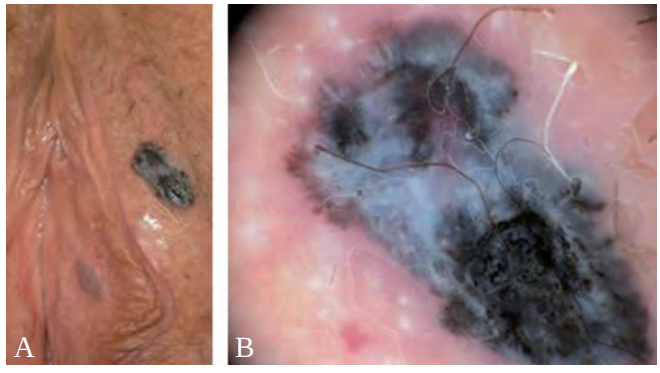

Figure 1.—Vulvar melanoma: clinical presentation (A) and dermatoscopic features (B, original magnification x 30).

a palpable mass is already present (28-72% of cases);[2] on the contrary, this finding is very rare in patients with CM. In most cases, VM is diagnosed at an advanced stage, when a polypoid, ulcerated, bleeding and sometimes amelanotic tumor is observable;[3] however, flat pigmented areas may be the only clinical finding at an early stage,[3] therefore a prompt diagnosis is crucial. The main dermatoscopic characteristics of VM (Figure 1B) are a multi-component pattern, with three or more colors, a blue-whitish veil, irregular brown-to-black dots, atypical vessels, reticular depigmentation.[3] In particular, Ronger-Savle *et al.*[3] found that the dermatoscopic variables that were strongly statistically related to VM were: multicomponent pattern, blue-whitish veil, three or more colors and atypical vessels. In another study, Blum *et al.* found that the most important dermatoscopic criteria that may help in the differential diagnosis between malignant and benign mucosal lesions are the combination of blue, gray and white color and the presence of structureless areas.[4] VM is multifocal in approximately 20% of cases. It is known that, in postmenopausal age, vulvar melanosis represents the main differential diagnosis of VM,[5] for the presence, in some cases, of asymmetry, irregular borders, an uneven pigmentation and sometimes a large size and wide extent of the lesion. Vulvar melanosis may also present a blue-white veil; as a result, vulvar pigmented lesions dermatoscopically characterized by the presence of a blue-white veil should be excised or biopsied, in order to rule out VM.[5]

Disease management

Vulvar melanosis usually shows all the criteria of ABCD rules;[5] therefore, the classical ABCD rules, that are used for CM, are not enough to differentiate early melanoma from melanosis.[5] As VM at an early stage may resemble vulvar melanosis, the challenge for clinicians is to detect thin VMs, with a better prognosis.[3] Another differential diagnosis of VM is represented by atypical melanocytic nevi of the genital type (AMNGT); however, AMNGTs usually affect younger women and, although the clinical, dermatoscopic and histologic features are sometimes alarming, they are characterized by a benign clinical behavior and a low malignant potential. The long-term prognosis of VM is poor, due to the high rate of recurrence.[5] The management of VM is complex; the treatment is primarily surgical, though there is no evidence that radical vulvectomy may increase the average survival rate compared to wide surgical excision.[6] According to current guidelines, if the histological examination reveals a Breslow thickness >1 mm and/or ulceration and/or regression and/or mitotic rate $\geq 1/mm^2$, the surgical resection will be followed by the analysis of the sentinel lymph node (SLN) and, in case of positivity of the latter, by lymph node dissection. The role of an immediate lymphadenectomy in patients with positive SLN is still debated, because in about 70% of cases the disease is localized only in the SLN itself. In cases of metastatic VM, instead, the therapeutic approach consists primarily in immunotherapy, since mutations in the c-kit gene are detected in a variable percentage of cases (up to 35% of cases). Finally, topical therapy with imiquimod has been reported in the literature, in cases of inoperable or relapsing disease, or when the surgical margins were affected by the tumor, with a variable clinical response (sometimes obtaining a complete remission, but also frequent local recurrences), showing promise in the control of local disease, even though it appears unlikely to affect the development of distant metastases.[7]

In male patients, melanoma of the penis (PM) is very rare and accounts for about 0.2% of all melanomas.[8] The literature data about PM are still lacking, as many of the published data concern small case series, in which the clinical and demographic data often concern different mucosal sites grouped together (male genital mucosa, female genital mucosa and oral mucosa). Among all melanomas, less than 1% of cases are located in the male genitourinary tract. The most affected sites are the glans (55%) and the prepuce (28%), less frequently the penile shaft (9%) and the urethral meatus (8%); the average Breslow thickness at the time of diagnosis is equal to 3.5 mm.[9] The average age at diagnosis of patients with PM is about 60 years; in the majority of cases, patients seek medical attention for the presence of a nodule (34.8%), as in women, but also for the onset of a pigmented macule (33.3%).[10] Similarly to VM, the diagnosis of PM is often delayed. In a previous work published in 2007,[10] 77% of patients with PM presented metastases at diagnosis; all patients with lymph node metastases (14%) or distant metastases (9%) at diagnosis died within two years; the 5 year survival was 31%. Dermatoscopy of PM often shows a multicom-

ponent pattern, the presence of multiple colors (brown, black, white, blue, red), a blue-whitish veil, regression structures and sometimes streaks in the periphery of the lesion.[9] The main differential diagnoses of PM are melanocytic nevi and genital melanosis, which sometimes, as in women, may mimic melanoma, presenting a variegated pigmentation with multiple colors, asymmetry of structures, irregular borders [8] or even a whitish veil. Another possible differential diagnosis of PM may be represented by pigmented Bowen disease (BD), that may present alarming dermatoscopic features, such as structureless hypopigmented areas, irregular brown areas, gray blotches, as described in a previous work by Ishioka *et al.*;[8] however, the presence of brown dots arranged in a linear fashion may help to recognize pigmented BD. The management of PM primarily consists in a wide local surgical excision, similar to VM, or in a partial or total penectomy in the case of wide lesions. Given the peculiarities of the anatomical site (lack of subcutaneous fat tissue and rich lymphatic vascularity), early diagnosis is very important. The analysis of the SLN plays a staging role, but its performance is still debated. Lymph node dissection in this body district would cause a considerable morbidity, whereas there is no evidence of a benefit in terms of survival.[10] Some cases of *in situ* PM treated with topical imiquimod (off-label) have been reported.[11, 12] It is known that the standard of care of melanoma *in situ* is surgical removal by excision with a 5-mm margins or Mohs Surgery;[12, 13] however, surgical resection of PM is not always possible due to many reasons, for example the patient's comorbidities, the wide extent of the disease, a potential aesthetic and functional impairment.[12] This treatment has proved effective in some cases, however it appears to be associated with a higher rate of recurrence compared to surgery, therefore a close follow-up of the patients (every six months) is recommended.[11] Dermatoscopy may be useful in the follow-up of *in situ* PMs treated with topical imiquimod, in order to monitor the clinical response to treatment.[12]

Conclusions

In conclusion, melanoma of the genital mucosa appears to be a more aggressive tumor than its cutaneous counterpart, both in male and in female patients. The diagnosis is often delayed, due to the peculiarities of this anatomical site and the difficulty in patients' self-observation. The real challenge for clinicians is to detect mucosal melanomas of the genital tract at an early stage, in order to perform a prompt therapy and to obtain a better outcome, in terms of survival, functional impairment and morbidity. A useful diagnostic tool may be videodermatoscopy (in particular high-resolution videodermatoscopy), paying particular attention to the observation of the different colors, which are more easily detectable in the mucosa than in the skin and which can change in the presence of inflammatory diseases (*e.g.* Lichen) or traumas. Mucosal melanoma, unlike MC, rarely presents dermatoscopic parameters such as an atypical pigment network or streaks.[14] On the contrary, it appears easier to find other dermatoscopic parameters such as a blue-whitish veil and an atypical vascular pattern.[14] Finally, the collaboration of Dermatologists with Gynecologists and Urologists is crucial for the diagnosis, management and treatment of mucosal melanoma of the genital tract.

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REVIEW

PRACTICAL RECOMMENDATIONS FOR THE MANAGEMENT OF SKIN CANCER PATIENTS

Melanoma and pregnancy

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ABSTRACT

The last decades were characterized by a worldwide increasing incidence in melanoma. Almost 35% of diagnosed with melanoma women are in childbearing age. Malignant melanoma is the most common malignancy during pregnancy. Considering this background it is clear how melanoma and pregnancy has becoming one of the main topic of discussion. Current knowledge about pregnancy and melanoma is characterized by many controversies and divergences. The real incidence of melanoma in childbearing and the impact of pregnancy on the prognosis of melanoma is still unclear. There are many uncertainties regarding other aspects of women with melanoma during childbearing, such as the changing in moles, the prognosis and the management. Every changing nevus that would raise concern for malignancy in a pregnant patient should be investigated and surgery should be performed safely using local anesthetic. Pregnancy can affect the staging and treatment of melanoma especially in advanced stage, the decision about introduction or continuation of treatment in the event of pregnancy should be preceded by an analysis of the potential benefits and risks. The role of hormonal changes during pregnancy on melanoma is continually debated. At present, there is a lack of a European guideline on this topic and this review aims to address the most controversial issues such as the roles of hormones, staging and therapeutic difficulties of melanoma during pregnancy. The authors' aim is to help the clinician in the difficult decision-making process concerning the woman suffering from melanoma and her child.

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Key words: Melanoma - Pregnancy - Hormone replacement therapy - Contraceptives, oral - Prognosis - Nevus.

As well known, the last decades were characterized by a worldwide increasing incidence in melanoma. Almost 35% of diagnosed with melanoma women are in childbearing age. Considering this background it is clear how melanoma and pregnancy has becoming one of the main topic of discussion among international scientific literature. Malignancies in pregnant women are a little percentage, accounting for 0.05-0.1% of all cancers, but melanoma is always among the first three most common neoplasms diagnosed in pregnancy. Current knowledge about pregnancy and melanoma is characterized by many controversies and divergences about how to deal properly with pregnancy related melanoma patients. These uncertainties extend to many aspects of women during childbearing, such as the changing in moles, the prognosis, the management, the hormone influence on melanoma. The effect of pregnancy on melanoma and also on melanocytic activity is not clearly established. Pregnancy could be associated with nevus changes that are not necessarily suggestive of melanoma but that may cause diagnostic difficulties especially in women with multiple or dysplastic nevi. Every changing nevus that would raise concern for malignancy in a pregnant patient should be investigated. Excision biopsy can be performed safely during pregnancy using local anesthetic.

In presence of melanoma the decision about the introduction or continuation of treatment in the event of pregnancy should be preceded by a detailed analysis of the potential benefits and risks for both mother and

fetus. There are no data to suggest that pregnancy termination alters the biological behavior of melanoma. All patients should be given appropriate advice and informed that at the moment there are insufficient scientific data to determine a general accepted consensus. It is always very important to respect the will of the patient. If the woman decides to undergo active treatment and maintain her pregnancy, it is necessary that the patient will be followed by a multidisciplinary team consisting of dermatologist, ginecologist, radiologist, oncologist and also a psychologist.

Here within we performed a critical review of the literature of the past decades to help the clinician in the difficult decision-making process concerning the pregnant woman suffering from melanoma and her child.

Hyperpigmentation, moles and pregnancy

Pregnancy induces strong metabolic, immunologic, and hormonal changes that affect also the skin. This period of women's life seems to increase melanocytic activity as showed by frequent presence of physiologic pigmentary changes: melasma, pigmentary demarcation lines, hyperpigmented areola, and *linea nigra*. It has been reported that 90% of women experience these hyper pigmentations during pregnancy. Although the molecular pathways are not well known, it is believed that the new hormonal assessment stimulates melanocytes to produce more melanin, potentially causing color changing of the skin. These changes may result from either a greater density of epidermal melanocytes in these body areas or their increased sensibility to estrogen, progesterone, and melanocyte-stimulating hormone. A current hypothesis is that the up-regulation by human placenta lipids of tyrosinase, an enzyme essential to melanin synthesis, may also contribute to skin hyperpigmentation. Pigmentary changes are more common among women with darker hair and skin color, who have a higher baseline level of melanin production. Although hyperpigmentation typically fades postpartum, it usually does not regress to pre-pregnancy levels.[1]

Pregnancy also could be associated with nevus changes that are not necessarily suggestive of melanoma, as actually scientific evidence suggests,[1-3] but it may cause diagnostic difficulties especially in women with multiple or dysplastic nevi, who are at higher risk.[4, 5]

As already well known, dermoscopy provides a more powerful tool than the naked-eye examination for clinicians to determine the need to excise a lesion, by allowing visualization of submacroscopic pigmented structures that correlate with specific underlying histopathologic structures.[5-8] Although dermatoscopy increases the diagnostic accuracy, it does not reach 100% sensitivity for melanoma. Nevertheless, digital dermatoscopy was shown to be an additional useful method in the evaluation of pigmented melanocytic skin lesions, since it allows the observation of morphologic changes over time. On the other hand, this method could carry a certain risk to miss a suspicious lesion when the patient is not compliant with follow-up visits. However, according to the patient's compliance, dermoscopy and digital dermatoscopic imaging together are actually consider the first choice methods to analyze pregnancy-related changes in melanocytic nevi.[5, 6] During pregnancy, nevi reveal reversible changes such as lightening or darkening, progressive reduction of thickness and prominence of reticular pattern, new appearance of dots or globules, increased vascularization, increase in size. In particular nevi on the breasts and abdomen increase in diameters, have new black dots and brown globules formation, changing their pigment network and architectural order-disorder. The majority of these findings might be the result of skin expansion during pregnancy.[7-10] It has been reported that these temporary moles changes are most evident during the third trimester and at the time of delivery and usually reverse themselves 3-6 months after childbirth, so it is recommended a patients' examination also after childbearing. These physiologic changes of nevi of the front body should be taken into account when performing short-term digital follow-up.[7] On the contrary, significant changes in nevi placed in more stable body areas such as the back or lower extremities are usually not found. However when considering these nevi in locations other than the chest and abdomen, it is also important to consider factors such as weight gain or edema that could contribute to skin expansion.[11] Concerning changes in nevi color during pregnancy, it is difficult to determine the reliability of these findings. Studies are most often assessed through photography of nevi, but many external factors can influence the color of the images. Study by Wyon *et al*. sought to objectively identify changes in color by using *in vivo* spectrophotometry to examine nevi pigmentation. This technology uses beams of light to detect the concentration and position

of melanin within the epidermis and papillary dermis. Spectophotometric analysis was performed during the first trimester and again at 37 weeks of gestation for the pregnant women and at matched time points in a control group. The authors attempted to avoid potential confounding effects of structural changes from skin stretching by including only nevi on the back or lower legs. Several nevi in both groups demonstrated changes in pigmentation, but none of the findings were statistically significant. Further investigation is needed to determine whether nevi naturally darken during pregnancy.[12] Presently, there is insufficient evidence to demonstrate that darkening of nevi occurs physiologically, and biopsy should be considered for nevi that have become darker. So, at present, there is no sufficient evidence to support the idea that nevi darken during pregnancy.[11, 12]

Histopathological studies, that have examined the changes in melanocytic nevi during pregnancy, showed no significant difference in the histopathologic features of the pigmented lesions from the pregnant women compared to those in age-matched controls.[2]

Any changing nevus that would raise concern for malignancy in a pregnant patient should be investigated. Earlier studies have shown that melanomas diagnosed during pregnancy have a greater thickness and thus a potentially worse prognosis.[13] Although this finding is at present more controversial, one explanation for this may lie in delay in diagnosis as a result of dismissed concerns of changing nevi during pregnancy. As such, pregnant patients who have nevi with features suggestive of melanoma should promptly be referred to a dermatologist for further examination. In fact, morphologic changes seen in benign melanocytic nevi differ clearly from those in melanomas. Hence symmetrical enlargement without important morphologic modifications was found in most of the benign melanocytic lesions, whereas the majority of melanomas revealed asymmetrical enlargement associated with marked changes and appearance of pattern, such as irregular globules, atypical pigment network, regression structures, irregular streaks and multi-component pattern, highly suggestive for melanoma.[7] Excision biopsy can be performed safely during pregnancy using local anesthetic. One of the most commonly used local anesthetic in dermatologic procedures is lidocaine with or without epinephrine. Although lidocaine is known to cross the placenta, it is classified as category B by the United States Food and Drug Administration (that means: animal reproduction studies show no fetal risk, or controlled human studies in pregnant women do not confirm a potential fetal risk previously observed in animal reproduction studies) and it is considered safe in pregnancy. Epinephrine is classified as category C (that means: animal studies have shown adverse effects on the fetus; there are no human studies to confirm or eject this possibility; drug is only to be used on strict indication when benefits outweigh risks), because it may lead to uterine artery spasm at high doses. However, many consider it safe for use at low doses in association with lidocaine: this drug combination is classified as category B. Due to its vasoconstrictive effect, epinephrine may allow a lower dose of lidocaine to be used, reducing the risk of placental transfer to the fetus. Other local anesthetics considered safe for pregnancy associated melanoma surgery are: bupivacaine and prilocaine.[14] The criteria for the excision of a suspected lesion in pregnancy are the same as in non-pregnant women. Obstetrician's and patient's consent should be collected before surgery.[15-18]

Epidemiology and prognosis of melanoma related pregnancy

Worldwide almost 35% of melanomas among women occur during reproductive age and melanoma itself is the most common malignancy in women of 25-29 years old.[17-19] One in 1000-1500 pregnancies is complicated by the presence of cancer in the mother. According to a recent population-based Swedish cancer registry study 20, melanoma is the most common pregnancy-associated malignant neoplasm, responsible for 24-31% of all malignancies diagnosed during pregnancy.

The estimated incidence of melanoma during pregnancy varies extremely according to different casistics: from 0, 14 to 2, 8 cases per 1000 pregnancies.[20-22] Various studies show that melanoma is always among the first three most common neoplasms diagnosed in pregnancy. The real incidence of melanoma in childbearing is unknown and some epidemiologic data do not show differences between incidence in pregnant and not pregnant women,[22-24] while others lead to significant differences.[25] The great variability of data about pregnancy and melanoma are also due to the different definition of "pregnancy associated melanoma" that varies across studies concerning a period that lasts from childbearing

to at least 5 years postpartum. In the recent years, pregnancy-associated melanomas are typically defined as melanoma diagnosed during pregnancy and up to 1 year after delivery. In Italy skin melanoma among females ranked 3rd as number of cases newly diagnosed in 2003-2005 in the age group 0-44 years. Moreover malignant cutaneous melanoma represents 8.4% of all malignancies in Italian women between 20 and 44 years old.[26]

There is some controversy about the effects of pregnancy on the progression and prognosis of melanoma diagnosed during pregnancy. Due in part to hormonal and immunosuppressive effect of childbearing, pregnancy-related melanoma has been thought to be associated with poorer prognosis, as confirmed also in some studies.[27, 28] Byrom *et al*.[27] in a meta-analysis found that mortality risk from four studies showed increased risk of melanoma death after adjustment for patient age and stage of melanoma (pHR 1.56, 95% CI: 1.23-1.99) for pregnancy-associated melanoma compared with other melanomas. The study included 9 retrospective clinical cohorts and 5 population-based cohort studies. Of these, 7 studies assessed the melanoma recurrence risk after a pregnancy associated melanoma diagnosis and 4 of the 7 studies reported no significant difference in disease-free survival compared with non-pregnancy associated melanoma. However, the methodology of this study has come under scrutiny by several investigators.[29, 30]

This meta-analysis is strongly influenced by the study of Moller *et al.*[31] They found that survival was strongly reduced in women who gave birth in the year prior to melanoma diagnosis. The age-adjusted hazard ratios (HR) with 95% CI were 2.06 (1.42-3.01) in the first year postpartum. Instead, for melanoma diagnosed in the second through fifth year postpartum mortality is not significantly increased. Andersson *et al*.[20] found fewer melanoma diagnosed during pregnancy than expected and a higher rate diagnosed six months postpartum. This overview may represent a rebound effect, caused by a delay in diagnosis, and could explained the increased mortality remarked by Moller *et al*. in their first year postpartum group.

In another recent meta-analysis of studies evaluating prognosis for melanoma diagnosed during pregnancy, the authors found a non-significantly elevated risk of death for pregnant patients diagnosed with melanoma (HR 1.19 [95% CI: 0.96-1.48]).[30] A single institutional retrospective study of Tellez *et al.*[28] recently reported a mortality rate of 20% and a 5.10 greater odds of death (P 5 0.03) in patients with pregnancy-associated melanoma than in nonpregnant women. The mortality rate and odds ratio reported are substantially higher than all previous studies in the literature. In this study, one potential issue lies in the inconsistent reporting of staging. Another flaw is in the statistical method used by the authors (use of logistic regression instead of survival and progression-free analysis to evaluate mortality and recurrence).[30]

However bulk of evidence actually demonstrates that outcomes for melanoma in pregnant women do not appear to be poorer compared with non-pregnancy melanoma.[32-35]

More data are needed to resolve this controversy and to confirm the increased propensity for lymphovascular spread.[36] In a recent retrospective cohort study, Merkel EA *et al*. assessed tumor stage and proliferative activity through mitotic rate and immunohistochemical markers of proliferation, phosphohistone H3(pHH3) and Ki-67. The aim of this study was to evaluate the impact of pregnancy on tumor progression, in melanomas occurring in association with pregnancy, and in a series of non-pregnancy-related melanomas from women of gestational age. They found that among invasive melanomas, there was no difference in proliferative activity between groups and pregnancy did not have a significant impact on tumor proliferation in early-stage melanoma.[37]

Childbearing does not increase the subsequent risk of having melanoma[38] and there is no increased risk of melanoma developing during pregnancy.[39] As in all patients, the prognosis of pregnant women with melanoma is still primarily dependent on tumor thickness and ulceration status.[40, 41] Some studies have shown that thicker lesions are associated with pregnancy, presumably as a result of delayed diagnosis.[13] Pregnant women with regional or metastatic melanoma do not seem to have a worse prognosis.

However there is still some controversy about the effects of pregnancy on the progression and prognosis of melanoma diagnosed during pregnancy, but women diagnosed with melanoma do not appear to have a poorer prognosis than non-pregnant controls.

Melanoma diagnosed prior to pregnancy

As society trends toward delayed childbearing, cur-

rently there is an increasing proportion of melanoma survivor's women who have yet to complete family building. Several studies analyzed whether the course of melanoma in a woman may be altered by a subsequent pregnancy. Some studies reported no significant influence on melanoma survival rates in women who had a subsequent pregnancy compared with women with melanoma that did not or were nulliparous.[42-45] A recent meta-analysis of quantitative data available from relevant studies demonstrated no significant effect of subsequent pregnancy on the risk of melanoma death with adjustment for patient age.[43]

In summary actually there is no evidence of a significant impact on prognosis when melanoma is diagnosed before pregnancy.[17, 18]

Regarding the influence of a pregnancy after the diagnosis of melanoma, evidence shows no effect on melanoma prognosis in early stage of the disease. Because the majority of the studies included predominantly localized melanomas, an effect of subsequent pregnancy on the prognosis of a previously diagnosed advanced melanoma cannot be ruled out.

In case of high-risk melanoma (Breslow thickness of >1 mm and/or presence of ulceration) it is recommended to wait before childbearing. There are no standard, defined guidelines regarding family planning for clinicians managing women diagnosed with melanoma during their reproductive years but the consensus is to recommend that women avoid pregnancy for two to five years after a high-risk melanoma,[17, 18] as most recurrences are seen within this period of time. If recurrence develops during pregnancy, then it may be disastrous both medically and emotionally, potentially altering treatment options and with also a minimal risk of placental and fetal metastasis. The exact waiting time depends on tumor thickness, presence of ulceration and sentinel node metastasis. Women with <0.5 mm thick melanoma have a 1-3% risk of recurrence within five years, while those with >4 mm thick melanoma have a risk of recurrence of up to 50% within two years. Naturally it is not completely predictable who will develop recurrent disease so each patient should be approached individually, with the patient ultimately making her own informed decision.[17, 18, 46, 47] Furthermore, the melanoma survivors carry a 9-fold increased risk of developing a secondary melanoma, with the highest risk of developing a second melanoma within the first 2 years.[48]

Staging and management of melanoma related to pregnancy

Melanoma staging and treatment during pregnancy may be conflicting between providing an optimal maternal therapeutic management and protecting the fetus from dangerous effects of the diagnostic procedures and of the treatment. The diagnosis and treatment of malignant melanoma in pregnancy does not differ from the diagnosis and treatment of non-pregnant patients.[20] Nevertheless, pregnancy even dictates the choice of radiological examinations to be performed for staging of melanoma.

The key to treatment, as always, is based on a surgery involving excision with a margin of 2.5-5 cm (depending of the depth of lesion).[14] This will help to decrease the possibility of metastases, especially to the placenta (and possibly to the fetus) in pregnant women.

In order to limit fetal exposure to ionizing radiation and depending on the gestational age the choice of radiological examinations for staging could be different.[49-51] Ultrasound and magnetic resonance, non-ionizing technologies, are preferred. However, magnetic resonance imaging should be considered with caution during the first trimester for heating effects for the fetus and high-frequency hearing loss in newborns.[52] The fetal radiation dose from a CT-scan can be reduced by the use of lead shielding or by a low-dose spiral CT with dose modulation algorithms.[14] Contrast gadolinium is designated as a pregnancy class C drug and is not routinely recommended in the pregnant patient.

The status of the sentinel node is one of the most important prognostic indicators for patients with clinically localized melanoma. Sentinel lymph node biopsy can be considered in patients with a clinically localized invasive melanoma of Breslow thickness >1 mm and in selected patients with a melanoma of Breslow thickness <1 mm presenting at least one of the following characteristics: ulceration, mitotic rate >0, and regression with documented thickness of ≥1 mm or regression of more than 50-75% of the whole pigmented lesion. Breslow thickness is the most important factor determining the indication for this procedure.[53] Some authors question the validity of mitotic rate of 1/mm^2 as cut off point for performing sentinel lymph node biopsy in pregnant patients with thin melanoma. As pregnant women show higher mitotic and proliferative activities in their nevi compared with non-pregnant women, melanoma presenting during

pregnancy may show a pregnancy induced increase in the mitotic activity of its melanocytes.[54]

The harvesting of a sentinel lymph node entails a sequence of procedures with participation of specialists in nuclear medicine, radiology, surgery and pathology. Pregnant patients may be offered sentinel lymph node biopsy after careful counseling regarding the safety and efficacy of the procedure.

The two tracer substances commonly used to perform sentinel lymph node biopsy are blue dye and radioactive labeled sulfur colloid. Isosulfan blue and methylene blue are both classified as pregnancy class C compounds. Currently, insufficient data exist on the safety of blue dyes for use in pregnancy, and therefore, they should not be used in this patient population.[55] Isosulfan blue carries a rare, but serious risk of anaphylactic reaction, which is further contraindication for its use. In lymphoscintigraphy, the colloidal tracer has >95% retention at the injection site or in the sentinel node which are then resected at the time of surgery.

Lymphoscintigraphy using technetium-^{99m} (TC-^{99m}) is probably safer in pregnant women; however this procedure should be offered after careful counseling about its safety and efficacy.[56, 57] Gentilini *et al.* showed that ^{99m}Tc largely remains trapped in the injection site or within the lymphatic structures and only a small amount of the injected radiation is found circulating in the blood pool and urinary system.[58] According to the literature, the risk to the fetus is considered negligible for investigations exposing a fetus to <1 mSv.[59] Only in a melanoma located rather close to the fetus (over the lower abdomen or back) is the theoretical risk of exceeding 1 mSv.

Regarding the sentinel lymph node biopsy during breastfeeding it is good to remember that the presence of ^{99m}Tc in breast milk has not been reported, but it has been recommended that breast feeding should be suspended in nursing mothers for at least 4 h [60] and preferably for 24 h after radiopharmaceutical administration,[61] since the radiopharmaceutical will be excreted from the breast milk during this period.

The timing of the surgery is a controversy topic: guidelines suggest that these patients as well as those with metastatic melanoma should probably be treated in the setting of a comprehensive cancer center.[17, 18] Some authors maintain that sentinel lymph node biopsy may be safely performed during pregnancy and underline its importance for all future decisions regarding oncological management.[50, 57] Others suggest deferring this surgical procedure until the postpartum period.[62] However in all cases surgery should be avoided in the first trimester due to an increased risk for spontaneous miscarriages.[50, 57, 62]

Patients with a positive sentinel node should undergo regional lymphadenectomy. However considering that this procedure does not improve survival, some authors suggest postponing until the postpartum period in case of micrometastatic lymph node involvement.[50, 63] General anesthesia should be avoided if the mother is still in her first trimester of pregnancy. Nevertheless, several studies have demonstrated that major surgery can safely be performed throughout pregnancy. The modifications in maternal anatomy and physiology require an aesthetic and surgical adaptations. The administration of nitrous oxide, opioid, regional or local anesthetics to pregnant women is not considered dangerous for embryonic or fetal development or clinical significance for an adverse neonatal outcome.[50] Negative effects of surgery in general anesthesia for the fetus are more correlated with maternal hypoxia, hypotension, hypothermia, or abnormal glucose metabolism than with anesthesia. Preterm births usually occur in cases of abdominal surgeries and peritonitis. Since pain can also induce premature labor, it is important to administer appropriate postoperative analgesia. Moreover, prophylaxis of thrombosis should also be implemented.[64] Among therapeutic procedures used for metastatic melanoma, surgery seems to be the easiest and safest to use.

Sentinel lymph node biopsy should not be performed in patients presenting with primary melanoma and satellitosis or intransit metastases. These patients are already stage III.[53]

In the course of malignancies, metastases in the placenta or the fetus are quite rare. Melanoma is the cancer most frequently involved. Staging of melanoma during childbearing consists also in placenta examination to detect pathological lesions or to exclude metastases and a close observation of the newborn to search signs of cancer development (22% fetal risk of metastasis if there is placenta metastasis).[65] Most cases of placental metastases from melanoma occurred with maternal metastases to other viscera (stage IV); even then metastases to the fetus are rare. Since a concomitant placental involvement was documented in neonatal melanoma, careful microscopic

examination of the placenta of women with known metastatic melanoma should be thus recommended, as well as close observation and follow-up of the infant, especially in case of placental metastatic localization. The probability of fetal metastases when the mother is diagnosed with malignant melanoma during pregnancy is dependent upon her stage of the disease.[49, 65] Literature reveals that only 22-25% of infants with placental metastatic melanoma go on to develop the disease. Those statistics aside, maternally derived melanoma metastases in the infant are almost always manifested at the time of delivery and are almost invariably fatal.[49, 65]

Immunotherapy with interferon in childbearing women with metastatic disease appears to be safe.[66] Adjuvant radiation therapy in pregnancy could be used only for lesions of the head and neck, brain metastases, and palliative treatment at sites other than the central nervous system. When metastases are limited to the brain they are treated with radiosurgery or neurosurgical resection. As radiosurgery delivers very high doses to very small volumes in the brain, it is possible, with a careful treatment planning, to treat a pregnant patient with a minimal exposure to the fetus. Delaying the treatment of metastatic lesions in the brain is not recommended.[49, 50]

The use of chemotherapy in a pregnant woman affected with metastatic melanoma is a much more challenging situation. Chemotherapy in melanoma, as distinct from some other cancers such as breast cancer, is widely considered as palliative and has never demonstrated any increase in overall survival.[67] Chemotherapy should be avoided during the organogenesis (first trimester) and fetus should be monitored for long term also after delivery for the risk of malformations.

In brief important key points could be summarized as follows: primary treatment of pregnancy related melanoma does not differ from not pregnant women as well as for margins of excision; staging of melanoma during childbearing consists also in placenta examination to detect pathological lesions or to exclude metastases; lymphoscintigraphy using technetium-^{99m} could be performed in pregnant women. Surgery is the easiest and safest treatment to use in pregnancy (from second trimester).

Implications for the fetus

As already said, the prognosis for the fetus in a woman diagnosed with melanoma during pregnancy depends on her stage of disease. Generally, the prognosis for the fetus is excellent, unless the mother has widely disseminated disease. A Danish study found no increased risk of adverse birth outcome for the newborns born to women with a diagnosis of melanoma before pregnancy or for the newborns born to women diagnosed during pregnancy.[68] Therefore, there is no justification for therapeutic abortion as was historically recommended.

Fortunately, placental and fetal metastasis from maternal malignant disease is an exceptionally rare event. However, if one considers all types of cancer that occur during pregnancy, melanoma is the most common maternal malignancy to metastasize to the placenta. Such metastases may be under-reported since not all placentas are histologically studied. With placental involvement, as already remember, fetal risk of melanoma metastasis is approximately 22%, which portends a fatal prognosis.[69] The skin and liver were the most common sites for metastases. Given the rarity of metastatic melanoma to the fetus, there are no evidence-based guidelines on monitoring an at-risk infant. Some authors suggest a histological examination of the placenta in women with known or suspected metastatic melanoma and, if there is placental involvement, a close follow-up of the newborn with skin examination, but also with abdominal ultrasound and urine melanogen screening.[49, 65, 70]

Influence of hormones on melanoma

The underlying mechanisms by which childbearing may influence melanoma and melanocytic nevi are still not completely known. For many years, clinicians have been concerned about a potential adverse effect of pregnancy-associated hormones and exogenous hormones on melanocytic nevi and malignant melanoma. Epidemiological data pointed out a significant divergence in melanoma incidence between sexes. Gender differences are also observed regarding the age-dependent onset of melanoma, with slightly higher rates in women aged 20-45 that decrease after the age of 45 years. On the other hand, in males, melanoma incidence progressively increases after 45-50 years of age and dramatically increase in men aged 50-85. Moreover, a significant disparity has also been noted in the prognosis of this tumor between males and females, with women having a significant survival advantage over men.[71] Today, these data are more significant as

women have delayed childbearing into their 30's and 40's, and the likelihood of diagnosis with melanoma during pregnancy is enhanced. More recent clinical, epidemiologic, and laboratory studies have shed some light on the relationship among hormones, nevi, and melanoma in pregnancy. Similarities between the pathologic progression of cancer and the physiologic process of placentation (*e.g.*, proliferation, invasion, and local/systemic tolerance) have been searched for many years. Sex hormones such as human chorionic gonadotropin, estrogens, progesterone, and others contribute to induction of immunologic tolerance at the beginning of pregnancy. They have been shown to play contributory roles in the growth of cancers such as breast, prostrate, endometrial and ovarian cancer, but their involvement as putative mediators of the immunologic escape of cancer are still not completely known. Progesterone and estrogen are important regulators of the immune system and angiogenesis in several types of cancers but also in pregnancy, during which are physiologically overexpressed.[72, 73] Although melanoma is classically considered a non-hormone-related cancer, growing evidence supports a direct correlation between sex hormones (estrogens, in particular) and melanoma growth and progression. Question regarding the relationship between role of these hormones and melanoma have prompted studies examining presence of their receptors in melanocytic tissue. Estrogens exert their effects through estrogen receptors: Estrogen receptor alfa (ERα) and Estrogen receptor beta (ERβ). Both are members of the nuclear receptor superfamily of transcription factors and both can form either homo- or heterodimers. Upon activation, these receptors translocate into the cellular nucleus to bind with co-regulatory proteins and control the transcription of target genes through the binding to specific regions. The estrogen receptors are encoded by two different genes that are located on chromosomes 6 and 14. Synthetic or natural ligands bind to ERα or ERβ with different affinities according to their chemical structure. Moreover, at the same time the same ligand may have different binding affinity for ERα or ERβ subtypes. This difference in binding affinity seems to depend on the ERα/ERβ ratio as well as on the specific cell context. Increasing evidence supports a relationship between the perturbation of estrogen signaling and cancer initiation, promotion, and progression. Overall, it is now well accepted that ERα contributes to tumorigenesis by stimulating cell proliferation, while ERβ is endowed with a significant antitumor activity. ERα is the main ER in human skin; however, this receptor does not seem to play any role in the pathophysiology of melanoma precursor lesions or melanomas.[71] In fact, estrogen receptor alfa have never be found in benign or dysplastic nevi, in metastatic or primary melanoma, as well as in pregnancy-associated melanoma.[74, 75] On the contrary, ERβ has been reported to be the predominant ER subtype in melanocytic lesions such as moles, dysplastic nevi and melanoma.[76] Its expression levels also correlated with the tumor microenvironment. The majority of epidemiological studies clearly indicate that ERβ is immunohistochemically the main ER subtype to be expressed in melanoma. Moreover, ERβ expression seems to be more prominent in lesions close to the epidermis, such as severely dysplastic nevus or melanoma in situ, and that the expression diminished in deeper and thicker lesions, thereby suggesting that ERβ could be interpreted as a marker for metastatic potential and for prognosis in malignant melanomas. Evaluating ERβ with mRNA by reverse transcriptase polymerase chain reaction, De Giorgi *et al*. observed that the levels of both ERβ mRNA and protein were lower in thicker and more invasive tumors. So the loss of this receptor appears to be associated to an increasing in Breslow depth.[77] According to these data ERβ seems to have a protective role for in the metastatic process of melanoma cells, as a tumor suppressor. Similar data have been previously reported for tumors related to the reproductive system, such as breast and ovarian cancers, but also tumors unrelated to the reproductive system as prostate and colon cancers. Furthermore, ERβ has been found to be expressed in melanomas of pregnant women more frequently than in men and also a trend to a higher expression in women than in men has been reported. This result was obtained evaluating hormone receptor expression in the melanomas of stage and age-matched patients of pregnant women, non-pregnant women, and men.[78] Based on all these data, it is possible to postulate that ERβ might explain the different prognosis of melanoma between women and men. Moreover, cutaneous melanoma should be considered almost a hormone-related tumor. Studies of estrogen-sensitive cancers showed that loss or decreasing levels of ERβ or increased ERα to ERβ ratio may be involved

in carcinogenesis, thereby suggesting that ERβ has a tumor-suppressive function. The *in-vitro* ER antagonist tamoxifen was first shown to induce cell death in human malignant melanoma cells and to reduce melanoma cells metastatic behavior.[79] Despite these promising results the treatment of melanoma with tamoxifen has not shown effective benefit in advanced melanoma.[80] The effects of chemotherapy with and without tamoxifen for the treatment of aggressive melanoma were compared in different clinical trials. These studies describe that co-treatment with tamoxifen may provide improvements in response rates, although it is often accompanied by increased toxicity and no survival benefit. It has been proposed that this is due to the fact that tamoxifen may decrease cell proliferation when it binds to ERα while it may increase cell proliferation when it binds to ERβ. Thus, the antitumor *vs.* prosurvival effects of tamoxifen likely depend on the different ERα/ERβ ratios in a given tissue. In line with this observation, low levels of expression of ERβ were shown to correlate with tamoxifen resistance in breast cancer cells.[79, 80] There is also evidence that patients with breast cancer have increased risk of melanoma and this risk seems to be greater for patients who do not receive anti-estrogen therapy.[81] On the contrary others find no correlation between receptor beta and Breslow depth or disease stage at diagnosis and no difference in expression between pregnant and not pregnant women.[78] The linkage between melanoma risk and hormonal/reproductive factors in women it is still unclear. *In vitro*, sex hormones and gonadotropins stimulate melanogenesis with direct action on the melanocytes [82] and in an era of personalized medicine, a pretreatment evaluation of the ER isoforms expression in each melanoma patient, together with the concurrent oncogenic mutations, should be considered in order to anticipate the response of melanoma patients to novel therapeutic strategies. Another issue that needs to be solved is the expression/activity of aromatase in melanoma tissues. It seems that the skin has its own capacity to produce steroids (including estrogens). In line with this, some authors [83] reported that the aromatase enzyme is expressed in melanoma tissues; however, no correlation was found between the expression of this enzyme and clinical outcomes. Actually the first generation aromatase inhibitor aminoglutethimide was found to be ineffective in reducing melanoma progression. Further studies should be performed with newer aromatase inhibitors for helping clarify this issue.[78] Little is known on the expression of androgen receptor in human melanoma.[84] A study shows that cancer risk among infertile women with androgen excess disorders is higher and found that the standardized incidence ratio was statistically significant for breast cancer, uterine cancer, and melanoma. Apolipoprotein D, an androgen-regulated protein, has been found in cutaneous melanoma. An animal study showed that androgen blockade enhanced the response to melanoma vaccine in male mice. Richardson *et al*. found that 17β estrodiol and estrone inhibited invasion and dehydroepiandrosterone enhanced invasion in melanoma cell lines. The same authors also found that the serum estrogen/androgen index was decreased in female patients with stage IV disease, although this finding was not statistically significant.

Another important issue is about the possible link between sex steroid assumption and melanoma development. The majority of studies pointed out that the use of exogenous female hormones (either as oral contraceptives or as hormonal replacement) do not contribute to increased risk of cutaneous melanoma. Therefore concerning the hormonal therapies, such as hormone replacement therapy (HRT) or the use of the oral contraceptive pill (OCP), there is no convincing evidence that they are able to affect the natural history of melanoma.[85] Likewise, age at menarche, age at menopause, or duration of menstrual life does not affect melanoma risk.[88] In particular regarding OCP there is no relation with length of use, age at first use or current use.[85-87] Similarly no association is found between HRT or its duration and malignant melanoma.[88-90] Hormone replacement therapy and oral contraceptives are not contraindicated in women who have had melanoma. Finally several studies with conflicting results had focused on fertility drugs as drivers of melanoma. Earlier ones demonstrated an increased incidence of melanoma in women undergoing ovarian stimulation (COS),[91] whereas actually no association has been found between malignant melanoma risk and use of fertility drugs.[92] More recently a study investigated a possible mole modifications in women undergoing COS for assisted reproduction technologies. The conclusion raised by authors is that the results obtained do not support a causal relation between the supraphysiological hormone levels stimulation and worsening of clinical features of moles.[93]

Given the complexity of genetic and environmental factors underlying the development of melanoma, all studies on the relationship between exogenous and endogenous hormonal factors present multiple potential biases such as: body mass index, sun exposure, number of pregnancies, causes infertility, use by the same woman more categories of fertility drugs and many more.

Conclusions

International and European scientific communities are still divided on the management and on recommendations to be followed in patients with melanoma during pregnancy.[94]

In brief, the main important evidences are that the criteria for the excision of a suspected lesion in pregnancy are the same as in non-pregnant women. In addition to this, pregnancy should not be kept waiting in the removal of a suspicious lesion. Excision biopsy can be safety performed. In summary epidemiological data suggest that there is no evidence of different incidence between pregnant and not pregnant women. Moreover childbearing does not increase the subsequent risk of having melanoma. In brief: there is no evidence of a significant impact on prognosis when melanoma is diagnosed before pregnancy and according to the most recent guidelines, in case of high-risk melanoma it is recommended to wait before childbearing. However the patient ultimately makes her own informed decision. To resume important key points could be summarized as follows: primary treatment of pregnancy related melanoma does not differ from not pregnant women as well as for margins of excision. Staging of melanoma during childbearing consists also in placenta examination to detect pathological lesions or to exclude metastases. Lymphoscintigraphy using technetium-^{99m} could be performed in pregnant women. Surgery is the easiest and safest treatment to use in pregnancy (from second trimester). In summary the evidence emerging from this review is that progesterone and estrogen are important regulators of the immune system and angiogenesis in several types of cancer and also in pregnancy. Moreover estrogen receptors may be expressed in moles, dysplastic nevi and melanoma. Based on evidence, hormone replacement therapy and the use of the oral contraceptive pill do not affect the natural history of melanoma and, at present, no association has been found between malignant melanoma risk and use of fertility drugs.

Despite all these evidences further investigations are required to definitely solve all controversy still emerging from literature concerning melanoma and pregnancy.

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REVIEW

PRACTICAL RECOMMENDATIONS FOR THE MANAGEMENT OF SKIN CANCER PATIENTS

Practical indications for the management of non-melanoma skin cancer patients

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ABSTRACT

Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), together encompassed in the term non-melanoma skin cancers (NMSC), are the most common cancers among fair-skinned populations. Individuating accurate risk stratification of NMSC patients is crucial to select different options among various treatment strategies. The majority of low risk NMSCs are easily treated with surgery, offering excellent cure rates and cosmetic results. Other treatment modalities include physical destruction (curettage, cautery and cryotherapy), chemical destruction (photodynamic therapy and topical 5-flurouracil) and immunomodulatory therapy (topical imiquimod). However, there is a subset of "high-risk" NMSC characterized by prognostic factors associated to aggressive behavior, such as tumor location and size, clinical margins, histopathological variants, recurrence or previous treatment. These lesions need to be treated accordingly also by mean of adjuvant treatments. The contribution of a multidisciplinary team is necessary to appropriately manage patients affected by advanced NMSC. The aim of these practical indications is to provide a useful guidance for risk stratification of NMSC patients in clinical setting and for consequential treatment choice, resulting in individualized management strategies.

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Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are referred to as non-melanoma skin cancers (NMSC). They are the most common malignancy in the fair skin population and their frequency continues rapidly to increase worldwide.[1, 2] Even though NMSC generally has a good prognosis and infrequently metastasize, both BCC and SCC can cause substantial morbidity due to their potential to invade and destroy local tissues, their tendency to recur and their multiplicity. Therefore, NMSC exert an appreciable decrement in quality of life, arising from the tumor itself or as a result of treatment, or through symptoms, functional limitations, cosmetic burden and disturbance to the everyday activities.[3] These practical indications are based on current published data and guidelines [4-7] and aim at apprising the most appropriate management for individual patients with NMSC.

Risk factors

Several risk factors are associated with NMSC.[4-5, 8, 9] Cumulative chronic UV radiation sunexposure is accepted as a major environmental risk factor for the development of the NMSCs.[10] The risk for SCC increases in direct proportion to cumulative lifetime sun exposure, while BCC risk appears to be more related to intermittent and childhood sun exposure.[9] Moreover, proximity to equator and artificial sources of UV radiation, such as PUVA therapy and indoor tanning devices, have also been implicated in the pathogenesis of skin cancers.[9]

Other environmental factors include X-ray radiation but also chemical factors such as arsenic and polycyclic hydrocarbons, mostly in the context of occupational exposure. Fair skin type, older age (80% of cases occur in people aged 60 years or older) and male sex, are key individual factors associated with risk for NMSCs.[9, 11, 12] A number of special conditions predispose to NMSC, namely immunosuppression, including allogeneic organ transplantation, therapy of immune-mediated and oncologic diseases and human immunodeficiency virus infection, or genetic syndromes such as albinism, xeroderma pigmentosum and nevoid BCC syndrome.[9] Finally, actinic keratosis, once classified among pre-cancerous lesions, is nowadays better defined as a common skin lesion of sun-damaged skin which can potentially progress to invasive SCC.[13] Even though some authors firmly believe that actinic keratosis already represents an early in situ SCC,[13, 14] it is not unanimously regarded as a NMSC,[13, 15] accordingly we consider its management beyond the scope of this paper.

Diagnostic pathway

Total body physical examination together with accurate anamnesis, are the first step in the approach to an individual with a suspicious lesion.[4, 5] It is mandatory to perform a complete skin check since patients with a NMSC are at increased risk of having additional precancers or concurrent cancers located at other body sites.[4, 5] These individuals are also at increased risk of developing cutaneous melanoma.[16, 17] Over the past two decades, dermoscopy, a widely used non-invasive technique, has remarkably enhanced the diagnostic accuracy of NMSC.[18-20] In particular, it can allow a better assessment of the various clinical type of BCC,[21] and of the different stages of progression from actinic keratosis to invasive SCC.[22] Besides its relevance for diagnostic purposes, dermoscopy can be useful in the management of NMSC for preoperative evaluation, in monitoring the outcome of treatments and in the post-treatment follow-up.[20] More recently it has been shown that also reflectance confocal microscopy, allowing *in-vivo* tissue imaging, may contribute to a more accurate diagnosis of NMSC, to the selection of the biopsy site or definition of the surgical safety margins.[23, 24] Any suspicious lesion should be confirmed by skin biopsy and histological examination.[4, 5] If extensive disease such as bone involvement, perineural invasion or deep soft tissue involvement is suspected, additional imaging studies can be performed.[4, 5] MRI is more sensitive than CT scan in the case of possible perineural disease. For SCC, regional lymph node palpation should always complete the clinical examination, followed by ultrasound imaging and a fine-needle aspiration when abnormal lymph nodes are identified.[5, 25] A staging system for NMSC has been recommended by the American Joint Committee on Cancer but actually it is not widely used in clinical practice, lacking the inclusion of other important risk factors, so that alternative staging systems have been successively proposed.[7, 26-28]

Identification of low- or high-risk NMSC patients

Treatment strategies must include different options according to accurate risk stratification of patients. Multiple prognostic factors involved in risk of recurrence and metastasis are shared by both BCC and SCC (Table I).[4-7, 25] When a high-risk feature is identified, the management of patient should be performed according to the high-risk pathway.

Common features for risk stratifications of NMSC

Anatomic site and size

Some locations, such as the head and neck area rather than the trunk and extremities, have been shown to be a risk factor for NMSC recurrence and metastasis.[4, 5] In addition for SCC the anatomic sites such as mucosae and ears are considered high-risk of metastasizing.[5] Increasing size also confers higher possibility of recurrence.[29] Three different areas of the body have been recognized with a distinct risk profile: 1) low-risk area including trunk and extremities (excluding pretibia, hands, feet,

Table I.—*Common features for risk stratifications of NMSC (modified from the NCCN Practical Guidelines in Oncology).*[4, 5]

	Low risk	High risk
Location/size	Area L-risk <20 mm Area M-risk <10 mm Area H-risk <6 mm	Area L-risk ≥20 mm Area M-risk ≥10 mm Area H-risk ≥6 mm
Clinical margins	Well defined	Poorly defined
Perineural invasion	–	+
Immune status	–	+
Radiotherapy	–	+
Recurrence	Primary	Recurrent

nail units, and ankles); 2) moderate-risk area including cheeks, forehead, scalp, neck, and pretibia; 3) high-risk area including "mask areas" of face (central face, eyelids, eyebrows, periorbital, nose, lips, chin, mandible, preauricular and postauricular skin/sulci, temple, ear), genitalia, hands, and feet.[4, 5] A different cut-off size was fixed for the definition of the risk of recurrence in each one of these anatomical locations: 20 mm for the low-risk area; 10 mm for the moderate-risk area and 6mm for the high-risk area.[30]

Clinical margins

Many studies document the increased risk of clinically ill-defined tumor borders compared with well- circumscribed lesions.[31]

Perineural invasion

Perineural involvement is linked to an aggressive course in cutaneous BCC and SCC and to increase risk of metastasis in SCC.[8, 31] Although the incidence of perineural spread ranges from 2% to 6% in any NMSC, it is more common with SCC than BCC.[32] If large nerve involvement is suspected, MRI studies are recommended.[33] Tumors with small nerve (<0.1 mm in caliber) invasion may have a low risk of poor outcomes in the absence of other risk factors.[28, 34]

Immune status

Immunosuppression induced by organ transplantation or by high-dose exposure to PUVA is associated with a persistent, time-related increase in the risk of SCC and BCC.[35, 36] Exposure to PUVA has far less effect on the risk of BCC.[36] In organ transplant recipient, SCC show more aggressive tumor behavior whereas this difference was not reported for BCC.[37, 38] Although the role of immunosuppression in promoting the recurrence or metastasis of BCC has not been already clarified in the literature, current guidelines classify both BCC and SCC developing in settings of immunosuppression as high-risk tumors.[4-7, 25, 39]

Radiation therapy

Primary NMSCs developing in sites of prior radiation therapy (RT) are considered at high risk for recurrence or metastasis.[40, 41]

Recurrence

Previously untreated (primary) NMSCs show a lower long-term recurrence rate than recurrent tumors after failure of previous treatments.[31]

Additional prognostic factors for BCC

Histopathological variants

Some histopathological features of BCC such as growth pattern and differentiation are considered as predictor of recurrence risk.[39, 42, 43] Micronodular, infiltrative, and morpheiform variants have more aggressive behavior than the nodular, superficial and fibroepithelial BCC.[39, 42, 43] Basosquamous carcinomas is a term used to describe BCC that are associated with squamous differentiation (metatypical carcinoma is mainly used synonymously).[42, 43] Data suggest that basosquamous carcinomas have a particularly aggressive nature showing an increased likelihood of recurrence and a potential for metastasis that is more similar to that of SCC than BCC.[43] Involvement of the subcutaneous fat, perineural and intravascular invasion, and larger tumor size also correlate to aggressive behavior (Table II).[42]

Table II.—*Additional prognostic factors for risk stratifications of NMSC.*

	Low risk	High risk
Additional prognostic factors for BCC		
Histopathological variants	Superficial, nodular, fibroepithelioma of Pinkus	Micronodular, infiltrative, morpheiform, basosquamous
Additional prognostic factors for SCC		
Degree of differentiation	Well differentiated	Poorly differentiated
Histopathological variants	Common variants, verrucous, Bowen's disease	Acantholytic, adenosquamous, desmoplastic or spindle
Thickness/Clark level	<2 mm / I, II, III	≥2 mm / IV, V
Rate of growth	–	+
Site of a burn scar	–	+
Neurologic symptoms	–	+

Additional prognostic factors for SCC

Degree of differentiation

Patients with well-differentiated and keratinizing tumors have a better prognosis than those patients with poorly differentiated lesions, being the latter significantly more associated with recurrence.[25, 31, 33, 44]

Histopathological variants

The histologic subtypes of acantholytic (or adenoid), adenosquamous (or mucin-producing) and spindle (sarcomatoid) SCC are markers for an increased risk of recurrence or metastasis.[5, 7, 25, 44] Other high-risk histopathological feature is the presence of desmoplasia, with a high rate of recurrence of 80%.[45]

Depth of invasion

Maximum vertical tumor thickness and Clark level have been included in the T classification of the most recent AJCC staging for SCC.[26, 46] A modified Breslow measurement should exclude parakeratosis or scale, crust, and should be made from base of ulcer if present.[47] Tumors with less than 2-mm thickness have 0% metastatic rate compared to tumors of more than 2-mm thickness which carry a metastatic rate of more than 4%.[7] A small, somewhat older body of literature found an association between invasion of SCC into the deep reticular dermis or subcutaneous adipose tissue (corresponding to a Clark level IV or V) and aggressive behavior.[25] A meta-analysis of SCC risk factors for recurrence and metastasis found that both types of depth measurements have prognostic value.[31] The presence of perineural invasion is an adverse prognostic factor for SCC and should also be included in histology reports as long as lymphatic or vascular involvement.[5, 7]

Rate of growth

Fast growing SCC, reported in the literature as acute epithelioma, is indicative of high-risk behavior.[48]

Site of burn scar

Having high aggressive features, the suspicion of development of SCC in burn scar should be kept in mind.[49]

Neurologic symptoms

Any suggestion of neurologic involvement requires a high index of alert. Symptoms include numbness, formication, stinging, burning, pain, and/or motor deficits.[5] Approximately 30-40% of SCC with perineural invasion have clinical evidence of sensory or motor nerve involvement.[50]

Management of low-risk BCC and SCC

Primary treatment

Standard excision

Standard surgical excision, followed by postoperative margin assessment, has been reported to achieve 5-year disease-free rates of over 98% for BCC and 92% for SCC.[51] For well-circumscribed BCC lesions, less than 2 cm in diameter, excision with 4-mm clinical margins should result in complete removal in more than 95% of cases.[52] In case of SCC, any peripheral rim of erythema around the tumor must be included in the excision and it is safer to expand the clinical margins to 6 mm in order to achieve complete removal.[5, 7] If lesions can be excised with the recommended margins, then linear closure, skin grafting, or second intention healing are all appropriate reconstructive approaches.[4, 5] Surgical approaches often offer the most effective cure, but attention on function, cosmesis, and patient preference should be also considered.[4, 5]

Curettage and electrodesiccation

Curettage and electrodesiccation showed a good cost-effect rate for superficial lesions: observational and retrospective studies have reported overall 5-year cure rates of 92% and 96%, in patients with BCC and SCC selected for curettage and electrodesiccation, respectively.[53] However there are some cautions to take in consideration: 1) curettage and electrodesiccation should not be used to treat areas with terminal hair growth (such as the scalp, pubic and axillary regions, or beard area in males), since a tumor extending down the follicle might not be adequately removed; 2) if the adipose tissue is reached during the procedure, surgical excision should be at that point performed.[4, 5, 54] Carbon dioxide (CO_2) laser ablation may be effective in the treatment for low-risk BCC.[6]

Radiation therapy

RT is usually used when surgery is not indicated, often for patients older than 60 years because of possible long-term sequelae.[55] Two meta-analyses reported 5-year recurrence rates of 8.7% and 10% after RT on primary BCC and SCC, respectively.[4, 5] Furthermore RT is contraindicated in genetic conditions predisposing to skin cancer (*e.g*., basal cell nevus syndrome, xeroderma pigmentosum) and connective tissue diseases (*e.g*., lupus, scleroderma).[4-7] This option is also not indicated in verrucous carcinoma because several reports in the literature documented an increased metastatic risk thereafter.[7] RT is also an effective treatment option for selected patients with Bowen's disease who have large or multiple lesions.[5]

Superficial therapies

In patients with low-risk, superficial BCC cryotherapy, photodynamic therapy (PDT) and medical treatments (topical imiquimod, topical 5-fluorouracil) can be indicated.[4, 6] In particular, superficial therapies have good cosmetic outcomes and patient compliance. However reported recurrence rates after one year are 10% for imiquimod, 55% for 5-fluorouracil, 11-24% for PDT.[6, 56] Cryosurgery is a good treatment for low-risk BCC.[6] In nodular BCC (less than 2 cm in diameter and in low-risk areas), superficial therapies may play a role as second-line modalities.[6] In patients with low-risk *in-situ* SCC (*i.e*. Bowen's disease), where surgery and radiation are contraindicated or impractical, alternative therapies such as vigorous cryotherapy, PDT, and topical imiquimod/topical 5-fluorouracil may be considered, even though the cure rates is lower than surgery or RT.[6, 57]

Adjuvant treatment

If margins are positive after excision, patients should receive the following adjuvant treatments:[4, 5] 1) standard re-excision, recommended for low-risk L areas; 2) Mohs micrographic surgery (MMS); 3) excision with complete circumferential peripheral and deep margin assessment (CCPDMA) executed with intraoperative frozen or permanent sections; 4) RT, as a valid alternative for non-surgical candidates.

Management of high-risk BCC and SCC

Primary treatment

Standard excision

Standard excision with postoperative margin assessment is recommended for high-risk NMSC. However, wider surgical margins than those for low-risk lesions are needed.[4-7] Due to the wide variability of clinical characteristics, there is no consensus about a defined margin.[4-7] Linear or delayed repair are recommended.

MMS or excision with complete margin assessment

MMS is the preferred surgical technique for high-risk NMSC because it allows intraoperative analysis of the whole excision margin.[4-7] Two meta-analyses associated MMS with a 5-year recurrence rate of 1.0% for primary BCC, and 5.6% for recurrent BCC. In both of these meta-analyses the recurrence rate for MMS was lower than that for standard surgical excision (10.1% and 17.4% for primary and recurrent BCC, respectively), and lower than the recurrence rate for any other treatment modality included in the analysis (curettage and electrodesiccation, cryotherapy, and RT).[4-7] CCPDMA using intraoperative frozen or permanent section assessment is acceptable as an alternative to MMS.

Radiation therapy

RT is usually used for non-surgical candidates, especially if older than 60 years.[55]

Adjuvant treatment

If negative margins are not achieved after treatment with standard excision, MMS or resection with CCPDMA, patients should receive adjuvant therapy.[4-7] If the disease is still present after adjuvant treatment, and further surgery or RT are contraindicated, multidisciplinary consultation should be considered in order to consider systemic treatment or clinical trial.[4-7] Adjuvant RT is also recommended for patients with negative margins after surgery but with large nerve or extensive perineural involvement.[4-7] For high-risk SCC lesions, sentinel lymph node mapping may be considered.[5, 7, 58]

Management of recurrence, regional lymph node involvement, metastasis and advanced tumors

Recurrence

For the management of local tumor recurrence is recommended to follow the pathway for primary treatment.[4, 5]

Regional lymph node involvement

Lymph node involvement in patients with primary SCC is not infrequent and it significantly increases the risk of recurrence and mortality.[5, 7, 58] For the management of lymph-node involvement, patients with palpable or suspicious lymph nodes on ultrasound imaging should receive a fine-needle biopsy or core biopsy. If there are positive histopathological involvement of a lymph node, CT with contrast should be performed, in order to determine the size, number, and precise locations of the nodes and to rule out distant disease.[59] Once documented the nodal involvement, in patients with an operable disease, the preferred treatment is regional lymph node dissection according to specific surgical modalities based on clinicopathological characteristics of the nodal basins involved.[5, 60] Although the use of sentinel lymph node biopsy has been investigated in several studies, there are no conclusive data on its prognostic information or the possible therapeutic value.[5, 7, 58]

Metastasis and advanced tumor

Even if the behavior of cutaneous BCC is characteristically indolent, the disease does very rarely metastasize to distant sites, with an incidence ranging from 0.0028% to 0.55% of cases.[6] Nodal or distant metastases should be treated with surgery and managed by a multidisciplinary tumor board.[4, 6] Advanced BCC is a definition that encompasses both metastatic BCC and locally advanced BCC (a lesion no longer amenable to surgery or RT). Treatment of advanced BCC is still very challenging for the lack of randomized controlled trials/guidelines and the scarcity of available therapeutic options.[61, 62] Based on current data, surgical procedures and RT remain the treatments of choice although often associated with substantial morbidity and/or deformity.[4, 6] Alternatively, systemic chemotherapy and electrochemotherapy can be used but standardized treatment schedules and randomized clinical trials are not available for both treatments.[4, 6] In recent years, novel tumor-specific and pathogenesis-based molecules have been developed for the targeted therapy of advanced BCC.[61-63] A number of clinical trials have recently demonstrated the efficacy and tolerability of vismodegib, the first Hedgehog pathway inhibitor, showing objective response in 48% and 33% of patients with locally advanced and metastatic disease respectively, with median response duration of 9.5 months and 7.6 months, respectively.[64] Sonidegib, another hedgehog pathway inhibitor, has also been approved by the FDA for the treatment of patients with locally advanced BCC that has recurred following surgery or RT, or who are not candidates for surgery or radiotherapy, although it is currently still not approved in Europe.[62, 65]

Cutaneous SCC with distant metastasis, while infrequent, is more common than metastatic BCC, having 3.7% risk of distant metastasis and 2.1% risk of disease-specific death.[4] Unfortunately, there is no evidence available regarding systemic therapy for this condition and there are no prospective phase III studies available.[66] Possible agents for patients with metastatic SCC include cisplatin monotherapy, cisplatin plus 5-fluorouracil, epidermal growth factor receptor inhibitors such as cetuximab, or PD-1 inhibitors: all these treatments have occasionally produced useful responses, but data supporting efficacy are limited.[67, 68] The introduction of novel therapeutic options requires continuous updates of recommendations and adaptation to national contexts. Remarkably, the contribution of a multidisciplinary team composed of dermatologists, surgeons, oncologists, pathologists, radiologists, and radiotherapists is required to properly manage patients affected by advanced NMSC.[4-7]

Follow-up

Individuals with personal history of NMSC are at heightened risk of developing additional primary skin cancers.[69] About 30% to 50% of patients with a prior SCC will develop another NMSC within 5 years.[70] These patients are also at increased risk of developing cutaneous melanoma.[16] Therefore, it is important to carry on a long-term surveillance, with physical exam and complete skin examination.[4-7] A prospective population-

TABLE III.—*Recommended follow-up intervals for NMSC.*

	Skin examination	Lymph-nodal investigation
Low-risk primary BCC	First 2 years: every 12 months	
High-risk primary BCC	First 2 years: every 6 months	
Low-risk primary SCC	First 5 years: every 12 months	
High-risk primary SCC	First 2 years: every 3-4 months 3rd-5th year: every 6 months After 5th year: every 12 months	First 2 years: lymph-nodal ultrasound every 3-4 months
Advanced or regional SCC	First 5 years: every 3 months Thereafter follow the schedule of high risk primary SCC	First 5 years: lymph-nodal ultrasound every 3-4 months and imaging every 6 months
High-risk patients	Every 6 months lifelong + follow the schedule depending on the primary tumors	

based cohort study found that development of a second NMSC is most likely during the short-term follow-up period after diagnosis of the first lesion.[4-7] Therefore, close follow-up of these patients during this time period is critical. For both BCC and SCC, the frequency of follow-up should be based on risk (Table III).[4-7] For BCC, exams should occur at least every 6 to 12 months during the first 2 years; if no further skin cancer develops in the first 2 years, then it may be appropriate to reduce exam frequency.[4, 5] For low-risk primary SCC physical exam and complete skin examination should occur every 12 months in the first 5 years, since the low risk of development of new skin cancers.[4, 5] For the high-risk primary SCC physical exam and complete skin examination should occur every 3-4 months in the first 2 years, then every 6 months for the following 3 years, then annually starting from the 5th year. In this case, it is also important to perform a lymph-nodal ultrasound exam at the time of the clinical examination in the first 2 years.[4, 5] For advanced or regional SCC physical exam and complete skin examination should occur every 3 months in the first 5 years, thereafter clinicians should follow the schedule for high-risk primary SCC. In this case, it is also important to perform a lymph-nodal ultrasound exam every 3-4 months and imaging every 6 months during the first 5 years, since the high risk of regional and distant metastases.[4-7]

For high-risk patients (affected by genetic conditions predisposing to skin cancer, like basal cell nevus syndrome or xeroderma pigmentosum, or immunosuppressed patients), physical exam and complete skin examination should occur every 6 months lifelong, since the very high risk of new skin cancers.[4, 5]

Prevention

Finally, it is crucial to highlight the importance of prevention and patient educations.[4, 5, 9, 71] The value of sun avoidance and protection methods should be extensively explained to NMSC patients, along with periodical self-examination.[4, 5] The use of sunscreens may protect from the development of subsequent BCC, but currently, insufficient evidence supports the use of sunscreens in BCC prevention.[6] In patients with precancerous lesions, early detection and intervention are critical in order to prevent the development of invasive SCC.[7] The use of nicotinamide or oral retinoids (acitretin, isotretinoin) has been effective in reducing the development of actinic keratosis and SCC in some high-risk patients.[4, 5]

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Surgical approach to primitive melanoma

Dear Editor,

The optimal surgical approach to melanoma and suspicious skin lesions has been the subject of debate for many years. The current trend, which is universally accepted, is to excise the lesion as closely as possible to maximize preservation of the surrounding normal skin. The exact approach will depend on the lesion site and clinical features, and includes excisional biopsy with possible enlargement of the surgical incision or incisional biopsy, if necessary.

Excisional biopsy is the first choice of surgical approach in the case of a melanoma or a suspicious pigmented lesion. The excision of the lesion must be complete, include the major axis of the anatomical site in which the lesion is located as well as margins of 1-2 mm of the healthy skin around the lesion, and extend into the subcutaneous tissue (Figure 1).[1]

This type of limited intervention is necessary to obtain a clear diagnosis of the excised lesion and an accurate description of the histopathological characteristics. The decision to enlarge the surgical biopsy incision and perform a sentinel node biopsy will be based on the findings of the excisional biopsy.[2] Even when the clinical diagnosis is certain, the excision biopsy should include margins of at least 2 mm, so that the surgeon can decide how much skin must be eliminated on the basis of the histopathological data. The margin also helps ensure that the lymphatic drainage pathways are not disrupted and allows the proper identification of the sentinel lymph node.

Enlargement of the biopsy incision or radicalization is the second step in the surgical treatment of melanoma and must be done preferably within 4-6 weeks of initial diagnosis.[2] The distance of the incision depends on lesion thickness (Table I). Excisions should be performed with vertical edges to ensure regular margins. Excision of the lesion must extend to the wing, but without remove it, unless it is not also involved. However, in some cases, a high number of mitoses are associated with a low lesion thickness as well as the presence of ulceration and the age of the patient. In this scenario, the distance from the biopsy margin should be determined on a case-by-case basis (Figure 2).[3-6]

In excisional biopsies, often, the histological reports describe a distance from the margin of 4 or 5 mm, and depending on the anatomical site, this would correspond to 7-8 mm *in vivo,* as the cut skin retracts. When satisfied with the diagnosis and defined histological parameters, the surgeon must determine how much healthy skin should be discarded during surgical enlargement.

The Italian and European guidelines recommend making the enlargement by marking the adequate distance from the edge of the previous scar with a ruler and a marker. The margins must be measured prior to excision, without taking into consideration the skin already eliminated.[1, 3-10] The optimal enlargement distance is a topic on which no consensus has yet been reached.

In particular cases and special locations, the surgical excision margins can be reduced to preserve the noble structures of the face or the anatomical and functional integrity of the affected site,[10] for example, in the case of lesions involving the face, nose, ears, eyelids, nail beds, or the palmar and plantar surfaces, or in the case of

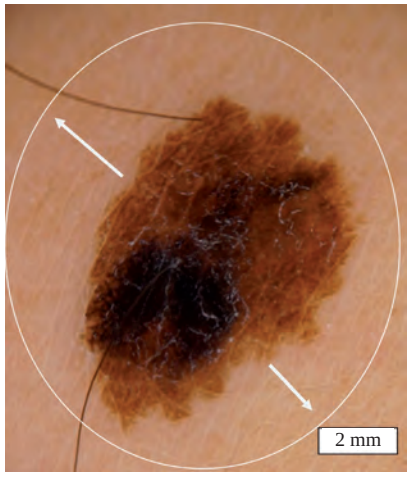

Figure 1.—Excisional biopsy.

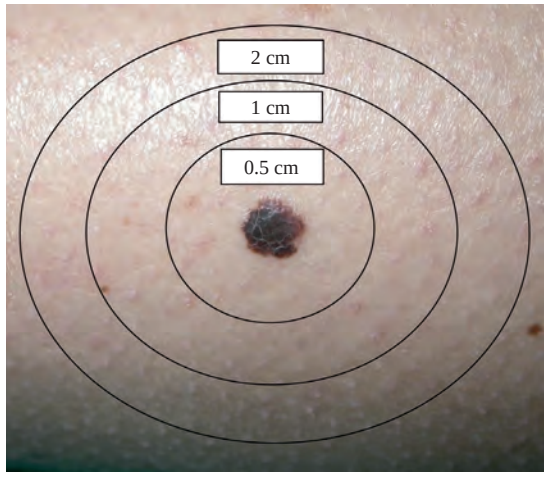

Figure 2.—Surgical margins.

TABLE I.—*Surgical margins for the radicalization of primary melanoma.*

Tumor thickness	Recommended margins *	Strength of recommendation
In situ	0.5 cm	III B
0-1 mm	1 cm	I A
1.01-2 mm	1-2 cm	I A
2.01-4 mm	2.0 cm	I A
>4 mm	2-3 cm	II B

*The margins can be modified based on the location of the melanoma.[6]

very large lesions.[10, 11] Flaps and grafts are not recommended and should be used only when primary closure is not possible.[12]

Incisional biopsy, such as the superficial "shave" biopsy and the deep "punch" biopsy, is usually not performed in cases of melanoma because it does not give reliable results, especially, with regard to the regression and thickness of the lesion.[1, 2] However, it may be used in particular cases where the clinical and dermoscopic diagnoses are uncertain, in the case of large lesions, in the case of lesions in aesthetically important sites, or when there is a need to document with certainty a lesion whose removal would imply a complex and mutilating surgery, for example, lesions involving the nail bed. Incisional biopsies are not associated with an increased risk of metastasis.[1, 2, 13]

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ORIGINAL ARTICLE

Efficacy of cyclosporine A as monotherapy in patients with psoriatic arthritis: a subgroup analysis of the SYNERGY Study

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ABSTRACT

BACKGROUND: The SYNERGY Study is an observational, multicenter Italian study, conducted in patients with diagnosis of psoriatic arthritis (PsA) treated from at least 3 months with cyclosporine and aimed at assessing patients' seropositivity for viral infections and efficacy and safety of cyclosporine, administered as monotherapy or in combination with other systemic drugs in the routine clinical practice. The aim of this subanalysis of the SYNERGY study was to evaluate the effects of CsA as monotherapy only in PsA over 12 months of observation.
METHODS: Psoriasis was evaluated by Body Surface Area and the Psoriasis Area Severity Index (PASI). PsA was evaluated by number of swollen and tender joints, painful entheses and fingers with dactylitis, the Bath Ankylosing Spondylitis Activity Index (BASDAI) and by patients' and physicians' global assessment on a 10-point Visual Analogue Scale.
RESULTS: Cyclosporine in monotherapy was effective in reducing all the measured disease parameters. The major indexes of cutaneous and spinal involvement, PASI and BASDAI were significantly reduced over the study period, as were the number of swollen and tender peripheral joints, and enthesitis and dactylitis.
CONCLUSIONS: Cyclosporine in monotherapy confirmed its efficacy in cutaneous psoriasis and suggested to be effective also on PsA, reducing spinal and peripheral joints' signs and symptoms.

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Key words: Cyclosporine - Observational study - Psoriasis - Arthritis, psoriatic.

The SYNERGY Study is an observational, cross-sectional and longitudinal (12 months) multicenter Italian study, which enrolled 238 consecutive patients with diagnosis of psoriatic arthritis (PsA), performed within 8 years from baseline and treated from at least 3 months with cyclosporine A (CsA) as monotherapy or in combination with one or more systemic drugs. The study was aimed at assessing the patients' seropositivity for former viral infection or suggestive of acute viral infection and the efficacy and safety of CsA administered in routine clinical practice alone or in combination with other immunosuppressants over 12 months. The cross-sectional phase showed that 56% of patients had 2 or more seropositivities indicative of former infections,

and 31 patients (13.8%) presented seropositivity for HCV, HBV, HSV-1 and -2, HHV-6, EBV, or parvovirus infection. None of these 31 patients developed virus reactivation. Significant reductions (P<0.001) in PASI, BASDAI, and VAS scores were observed at 6 and 12 months, suggesting that treatment of PsA with CsA as monotherapy or in combination with other immunosuppressants is safe and effective and might be the treatment of choice in PsA HCV-positive patients.[1]

CsA is recognized as an effective treatment for psoriasis also in monotherapy, administered with many different approaches: 1) intermittently as a short-course (12-16 weeks) therapy; 2) continuously to maintain remission in a minority of patients with refractory disease; 3) as a crisis intervention (4-8 weeks) to reduce flare or treat severe disease; 4) as a part of sequential and rotational therapy to help minimize toxicity and optimize efficacy.[2, 3] Particularly in skin disease, CsA alone was shown to be superior to adalimumab alone.[4] Therefore, the present subgroup analysis was conducted in patients treated with CsA alone, in order to evaluate the effects of CsA monotherapy in PsA over 12 months of observation.

Materials and methods

SYNERGY was a cross-sectional and longitudinal 12-month observational study, conducted in 24 Italian dermatology clinics, which included 238 consecutively enrolled patients with psoriatic arthritis. Patients and methods of the study are described in details elsewhere.[1] Patients evaluable for the SYNERGY cross-sectional analysis who had undergone treatment with CsA only during the 12-month observation period were considered in this sub-analysis. Psoriasis was evaluated by Body Surface Area (BSA) and the Psoriasis Area Severity Index (PASI).[5] PsA was evaluated on the basis of the number of swollen and tender joints, of painful entheses and fingers with dactylitis, and by the patients' and the physicians' global assessment of the disease, using a 10-point Visual Analogue Scale (VAS).[6] The Bath Ankylosing Spondylitis Activity Index (BASDAI)[7] was used to evaluate spinal inflammation. We also analyzed if there were disease characteristics affecting physicians' decision to prescribe CsA as monotherapy or in combination with other systemic medications.

Patients with missing data were not excluded from the cohort, but simply not analyzed for the missing variable. Statistical analyses were performed by SAS v. 9.2 (SAS Institute Inc., Cary, NC, USA) and Enterprise Guide 4.3 (SAS Institute Inc.).

The study was approved by the committee on research ethics at the institution in which the research was conducted and any informed consent from human subjects was obtained as required.

Results

Ninety-four patients of the whole SYNERGY cohort were included in this sub-analysis. Fifty-two (55.3%) were males, mean age was 48.2 (Standard Deviation 12.3), 43 (45.7%) had concomitant diseases, mainly cardiovascular (N.=25, 26.6%). Mean age at diagnosis of psoriasis was 37.3 (15.4) years and mean duration of disease was 11.3 (10.6) years. Seventy-one (75.5%) patients had plaque psoriasis, 4 had pustular, 3 had guttata, one each had inverse and erythrodermic psoriasis; 12 patients had plaque psoriasis combined with other types (guttata and erythrodermic, 4 each; inverse and pustular, 2 each). Mean age at the diagnosis of PsA was 46.5 (12.2) years, median duration of PsA was 3 years (25^{th}-75^{th} percentiles: 1.3-4.5), and the median delay between first PsA symptoms and diagnosis was 4 months (25^{th}-75^{th} percentiles: 2-12). Presence of CASPAR criteria at baseline are shown in Table I. At baseline, peripheral arthritis was observed in 73 (77.7%) of patients, enthesitis in 36 (38.3%), and spondylitis in 9 (9.6%). Peripheral arthritis was monoarticular in 7 (7.4%) patients, oligoarticular in 41 (43.6%), and polyarticular in 28 (29.8%).

During the 12 months prior to enrolment in the SYNERGY Study, 73 (77.7%) of the 94 patients of this analysis had already received CsA as systemic monotherapy, while the remaining 21 had received CsA combined with other non-biologic systemic treatments; 74 (78.7%) had received topical therapy for psoriasis, and

TABLE I.—*CASPAR criteria for psoriatic arthritis present at baseline.*

Criterion	N. (%)
Current psoriasis	83 (88.3)
Personal or family history of psoriasis	11 (11.7)
Nail psoriasis	70 (74.5)
Dactylitis	53 (56.4)
Negative rheumatoid factor	64 (68.1)
Rx evidence of new bone formation	12 (12.8)

TABLE II.—*BSA evolution during the 12-month follow-up.*

	Baseline % (SD)	6 months % (SD)	12 months % (SD)
BSA face and scalp	1.2 (1.1)	0.5 (0.7)	0.3 (0.6)
BSA trunk	1.3 (1.2)	0.6 (0.8)	0.4 (0.8)
BSA upper limbs	1.5 (1.0)	0.9 (0.8)	0.6 (0.9)
BSA lower limbs	1.4 (1.2)	0.9 (1.0)	0.6 (0.9)

one only had received local corticosteroid infiltrations for PsA. During the 12-month observation period, the number of patients on topical therapy for psoriasis decreased to 44 at 6 months and 45 at 12 months, while local infiltrations remained limited to 2 cases at both 6 and 12 months.

During the 12 months of the study with CsA as only systemic treatment, BSA progressively decreased (Table II). PASI, BASDAI and VAS on activity of disease, evaluated by both patients and physicians, significantly decreased from baseline at 6 and 12 months (Figure 1). The numbers of swollen and tender joints, painful entheses and fingers with dactylitis at each study visit are summarized in Table III. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) did not significantly change over the study period. Significantly higher percentages of patients with peripheral arthritis and spondylitis were treated with CsA in combination compared to monotherapy (92.0 *vs.* 77.7%, P=0.004, and 24.1 *vs.* 9.6, P=0.006, respectively), while the opposite was observed for enthesitis and dactylitis (22.3 *vs.* 38.3, P=0.012 and 34.8 *vs.* 56.4, P=0.002, respectively). No difference was observed in the allocation to CsA monotherapy or combination therapy on the basis of PASI, BASDAI, presence of current dermatological manifestations, and nail disease. The type of treatment received in the 12 months preceding the entry into the study was highly maintained during the study: 77.7% of the patients in this analysis had been treated with monotherapy also in the previous year.

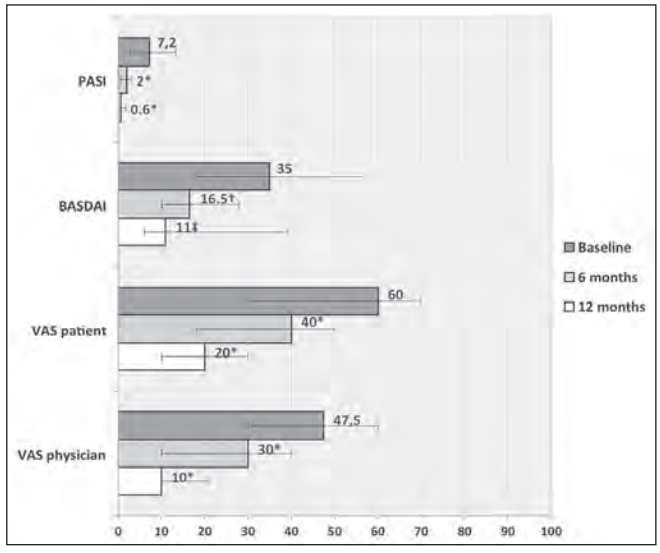

Figure 1.—PASI, BASDAI, and VAS on activity of disease assessed by patients and physicians at the 3 study visits.
Median values are represented by horizontal bars, 25th and 75th percentiles by error bars; 12-month VAS physician 25th percentile and median are both equal to 10.
*P<0.0001; †P=0.0007; ‡P=0.0034 Wilcoxon Test.

Discussion

CsA is approved for the treatment of moderate to severe psoriasis based on positive findings from a number of controlled clinical trials.[8-15] Less is known about its efficacy in PsA: three studies have reported the safety and moderate efficacy of CsA in PsA.[16-18] The SYNERGY study had among its objectives to assess the efficacy and safety of CsA administered in routine clinical practice, alone or in combination with other immunosuppressants.[1] The results showed a progressive improvement in patients' cutaneous and musculoskeletal clinical picture, and the authors hypothesized that such improvement was related partly to the progressive therapeutic effect of CsA, and partly to the increased number of patients who received biologics in combination with CsA.

TABLE III.—*PsA evolution during the study.*

Parameter	Baseline median (25-75 percentile)	6 months median (25-75 percentile)	P value*	12 months median (25-75 percentile)	P value*
Number of swollen joints	1 (0-4)	0 (0-3)	0.0281	0 (0-1)	0.0006
Number of painful joints	4 (2-10)	2 (1-8)	0.0057	1 (0-3)	0.0002
Number of painful entheses	0 (0-2)	0 (0-2)	0.0239	0 (0-2)	0.1946
Number of fingers with dactylitis	0 (0-4)	0 (0-2)	0.0573	0 (0-0)	0.0001

*from baseline, Wilcoxon Test.

In the present subgroup analysis, only patients treated with CsA alone during the 12-month study observation period were considered. We observed that CsA in monotherapy was effective in reducing all the measured disease parameters. The major indexes of cutaneous and spinal involvement, PASI and BASDAI were significantly reduced over the study period, as were all PsA signs and symptoms. It has to be pointed out that patients with peripheral joint and spinal involvement were more likely to be treated with CsA in combination with other systemic therapies; however almost 80% of patients in our cohort had peripheral arthritis, mainly oligo- or polyarticular. In these patients, CsA alone was able to significantly reduce the number of swollen and tender joints. There was no impact of CsA treatment on acute phase measures, but this is not surprising since CsA does not act as an anti-inflammatory drug, but rather through an immunosuppressive mechanism.

Concerning our analysis aimed at exploring possible criteria for allocating patients to either mono- or combination therapy, first of all it has to be underlined that nearly 80% of patients were maintained in the same treatment regimen before and during the study. It is not surprising that patients with peripheral and spinal arthritis were treated with combination therapy, as the addition of methotrexate or biologics is known to improve efficacy on the arthritic components of the disease. Interestingly, on the other hand, the presence of enthesitis and dactylitis did not discourage physicians to maintain the patients in monotherapy.

Limitations of the study

Obviously, this analysis suffers the limitation that it was not pre-specified in the study protocol and, even more importantly, the study had not been designed to specifically evaluate the efficacy of CsA monotherapy in treating psoriasis and PsA. Therefore, also the size of the CsA monotherapy sample is inadequate to draw conclusions about the efficacy of this treatment approach.

Conclusions

Taking advantage of the data available from the SYNERGY Study, we can conclude from this analysis that CsA in monotherapy confirms its efficacy in cutaneous psoriasis and suggests to be effective also on PsA, at least in this limited subgroup of patients, reducing BASDAI and articular signs and symptoms.

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REVIEW

"Active" photoprotection: sunscreens with DNA repair enzymes

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ABSTRACT

Ultraviolet (UV) radiation exerts different harmful effects on human health, being the main etiological agent of certain skin cancers and photoaging. In this context, photoprotection, intended as a set of measures adopted to limit and prevent the effects of UV radiation, plays a critical role in avoiding undesired sunlight outcomes. Traditional sunscreens represent a widely used photoprotective approach, even if they exert a "passive photoprotection" and are not effective once damage to skin cells has been generated after sun exposure. Conversely, "active" photoprotection is represented by topical sunscreens including also antioxidants and liposome-containing DNA repair enzymes, which may constitute a photostrategy filling the current gap in sun protection. In the current review, we focused on "active" photoprotection at a topical level, reporting present knowledge and future prospective regarding DNA repair enzymes such as photolyase, endonuclease and 8-oxoguanine glycosylase which are able to enhance the protective power of traditional sunscreens.

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Key words: Sun protection factor - Skin care - Endonucleases - Deoxyribodipyrimidine photo-lyase - Human oxoguanine glycosylase 1 - DNA damage - DNA repair.

Every day the earth is regularly irradiated by sunlight. Nevertheless, ultraviolet (UV) radiation (290-400 nm) represents only 5% of light coming from the sun and it exerts many different and significant either beneficial or harmful effects on human health. Particularly, UV plays a crucial role in vitamin D synthesis, regulation of circadian rhythm, and improvement of mood through the regulation of serotonin production.[1] On the other hand, UV radiation can be absorbed by diverse skin chromophores (melanin, DNA, RNA, proteins, trans-urocanic acid, etc.) leading to different photochemical reactions and alterations such as reactive oxygen species (ROS) production and consequent oxidative stress processes, DNA damage (formation of cyclobutane pyrimidine dimers, 6,4 pyrimidine-pyrimidones adducts, hydration products, etc.), cells apoptosis and/or necrosis.[1, 2] Furthermore, all these processes are also able to modulate the synthesis and release of both pro-inflammatory and anti-inflammatory cytokines such as interleukin (IL) -1, -6, -10, -17, and -22, interferons (IFN) α and γ, and tumor necrosis factor (TNF) α leading to biological response modifiers modulation with consequent immune suppression.[1, 2]

This variety of biological effects can deeply influence functional and structural components: immune system, cellular renewal and intercellular matrix production, thus leading to enhanced skin aging and induction of skin cancers, as well as ocular damage, and/or modification of the natural course of several inflammatory/autoimmune diseases (lupus erythematosus, dermatomyositis, polymorphous light eruption, etc.).[3-5]

In this context, photoprotection, intended as a set of measures adopted to limit and prevent the effects of UV radiation, reducing sun exposure and preventing the de-

velopment of acute and chronic actinic damage, plays a critical role in avoiding undesired sunlight outcomes.[6, 7] Among photoprotective measures, the best and most used is undoubtedly the application of sunscreens, products primarily designed to protect the skin from the dangerous effects of solar UV radiation.[1, 6] There are several types of sunscreens, which widely differ in composition, type of vehicle, mechanism of action, etc. Particularly, depending also on sunscreens features, photoprotection can be performed in an "active" or a "passive" modality.[8] "Passive" photoprotection is displayed by traditional topical sunscreens through filtering or scattering UV radiations; this is still the most widely used photoprotective approach, but it is ineffective toward damaged skin cells after sun exposure. Conversely, "active" photoprotection, whose development has shown a great progress in the last decade, is represented by substances able to reduce the oxidative insult or recover the DNA damage that occurs after sun exposure. These substances can be either administered through oral or topical compounds: antioxidants (carotenoids, vitamin C, vitamin E, selenium, *Polypodium leucotomos* extract, nicotinamide, etc.) and liposome-containing DNA repair enzymes.[9-17] In the current review, we focused on "active" photoprotection at a topical level, reporting present knowledge and future prospective regarding sunscreens with DNA repair enzymes.

Literature search

We searched for English-language literature describing "active" photoprotection in PubMed, Google, Google Scholar, and Scopus. The following key words were used: active photoprotection, DNA repair enzymes, sunscreens, photolyases, endonuclease, 8-oxoguanine glycosylase.

"Active" photoprotection with topical sunscreens

An ideal topical sunscreen should present different features: 1) protection against UVB radiation and long-wavelength UVA radiation; 2) stability and safety of the filters; 3) ROS scavenging capability; and possibly 4) inclusion of enzymes or active reagents that activate the cellular DNA repair systems.[1] In this context, the concept of "active" photoprotection should be considered as an attempt to fulfill all the requirements of an ideal topical sunscreen. Literature is deeply full of studies regarding topical sunscreens with antioxidants to fight against UV-induced ROS production, and diminish UV-related damage of the skin. Indeed, sunscreen formulations including classic antioxidants such as vitamin C, vitamin E, and betacarotene, whose photoprotective effects against UVB and UVA are well characterized, and have been widely investigated.[1, 9, 18-23] Moreover, plenty of additional and relatively newer antioxidants such as caffeic and ferulic acid,[24-26] flavonoids (equol,[27] genistein,[28, 29] quercetin,[30] silymarin),[31] polyphenols (green tea pholyphenols,[32, 33] ellagic acid,[34] resveratrol),[35, 36] *Polypodium leucotomos* extract [37-39] and pycnogenol [40] have also been extensively studied as possible components of topical sunscreens. Other than that, the newer and promising topical photoprotective agents contain also DNA repair enzymes such as photolyase, endonuclease and 8-oxoguanine glycosylase, designed to enhance the repair of damaged DNA after UV exposure.[1] The main features of these products are discussed in the following paragraphs.

Photolyase

Photolyase is a DNA-repair enzyme, which recognizes and specifically binds to cyclobutane pyrimidine dimers: exposure of the photolyase–dimer complex to photoreactivating light (300-500 nm) converts the dimerized pyrimidines to their monomeric form. Dimer-specific photolyase is present in an active form in numerous prokaryotes and certain eukaryotes, including fish and marsupials but not humans and mammals.[41, 42] Photolyase was first isolated from the cyanobacteria, *Anacystis nidulans*, which are a component of plankton.[41, 42] In 2000, Stege *et al.* showed that topical application of photolyase (prepared from *Anacystis nidulans)* was able to induce a 50% decrease in the number of UVB-induced dimers in human skin, with a maximum reduction after 22.5 hours post exposure.[43] Particularly, topical application of photolyase (encapsulated into liposomes), immediately after UVB exposure, was shown to be effective in partially removing UVB radiation-induced cyclobutane pyrimidine dimers from the epidermis, thereby diminishing erythema, sunburn-cell formation, and the suppression of production of intracellular adhesion molecule-1, a molecule required for immunity and inflammatory events in the epidermis.[43] In 2012, Berardesca *et al.* confirmed that the addition of photolyase to a traditional sunscreen enhanced the reduc-

tion of both cyclobutane pyrimidine dimers and apoptotic cell death in human skin after solar-simulated UV exposure (photolyase and traditional sunscreen were applied 30 minutes before UV irradiation).[44] Moreover, other than in DNA repair, photolyase aids in cell regeneration and reduces skin inflammation caused by exposure to sunlight, through reduction of pro-inflammatory cytokines such as IL-6, which is also involved in cell apoptosis.[45] An evidence regarding the effective role of photolyase in fighting against UV induced alterations and tumorigenesis comes from a survey on 8 patients with xeroderma pigmentosum, a rare genetic disease with clinical and cellular hypersensitivity to UV radiation, characterized by a defective DNA repair process.[46] Particularly, the authors observed that 12-month treatment with photolyase and very high-protection UV filters was associated with 65% reduction in appearance of new actinic keratosis, and with 56% and 100% reductions in the incidence of new basal cell carcinoma and squamous cell carcinoma, respectively. This suggested that the topical use of photoprotection and DNA repair enzyme could help lower UV induced skin cancer lesions.[46] These data are further supported by other experimental studies carried out in patients with actinic keratosis, through clinical and confocal microscopy observation, where the application of a medical device containing photolyase and high-protection UV filters was associated with an improvement of the cutaneous cancerization field, the skin area associated with genomic alterations due to the carcinogenic effect of sun exposure and where actinic keratosis and squamous cell carcinoma develop.[47, 48] Therefore, photoprotection and photorepair appeared to be a useful strategy in order to further reduce the risk of pre-malignant and malignant UV-induced skin lesions in comparison with simple photoprotection strategies. Exogenous application of photolyase differs from conventional photoprotection for its ability to remove damage that has already occurred. This enzymatic therapy approach could thus be ideally combined as an after-sun strategy with conventional sunscreens to provide photoprotection and repair at the same time.

Endonuclease

Endonuclease is a DNA repair enzyme form *Micrococcus iuteus*, a UV resistant bacterium found in marine waters and soils, which acts as a cyclobutane pyrimidine dimers glycosylase/abasic lyase but, differently form photolyase, does not require light energy activation.[49, 50] Similarly to photolyase, topical application of endonuclease was shown to promote dimer repair in skin of patients affected by xeroderma pigmentosum, presenting an average of enhanced dimer removal of approximately 20% in 6 hours;[51] moreover the same enzyme was able to decrease the development of actinic keratosis and basal cell carcinoma (68% and 30% respect to placebo, respectively) during one year of treatment, in a group of 20 xeroderma pigmentosum patients in a prospective, multicenter, double-blind study.[52] In addition, Wolf *et al.* observed that topical application of endonuclease nearly completely (>90%) prevented UV induced upregulation of TNF-α at RNA message level and IL-10 at both RNA and protein level, providing a new avenue for photoprotection against some forms of light-induced autoimmune inflammatory dermatitis.[53] Indeed, it is well-known that immunosuppression *via* TNF-α and IL-10 dysregulation contributes to cutaneous tumorigenesis, further underlying the protective effects of this enzyme and its ability to enhance the immune system.[54-56] In cosmetic preparations, endonuclease enzyme is encased in a multi-layer phospholipid coated envelope, which allows for the enzyme to easily enter cells, improving the efficiency and speed of DNA repair approximately fourfold.[54] Moreover, generally it also stimulates skin regeneration and reconstruction, alleviates skin irritation by reducing pro-inflammatory mediators, and prevents the destruction of extracellular matrix components.[57] Application of endonuclease liposomes immediately after UV exposure is also able to partially protect against sunburn cell formation.[58] In line with previous studies, DeBoyes *et al.*, evaluating 17 patients applying liposome lotion containing endonuclease over 48 weeks, demonstrated a reduced incidence of actinic keratosis in normal individuals with moderate-to-severe photodamaged skin.[54]

Photolyase + endonuclease

There are several studies investigating the beneficial and preventive effects of topical application of traditional sunscreens containing both photolyase and endonuclease. Recently, Carducci *et al.*, through a 6-month randomized clinical study, reported that the addition of these DNA repair enzymes to conventional sunscreens enhance the reduction of the cancerization field and cyclobutane pyrimidine dimers (61% *vs.* 35%), compared

to sunscreens alone, in actinic keratoses patients, despite a similar effect on hyperkeratosis.[59] In addition, other authors reported that topical application of xenogenic DNA repair enzymes, represented by photolyase from *Anacystis nidulans* and endonuclease from *Micrococcus iuteus,* was able to fight against UV induced skin aging and increased risk of tumorigenesis through abrogation of telomere shortening and c-FOS proto-oncogene hyperexpression in UV irradiated human skin.[60] Furthermore, Hofer *et al.* observed that the use of an after-sun lotion containing DNA-repair enzymes (photolyase and endonuclease) induced a significant reduction in symptoms of polymorphic light eruption (PLE), a photodermatosis whose pathogenesis may involve resistance to light-induced immune suppression and simultaneous immune reactions against skin photoneoantigens.[61]

8-oxoguanine glycosylase

8-oxoguanine glycosylase (OGG1) is a DNA repair enzyme derived from the mustard plant *Arabidopsis thaliana* able to repair DNA bases damage caused by ROS (oxidative damage to DNA bases such as the formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine [8OHdG] and 8-oxo-7,8-dihydroguanine [8-oxoG] which form helix-distorting photoproducts).[2] Indeed, Berneburg *et al.* showed that OGG1 was able to remove 8-oxoG DNA adducts within 2 hours, in photoexposed human keratinocytes, suggesting that topical exogenous OGG1 can dramatically reduce free-radical damage.[62] Furthermore, Emanuele *et al.* showed the effectiveness of a new topical product (TPF50) consisting of a traditional physical sunscreen (SPF 50) enriched with liposome-encapsulated DNA repair enzyme complex (photolyase, endonuclease, and OGG1), and a potent antioxidant complex (carnosine, arazine, ergothionine), compared to existing products.[63] Particularly, TPF50 showed the best efficacy in reducing cyclobutane pyrimidine dimers, 8OHdG and ROS induced protein carbonylation in human skin, reducing the risk of skin aging and development of skin cancers. The importance of OGG1 in fighting skin cancers is also highlighted by the fact that its levels are down-regulated in human basal cell carcinoma, the most common form of skin neoplasm,[64] but also by the fact that its application is able to reduce tumor size and tumor progression in mice treated with UVB 3 times weekly.[65]

Conclusions

UV light is able to exert several harmful effects. One of the most important is represented by DNA damage, which plays a crucial role in the induction of immunosuppression finally leading to photocarcinogenesis. Traditional sunscreens represent a widely used photoprotective approach even if they are not effective once damage to skin cells has been generated after sun exposure ("passive" photoprotection). In this context, the addition of topical DNA-repair enzymes constitute a new photostrategy which may fill the current gap in sun protection. Indeed, DNA-repair enzymes such as photolyase and endonuclease have been shown to reduce UVB radiation-induced cyclobutane pyrimidine dimers, apoptotic cell death, development of UV related skin cancers (actinic keratosis, basal and squamous cell carcinoma), as well as influence immune system action. Therefore, the future of sun protection agents will undoubtedly include a new generation of compounds with the ability of absorbing harmful radiation in combination with substances having the ability to repair damage caused within DNA.

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CASE REPORT

Recalcitrant cases of pyoderma gangrenosum, responding dramatically to systemic tacrolimus

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ABSTRACT

Pyoderma gangrenosum (PG) is an uncommon ulcerative cutaneous disease, without any well-known specific and effective treatment. Here we report two patients with severe recalcitrant perineal pyoderma gangrenosum, successfully treated with low dose systemic tacrolimus. Tacrolimus can be a safe effective drug in the management of recalcitrant PG.

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Key words: Pyoderma gangrenosum - Perineum - Tacrolimus.

Pyoderma gangrenosum (PG) is a serious ulcerative skin disease.[1] Its etiology is unknown. Several theories have been postulated, but none explains all the patients.[2] PG treatment is mostly empirical, and no single treatment exists. Only few controlled trials have been done,[1] and it is often refractory to standard treatments such as corticosteroids, cyclosporine and infliximab.[3]

We report two cases of PG refractory to standard treatments who responded dramatically to low dose systemic tacrolimus, and prednisolone with rapid tapering.

Clinical series

Case 1

The patient is a 26-year-old female who ten years ago presented with three pustules on the breast, shoulder and perineum, which gradually progressed to undermined ulcers. In examination, there were three undermined ulcers ranging from 2 to 3×3 cm in the breast and shoulder and 8×9 cm in the perineum with violaceous borders and purulent discharge. Two biopsy specimens of the ulcers showed tissue necrosis with surrounding mononuclear cell infiltrates and fibrosing inflammation at the edge of ulcer, in favor of PG. An extensive work up was done at that time to rule out secondary causes of PG. Colonoscopy with biopsy, rheumatologic and hematologic examinations were performed which turned out to be negative. History was negative for any drugs.

With the diagnosis of primary PG, prednisolone 30 mg per day (0.5 mg per kg) and cyclosporine 200 mg per day were started for her. Over two months, ulcerated lesions started to shrink, yet, during the tapering of prednisolone to 15 mg per day, the ulcers began to reappear.

During the last 7 years, various drugs such as dapsone 100 mg per day, mycophenolate mofetil 2 g, azathioprine 150 mg, colchicine 1.2 mg, clofazimine and potassium iodide 900 mg per day, each for various months, were added to prednisolone in an attempt to taper prednisolone, but the ulcers began to expand as the tapering was started with the largest one in the perineum up to 10 cm. The patient's quality of life was severely compromised.

Three years ago, two courses of infliximab 200 mg in the months 0, 2 and 4 were prescribed, with a four-month interval. The ulcers began to heal and prednisolone was gradually tapered to 5 mg per day. After the third infliximab infusion, the ulcers reappeared with more severity, in both two courses.

Therefore, infliximab was discontinued 1.5 years ago and prednisolone 30 mg per day and as the last resort, tacrolimus 2 mg per day were prescribed. In two months, prednisolone was tapered to 5 mg per day gradually, and the ulcers were healed during three months. Tacrolimus was maintained at the same dosage.

The ulcers healed completely with residual scarring and during these 10 years, for the very first time, the patient has remained free of the relapse for 16 months so far.

Case 2

The patient is a 58-year-old woman without previous medical conditions who referred with perianal and thigh ulcers 3 years ago. The ulcers had begun as tender bullas on a violaceous base which underwent necrosis leading to central ulceration for 1 month before admission. The examination showed one 4×5-cm ulcer in the perianal area and two other ulcers 1×2-cm in thighs with purulent discharge and undermined borders. Two skin biopsy specimens were in favor of PG. Pathergy at the site of the skin biopsy was positive. Extensive work up for the secondary causes of PG was negative. Therefore, the diagnosis of PG without underlying cause was made. Cyclosporine 200 mg and prednisolone 0.5 mg per kg per day was started.

Two months later she came with the same complaints and minimal change in the ulcer's size. Dapsone 100 mg per day was added to prednisolone with minimal response, and in the next admission methotrexate 10 mg weekly was started, increasing weekly to 20 mg. She took MTX for a few months and the changes were minimal. During the next three years, she was admitted in hospital three more times with recurrent infection of perianal PG ulcer still 4×5 cm in size. She continued to take cyclosporine 100 mg every other day and prednisolone with various dosages but never showed a favorable response.

During the last admission, prednisolone 30 mg and tacrolimus 2 mg per day were started. Prednisolone was rapidly tapered to 5 mg per day for 2 months and tacrolimus was continued with the same dosage. The perianal ulcer started to shrink at 2 months after discharge from the hospital, and 6 months later the lesion size decreased to a negligible size (0.5×0.5 cm), without discharge. The quality of the patient's life has now significantly improved.

Discussion

The pathophysiology of PG is poorly understood. Immune dysregulation plays a role in PG pathogenesis, including defects in the neutrophil hyperreactivity and over-expression of cytokines such as interleukin-8 (IL-8). These effects may be mediated by the pro-inflammatory cytokine TNF-α.[5]

TNF-α enhances neutrophil activation, upregulates the expression of adhesion molecules, and induces the release of chemokines and cytokines from the fibroblasts.[5] Therefore, anti TNF-α agents have been used in PG treatment. But there are also reports of PG appearing after treatment with infliximab.[4] Despite multiple therapeutic options, many cases are refractory to treatment.[5]

Tacrolimus (FK-506) is an isolated macrolide antibiotic. It inhibits the activation and proliferation of $CD4^+$ T lymphocytes.[6] Tacrolimus binds to FK-binding proteins in the cytoplasm. The complex causes the calcium-dependent calcineurin/calmodulin complexes to impede calcium-dependent signal transduction in the lymphocytes and results in the reduction of the transcription factors that promote cytokine gene activation. Tacrolimus has cytokine modulating effects; it potently inhibits T cell activation-induced TNF-α and IL-1β production *in vitro* by human peripheral blood mononuclear cells. Tacrolimus was also found to be more potent than dexamethasone and cyclosporine in that regard, and more effective than methotrexate in reducing elevated levels of inflammatory cytokines, TNF-α, IL-1β, and IL-6.[7]

Tacrolimus can also inhibit IL-8/IL-8 receptor (IL-8R) pathway *in vitro*.[8] As mentioned above, IL-8 overexpression is one of the mechanisms implicated in PG pathogenesis.[5] Tacrolimus can result in elevations of BUN, creatinine and blood pressure, but it can be minimized by following blood levels and blood pressure closely.

Topical tacrolimus has been used with success in the treatment of PG.[9] Also, there were encouraging results with systemic tacrolimus based on case report or series.[6, 10, 11]

Our patients did not show a satisfactory response to corticosteroid and cyclosporine with variable dosage and also other immunosuppressive medications, including infliximab. Ultimately, the patients responded rapidly and dramatically to systemic tacrolimus in low dosage, without any serious side effect.

Conclusions

PG can severely affect the quality of the patient's life, especially in our patients where the perineum was mostly affected. Systemic tacrolimus has never been studied in a randomized controlled trial and only limited published case reports or series exist in this regard, mainly due to the rarity of PG. It may be a valuable alternative treatment, especially for recalcitrant cases.

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IgA anti-epidermal transglutaminase autoantibodies: a simple test to improve differential diagnosis between *dermatitis herpetiformis* and atopic dermatitis

Dear Editor,

Dermatitis herpetiformis (DH) is a rare gluten-sensitive autoimmune blistering and intensely itchy rash, closely related to celiac disease (CD).[1] It is characterized by a chronic-relapsing course, with typical involvement of elbows, shoulders, forearms, buttocks, knees, central area of face and scalp. DH patients present polymorphic lesions, in particular erythema, urticarial plaques, maculopapular and/or papulovesicular lesions, vesico-bullous lesions, small erosions, excoriations and hyperpigmentations on typical sites.[1] Atopic dermatitis (AD) in adult patients is characterized by the appearance of intensely pruritic erythematous papules and vesicles, localized not only at flexural sites like in childhood, but also on the upper and lower limbs, face, buttocks and shoulders.[2]

The usefulness of serum anti-epidermal transglutaminase (eTG) antibodies, with a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit, has been recently developed as a new high sensitive and specific test for the diagnosis of DH.[3, 4]

The aim of this work was to confirm the absence of anti-eTG antibodies in a population of adults and young adults diagnosed as affected by AD.

Forty patients (17 men, 23 women), aged between 14 and 59 years, consecutively seen for mild-to-moderate AD, and treated only with topical therapy were included in the study, which was approved by the Ethics Committee of our Institution. Informed consent was signed by all the patients or by their parents.

Venous blood samples were taken from each patient to test the dosage of circulating anti eTG antibodies. The samples were centrifuged and further separated to obtain the serum that was stored at -80 °C. IgA eTG antibodies were tested in all serum samples with a commercial ELISA kit (Immunopharmacology Research-IPR, Valverde, Catania, Italy). The eTG antibodies kit provides an ELISA plate coated with recombinant eTG from human source. Patients' sera diluted 1:100 were added to the wells (100 µL) and incubated for 30 minutes at room temperature. After 4 washings, wells were incubated with 100 µL peroxidase-conjugated anti-human IgA specific antibody for 30 minutes at room temperature. After 4 washings, unbound antibodies were removed and the color was developed by adding 100 µL of tetramethylbenzidine and H_2O_2. After 30 minutes, the reaction was stopped with 100 µL of H_2SO_4–0.5 M. Absorbance (optical density, O.D.) was read on a Multiskan*EX Microplate Photometer at 450 nm.

Values under <2.6 IU/mL were considered negative, borderline between 2.6 and 3.5 IU/mL and positive >3.5 IU/mL, with cut-off equal to 3 IU/mL.

Anti-eTG antibody levels in the sera of all 40 patients affected by AD were negative, amounting in all cases to values between 0 and 1 IU/mL (mean value 0.36±0.52 IU/mL).

Differential diagnosis of fully developed DH lesions has to be done with different bullous dermatoses, autoimmune blistering itchy diseases, namely bullous pemphigoid and IgA linear disease, more rarely with *epidermolisis bullosa acquisita*.[1] However DH clinical presentation is sometimes elusive with no blisters, few tiny vesicles, some crusting and erythema, causing some difficulties in differential diagnosis with a common disease such as AD.[1] Palmoplantar involvement with hemorrhagic lesions is exceedingly rare, as well as hypopigmented spots on back and shoulders.[1]

Histopathology of DH skin lesion is diagnostic, albeit not exclusive, in several cases: subepidermal blisters or clefts and neutrophils and/or eosinophils at the papillary tips with fibrin deposition are of diagnostic value.[1] Direct immunofluorescence from perilesional affected skin is currently the gold standard for the diagnosis of DH, demonstrating pathognomonic IgA granular deposits localized either in the dermal papillae or along the dermal-epidermal junction.[1]

Circulating anti-tissue transglutaminase (tTG) IgA antibodies are the most sensitive marker for CD and also a diagnostic marker for enteropathy in DH patients.[5] In 2002, epidermal transglutaminase autoantigen (eTG), homologous of tTG within enzymatically active domains, was found to be involved in the pathogenesis of DH.[3] Sardy *et al*. demonstrated that eTG co-localizes with dermal IgA deposits and proposed it as the autoantigen in DH.[3] It has also been shown that antibodies to eTG are a more sensitive marker than anti-tTG antibodies in the diagnosis of DH.[3] Serum eTG antibodies have been recently demonstrated in discriminating between DH, CD, other gastrointestinal diseases and other not DH related vesicobullous itchy diseases, with 100% sensitivity and 97.1% specificity.[4]

In conclusion we found that this new test for the assay of circulating anti eTG antibodies proved negative in 100% adults and young adults patients affected by mild to moderate AD. Even if, so far, this test cannot replace histology and direct immunofluorescence for the diagnosis of DH, it represents a simple and non-invasive test that improves the differential diagnosis of *dermatitis herpetiformis versus* atopic dermatitis in patients with atypical clinical presentation.

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Acquired progressive hyperpigmentation

Dear Editor,

An otherwise healthy 72-year-old female complained of a 6-month-old diffuse hyperpigmentation. The lesions started in the inframammary folds, axillae, and groin (Figure 1A, B) and later spread to the back and trunk in a milder form (Figure 1C). No other skin lesions such as plugged follicles or periorificial pits were noted, and the nails and mucous membranes, including the genital skin, were spared. Her quality of life was seriously impaired by this condition. Her past medical history was unremarkable. She denied any drug consumption. None of her relatives were affected. Laboratory tests, including complete blood count, electrocardiogram, liver, pancreas, and kidney function tests, urinalysis, as well as extensive investigations to exclude paramalignant conditions (colonscopy, gastroscopy, gynecologic evaluation, thoracoabdominal CT scan, bone marrow aspiration) were within the normal range or noncontibutory. A skin biopsy was obtained from her left axilla (Figures 2, 3).

Histologic examination of an axillary lesion showed elongation of the rete ridges, melanin incontinence, and basal hyperpigmentation (Figure 2); branched rete ridges that intertwine at their bases ("antlerlike" appearance) and small intraepithelial horn inclusions (pseudocysts) were present (Figure 3). No acantholysis of the suprabasal keratinocytes was found. Biopsy examined under polarized light was negative for Congo red stain. A diagnosis of Dowling Degos disease (DDD) was made.

DDD [OMIM 179850] is a rare genodermatosis, first described by Dowling and Freudenthal in 1938,[1] as a form of *acanthosis nigricans* having a low risk of associated tumors. It was later termed *dermatose réticulée des plis* by Degos and Ossipowski.[2]

The disease is characterized by progressive hyperpigmented macules and papules on flexural sites, facial comedo-like lesions, pitted perioral scars, or, less often, palmar pits. Although it is usually a harmless and benign disorder, in our case it was perceived as esthetically disfiguring.

Narrowing the differential diagnosis to those diseases characterized as adult-onset, reticulate hyperpigmentation (*i.e.*, hyperpigmented, ephelideslike lesions), we should consider Galli-Galli disease (GGD), Kitamura acropigmentation (KA), Haber's Syndrome, and reticulate acropigmentation of Dohi.

Although all these entities share strikingly similar clinicopathologic features, they have been described in the past as distinctive diseases based on age of onset, distribution of the lesions, and associated conditions. Nevertheless, since many confusing, overlapping cases have been reported, the term *reticulate pigment disorders of the skin* has been recently introduced to include, under one underlying genodermatosis, all the aforementioned historically defined phenotypical variants.[3] KA is included by the Online Mendelian Inheritance in Man as a synonym for DDD, whereas other authors distinguish it from DDD because of its exclusive localization on the hands and feet, presence of palmar pits, and histologically broken epidermal ridges. GGD might be differentiated from DDD on a histological basis for suprabasal acantholysis, although genetically, GGD is considered the acantholytic variant of DDD.[4]

In our case, differential diagnosis included the so-called disorders with mottled pigmentation characterized by the concurrent presence of hypo- and hyperpigmentation. This group includes dyschromatosis symmetrica hereditaria (acropigmentation of Dohi) and *dyschromatosis universalis hereditaria* (DUH). Both have hyper-and hypopigmentated macules, but they lack the lacy epithelial strands and have a childhood onset. On the other hand, cases showing features of both DDD and DUH have been recently reported.[5]

TABLE I.—*Genetic inheritance of reticulate pigment disorders.*

Location	Phenotype	Inheritance	Gene/Locus
12q13.13	Dowling-Degos disease 1	ad	KRT5, DDD1
20q11.21	Dowling-Degos disease 2	ad	POFUT1, OFUCT1, KIAA0180, DDD2
17q21.3-q22	Dowling-Degos disease 3	ad	DDD3
3q13.33	Dowling-Degos disease 4	ad	POGLUT1, CLP46, KTELC1, RUMI, C3orf9, DDD4
1q21.3	Dyschromatosis symmetrica hereditaria	ad	ADAR, DRADA, DSH, DSRAD, IFI4, G1P1, AGS6
15q21.3	Reticulate acropigmentation of Kitamura	ad	ADAM10, MADM, RAK, AD18

ad: autosomal dominant.

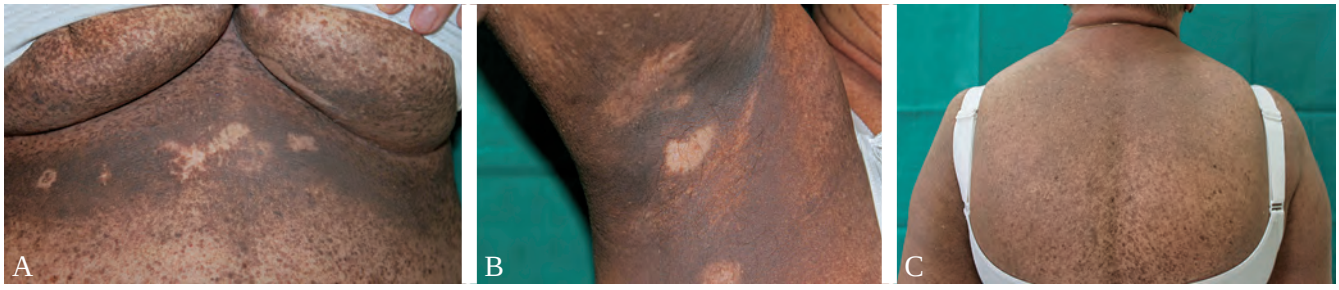

Figure 1.—A, B) Reticulate hyperpigmented macules in the flexures and (C) on the trunk.

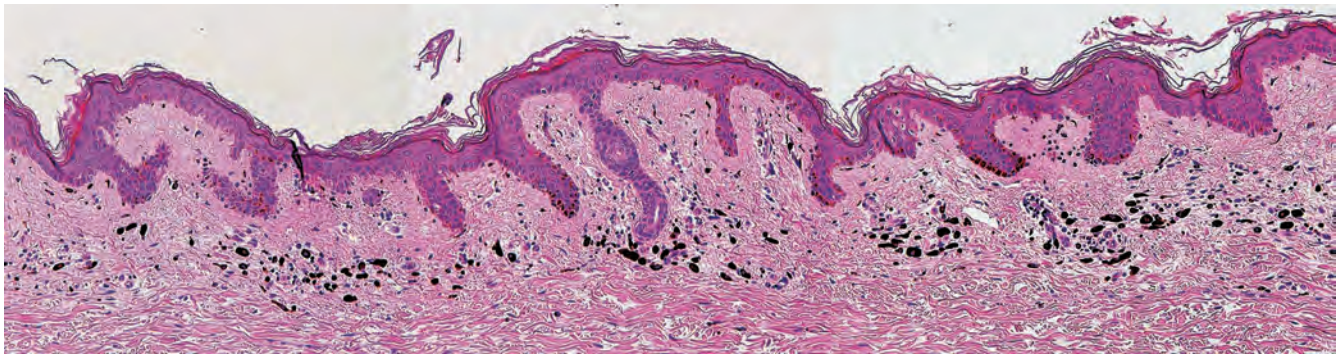

Figure 2.—Hyperkeratosis; thinning of the suprapapillary epithelium; elongated rete ridges with basal hyperpigmentation; and dermal melanosis (hematoxylin and eosin, original magnification ×10).

Finally, two acquired reticulate pigmentary disorders deserve mention: acanthosis nigricans and confluent and reticulate papillomatosis (Gougerot-Carteaud disease). Histologically, these two conditions can easily be differentiated from DDD by the churchspire papillomatosis with hyperkeratosis, but without increased melanin.

From a genetic point of view, the disease is heterogeneous. Betz identified mutations in the keratin-5 (DDD1, Table I).[6] On the other hand in a Chinese family with DDD, in which mutation in the KRT5 gene had been excluded in the proband, Li *et al.* found other genetic alterations: a mutation in POFUT1 gene, encoding protein O-fucosyltransferase 1 (DDD2, Table I),[7] and a linkage to a locus on chromosome 17p13.3 (DDD3, Table I).[8] Finally, In 5 unrelated patients with DDD known to be negative for mutation in the KRT5 with prominent involvement of non-flexural areas gene, Basmanav *et al.* identified genetic alterations in the POGLUT1 gene (DDD4, Table I).[9] Furthermore, although no genetic evaluation has been

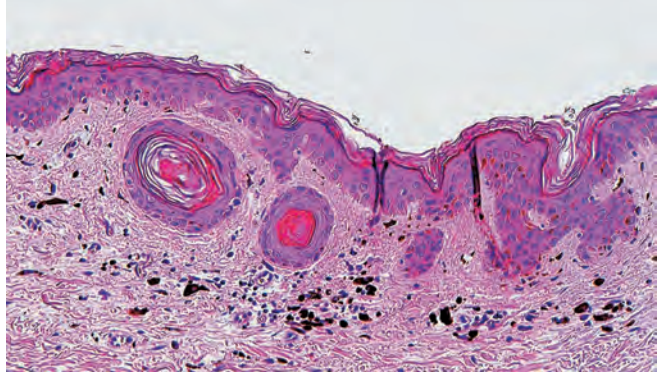

Figure 3.—Fibrosis along rete ridges and comedo-like follicular cysts; acantholysis of the suprabasal epidermis is absent (hematoxylin and eosin, original magnification ×40).

performed in the present case, some other sporadic cases of DDD have also been reported. Regrettably, DDD has limited treatment options. Our patient was treated with a 2% hydroquinone cream, resulting in some improvement, yet not satisfactory. Treatment with topical retinoids has been disappointing.[10] Erbium-doped yttrium aluminium garnet (Er:YAG) might represent a valuable option.

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Therapy of cutaneous *larva migrans* in pregnancy

Dear Editor,

Cutaneous *larva migrans* (CLM) is an infestation caused by penetration and migration in the epidermis of larvae of nematodes. *Ancylostoma braziliense* and *Ancylostoma caninum* are the species most frequently involved.[1] CLM is characterized clinically by erythematous and slightly raised tracks: they may be single or multiple, linear or, more often, serpiginous, ramified and intertwined. The length of tracks is extremely variable (sometimes many cm); the width ranges from 2 to 4 mm. Tracks are very often accompanied by pruritus.[1]

We present a case of self-healing CLM in a pregnant woman.

A 27-year-old Caucasian pregnant (10th week) woman was admitted because of a rash located on the right nipple. The patient stated that she was in good general health and that she was not undegoing treatment with systemic drugs. She also stated that she had just returned from a trip to Mexico, where the rash had appeared approximately two weeks earlier. The patient complained of severe pruritus.

Dermatological examination revealed the presence of several erythematous, serpiginous and raised tracks (Figure 1). No other similar lesions were observed elsewhere. On the basis of history and clinical picture (typical tracks accompanied by pruritus), a diagnosis of CLM was made.

General physical examination did not reveal anything pathological.

All laboratory examinations, including complete blood count, inflammatory tests, total IgE and copro-parasitological examinations, were within normal ranges or negative.

We proposed the cryotherapy, but the patient refused because she was afraid of scar formation. In order to reduce pruritus, we prescribed a cream containing 8% calamine, that was applied three times/day. The patient was again examined four weeks later: both the rash and pruritus had disappeared (Figure 2). A six-month follow-up was negative.

The therapy of CLM is currently based on cryotherapy,[2] topical drugs (thiabendazole [3] and albendazole [4]) and oral drugs (thiabendazole,[5] albendazole [6] and ivermectin [7]). The use of ethyl chloride,[8] and oral fluoromebendazole [9] and mebendazole,[10] has been abandoned.

Cryotherapy may be taken into consideration in single and small lesions; however, it is often ineffective;[11] in addition, it is painful and can induce the development of vesicles, blisters, erosions, ulcers and scars.[12] Thiabendazole is teratogenic in mice and rats,[13] albendazole is teratogenic in mice [14] and ivermectin is teratogenic in rats.[15]

CLM can be a self-limiting infestation: usually, its duration

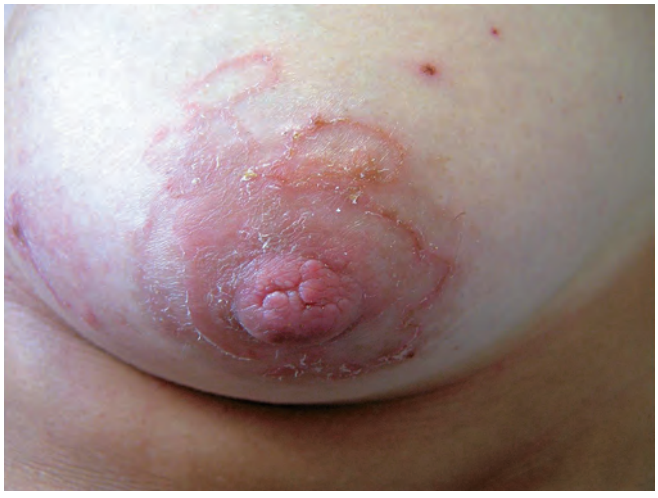

Figure 1.—CLM located at the right nipple.

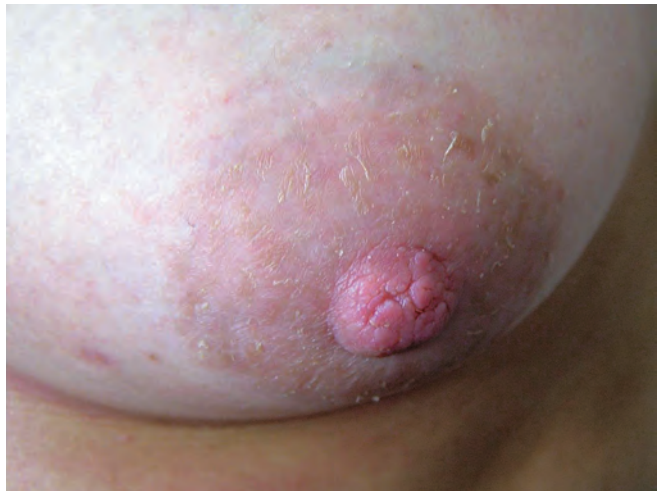

Figure 2. —Four weeks later.

is 2 to 8 weeks,[1] and the case we have described confirms this statement. However, an uncommon variety of CLM ("chronic" or "persistent" CLM), characterized by a typical clinical presentation but long/very long duration (from 5 to 14 months) has been described.[1]

In conclusion, the case we have reported confirms that CLM can be a self-healing infestation. In pregnancy, the therapy of CLM is hard, because all oral drugs commonly used are teratogenic. To our knowledge, only two cases of CLM in pregnancy were reported. The first patient, a 32-year-old woman who acquired the infestation in Thailand, developed sacroileitis, optic neuritis and panuveitis, but was successfully treated with albendazole and ivermectin.[16] The second patient, a 31-year-old woman, was successfully treated with cryotherapy.[17]

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Natural weight-loss products and acne induction in a patient with thyroid dysfunction

Dear Editor,

A 24-year-old female presented with a microcystic comedogenic acne on the face (Figure 1). Acute clinical onset was referred 6 months before, after consumption for 3 months of a product to lose weight, containing the brown alga *Ascophyllum nodosum*. Patient had never suffered from acne and her menstrual cycle was regular. However, since childhood she was affected by thyroiditis, requiring levothyroxine (75 mg/day) and liothyronine (20 mcg/day) treatment.

The discontinuation of alga consumption and the use of topical retinoids were initially ineffective. The successive adjustment of thyroid replacement therapy (levothyroxine, 50 mg, and liothyronine, 20 mcg), and the wash out from the brown alga enabled topical benzoyl peroxide to produce gradual clinical improvement. Seven months later, approaching summer time, benzoyl peroxide was tapered until discontinuation. By then, the clinical condition was markedly improved with few comedones left (Figure 2).

Endocrinological abnormalities, bacterial colonization, cosmetics and smoking have been implicated in late-onset acne pathogenesis, mainly in women. In case of thyroid diseases, many symptoms arise on the skin, but the role of thyroid in the pathophysiology of adult acne is not well-defined. Thyroxine decreases mitotic activity and increases lipid synthesis, while thyrotropin stimulates sebaceous gland activity.[1] Marine algae, mimicking the action of thyroid hormones, when assumed in systemic preparations, can affect thyroid homeostasis, and elicit multiple alterations, including acne. Conversely, when included in topical products, these algae seem to have antibacterial efficacy against *P. acnes*.[2]

Our case highlights the necessity to accurately collect personal history from adult patients with acute-onset acne, enquiring also about the use of the so-called natural weight-loss products. Moreover, the presence of predisposing factors, such as an underlying thyroid dysfunction, requiring hormone replacement therapy, needs to be considered and a possible link to the skin condition should be hypothesized.

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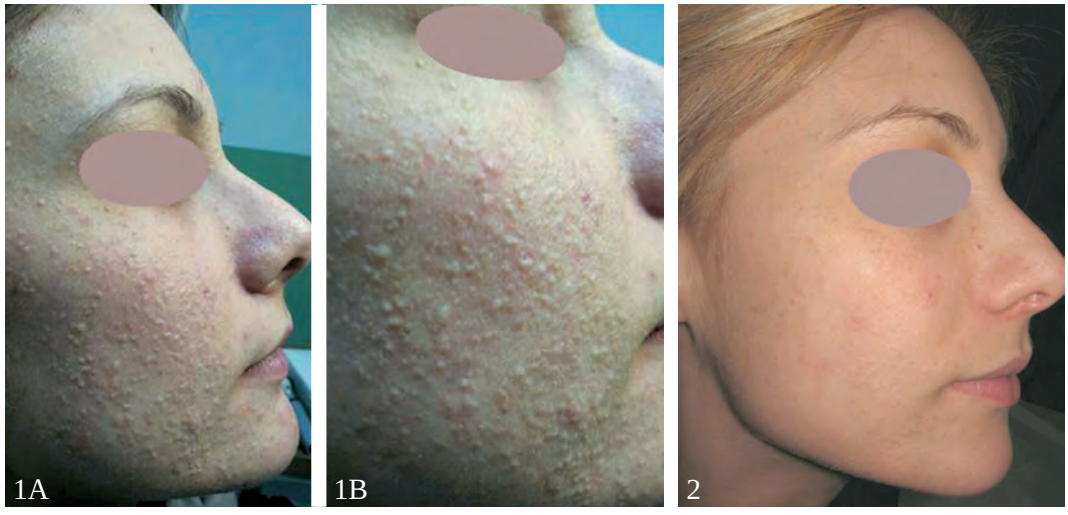

Figure 1.—Clinical appearance of microcystic comedogenic acne. Closed comedones can be appreciated in A and, more in detail, in B.

Figure 2.—After 7 months, marked clinical improvement with few comedones left. During this time, the patient had discontinued the alga, decreased thyroid replacement therapy, and applied topical benzoyl peroxide.

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Osteoclastic-like giant cells in a cutaneous squamous cell carcinoma

Dear Editor,

Cutaneous squamous cell carcinoma (SCC) is the second most common type of skin cancer. It is mainly non-aggressive and it manifests as a consequence of sun exposure in middle-aged and elderly individuals. A wide range of clinical and pathological presentations have been described.[1]

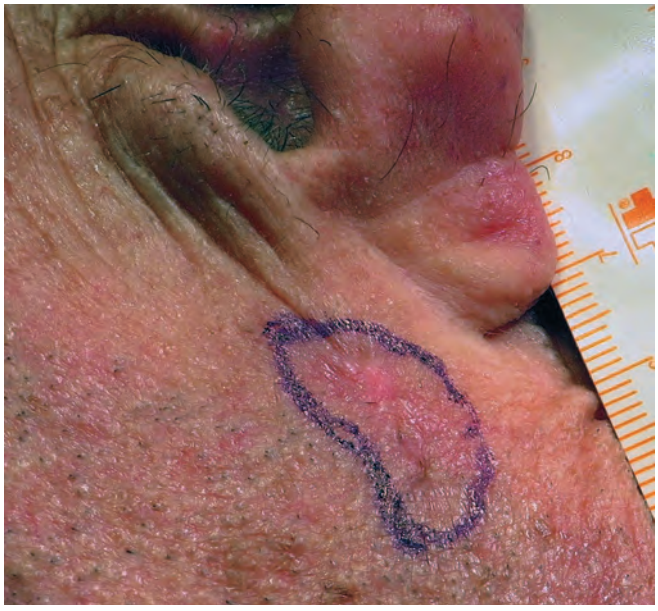

Figure 1.—Scarring and micronodular cutaneous lesion on the left mandible.

An 80-year-old male came to us with a cutaneous scarring lesion that appeared 11 months before. On clinical examination, a 1-cm scarring and partially nodular lesion was seen on the left mandible. His history was positive for skin cancers (malignant melanoma T2a N0 M0 and basal cell carcinoma both on the trunk, so far in regular follow-up). Moreover, the patient had a prostatic and bladder neoplasm treated with surgical excision. As the differential diagnosis included a basal cell carcinoma on the left mandible, a surgical excision for histological examination was performed. The specimen consisted of an ellipse of skin measuring 2×1.4 cm with a depth of 0.3 cm, with colored sides marked. Histopathological examination of the lesion showed two distinct tumoral populations: the lesion was mostly composed by moderately-differentiated (G2), keratinizing, cutaneous squamous cells carcinoma with deep dermal infiltration (Breslow depth 1.8 mm, Clark level III) (Figure 1). At higher magnification, the examination revealed osteoclast-like giant cells (OLGCs) in the central tumoral zone, characterized by nuclei placed like a crown in peripheral and central area (Figure 2).

On immunohistochemistry, we showed that OLGCs were negative for p63 and pan-cytokeratin, on the other hand the SCC was positive for pan-cytokeratin and p63. The CD68 immunostaining revealed many positive cells, instead neoplastic cells were negative (Figure 3). Finally, we diagnosed cutaneous SCC with OLGCs. Complete local excision of the lesion was performed. On clinical examination and US sonography of cervical and axillary lymph nodes, there was no evidence of metastatic spread. Thus, regular follow-up according to the guidelines for cutaneous SCC was recommended.

OLGCs have been described to occur in rare variants of diverse extraosseous, visceral, usually moderately to poorly differentiated malignant tumor. Cutaneous malignant neoplasms with infiltration of OLGCs are extremely rare.[2] To the best to our knowledge, only eleven cases of cutaneous SCC with OLGCs have been reported.[1-4, 6]

Osteoclast-like epithelioid giant cells in extraosseous sites have a clinical phenotype and immunohistochemical profile which are indistinguishable from those of osteoclasts. The origin of such cells was a matter of debate — in the pancreas, an acinar origin was initially suggested, whereas others favored a mesenchymal or-

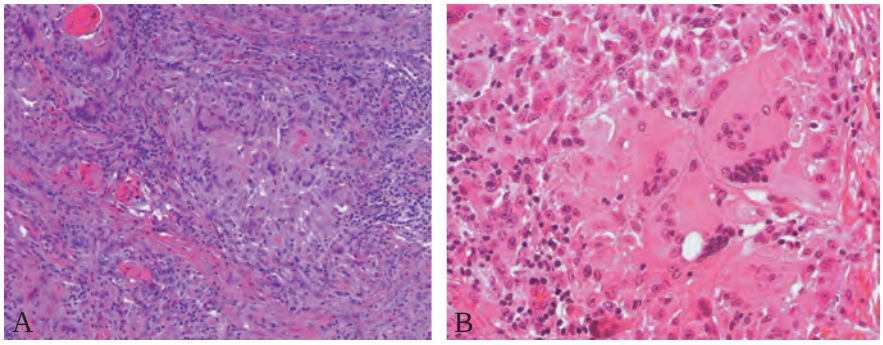

Figure 2.—A) Squamous cell carcinoma with osteoclast-like giant cells in the central zone; (220×, H&E stain); B) at higher magnification, osteoclast-like giant cells with nuclei placed in a crown-like pattern (440×, H&E).

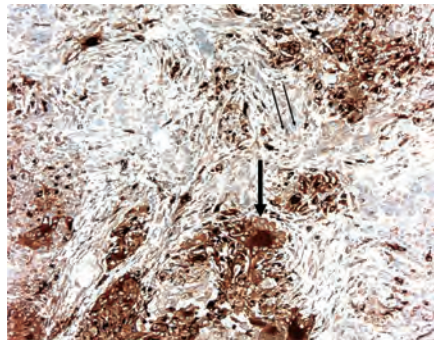

Figure 3.—CD68 immunostaining reveals a lot of positive cells and osteoclastic like giant cells (single arrow); neoplastic cells are negative (double arrows) (Endvision immunoassay, 220×).

igin.[1] Some SCC with OLGCs case reports showed a patient with organ transplantation, that our patient did not have.[3, 5, 6]

There is also disagreement with respect to the origin of the OGCs. The OLGCs have been considered by many authors as bone-marrow-derived monocytes that were secondarily recruited into the tumor. On the other hand, other authors speculated that the cells are generated by the fusion of adjacent monocytes/macrophages. Giant cells are composed by a large mass of cytoplasm containing multiple nuclei arranged peripherally (Langhans-type giant cell) or haphazardly (foreign-body-type giant cell). The giant cells in the current case had uniformly small, regularly dispersed central and peripheral nuclei (in a crown-like shape) and were morphologically indistinguishable from osteoclasts. Furthermore, discrimination from other OLGCs-containing conditions (named dermatofibroma, giant cell malignant fibrous histiocytoma, leiomyosarcoma with giant cells, atypical fibroxanthoma, malignant melanoma) is important. The mechanism underlying OLGCs infiltration in SCC is still unknown.[2-4, 6]

Moreover, regarding the singularity of our case report, we would like to highlight that in this patient the squamous cell carcinoma with osteoclast-like giant cells was diagnosed after the diagnosis of two other malignant skin tumors (melanoma and basal cell carcinoma).

In conclusion, we here reported a rare phenomenon in a cutaneous tumor. Moreover, we would like to remind the readers that the knowledge of differential diagnosis and a proper classification of OLGCs is essential for patient prognosis.

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Long-term follow-up assessment of daylight photodynamic therapy with methyl aminolevulinate in patients with actinic keratosis

Dear Editor,

Topical photodynamic therapy (PDT) is a highly effective non-invasive treatment for actinic keratoses (AK). Daylight PDT (D-PDT) is a novel simplified PDT procedure that is emerging as a treatment of choice for grade I and grade II AK on the face and scalp. Consistent data show that, in comparison with conventional PDT, D-PDT is as effective, better tolerated, and associated with lower pain intensity and high patient satisfaction.[1]

Our preliminary experience with D-PDT using topical methyl aminolaevulinate (MAL) included 53 patients with AK consecutively treated with a single session of D-PDT, as described in a previous report.[2] At the 3-month assessment following treatment, the clearance rate was 82.7% for the total lesions, reaching a value of 93% for grade I AK.[2] At 3 months, a complete response (defined by an AK number equal to 0) was obtained in 29 patients (54.7%), 15 females and 14 males, aged 55 to 95 years (mean age, 77.7 years), whose AK were mild (grade I) in 74.5% and moderate (grade II) in 25.5% of cases, and were mostly located on the face and/or the scalp. Cosmetic outcome was excellent in all patients.

Patients completely cleared after 3 months from the D-PDT session entered an observational follow-up phase to assess the long-term efficacy of D-PDT. A patient was lost to follow-up so that the follow-up analysis included a total of 28 patients.

Prospective clinical assessments, supported by photographic documentation, were performed at 3-month intervals until recur-

rence of lesions. During the follow-up period, topical active therapies for AK on the lesional area and/or systemic therapies that could have altered the course of AK were not allowed, while high photoprotection measures were recommended in each patient.

Lesion recurrence was observed in 13 patients (46.5%), 7 females and 6 males (mean age, 75 years), after a period of 6-21 months (mean, 11 months) from the D-PDT session. Of note, at the last follow-up visit when the recurrence was noted for the first time, 25% of the baseline lesions recurred, and 8 patients however showed a number of AK consistent with ≥75% reduction from baseline. A complete clearance was still maintained in 15 of the 28 responders (53.6%) after a post-treatment follow-up period of 18-29 months (mean, 23.5 months).

Risks of recurrence and malignant transformation are challenging problems in the management of AK in clinical practice. Therefore, sustained clearance is an important objective required for an optimal care of patients with AK.

Data on the recurrence rates of conventional treatment for AK are scarce.

A few studies evaluated the persistence of response of AK after conventional PDT.

In an Italian study,[3] patients with multiple AK of the face and scalp with variable thickness (from grade I to grade III) who had a complete response after 90 days were followed up, and at the 12-month evaluation a complete response still persisted in 55% of patients treated with MAL-PDT.

In Korean patients with facial AK, conventional PDT with MAL cream was found to induce a complete response rate of 50.7% at 12 months.[4]

Dirschka *et al*.[5] reported that, 12 months after treatment of mild-to-moderate AK on the face or scalp with MAL-PDT, the recurrence rate was 25.4% and the proportion of responders who remained completely clear was 55.2%.

Our preliminary results suggest that D-PDT is a valid treatment for superficial AK and can produce a sustained clinical improvement, with a complete response detected in nearly half of the examined patients during an average follow-up period of 23.5 months. Limitations of our study should however be taken into account for the interpretation of results, including the small sample size, the open-label design, and the clinical characteristics of patients, who showed mild lesions on the face and/or scalp in the majority of cases.

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Atypical syphilitic chancre of the tongue in a HIV-positive patient: the diagnostic role of NAATS

Dear Editor,

Syphilis is a sexually transmitted infection (STI) caused by *Treponema pallidum subspecies pallidum* (TP) and characterized by a triphasic course: the first stage of the disease is typical, with a chancre appearing in the site of TP inoculation; secondary syphilis is very polymorphic,[1] whereas the third stage, occurring in 30% of untreated patients, is characterized by both cutaneous and systemic involvement.[1] We describe an atypical syphilitic chancre of the tongue in a HIV-positive homosexual male, and we discuss the importance of nucleic acid amplification techniques (NAATs) in the diagnosis of syphilis. The patient was diagnosed positive for HIV 25 years before and he was taking highly active antiretroviral therapy (HAART); T-helper lymphocytes were 450 and viral load was

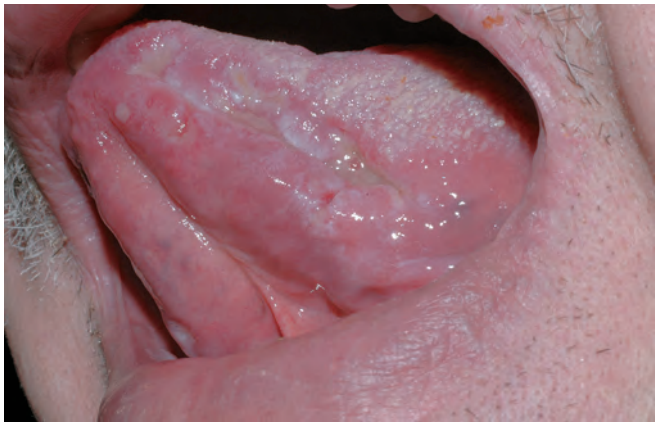

Figure 1.—Syphilitic chancre of the tongue presenting as an indurated long-shaped ulceration with clear-cut edges.

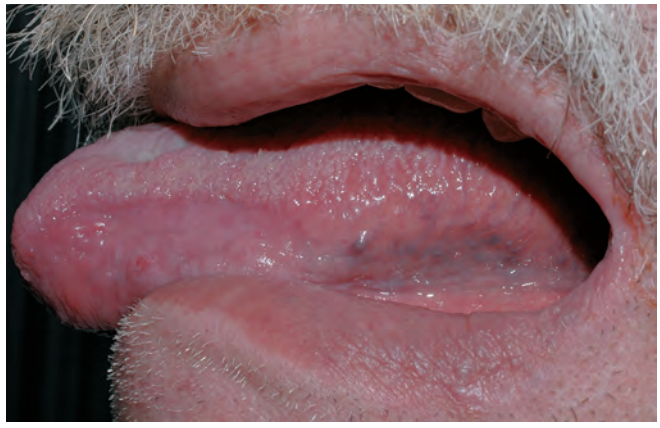

Figure 2.—Complete healing after the treatment.

<37 copies/mL. During periodical routine laboratory examinations, serological tests for syphilis resulted positive: TPPA was reactive at a titre of 1:80 and RPR at a titre of 1/64. Physical examination revealed only an indurated ulcer on the left side of the tongue associated with submandibolar adenopathy (Figure 1). The lesion was long-shaped with clear-cut edges and it was completely asymptomatic; the patient was aware of its presence. No other muco-cutaneous lesions were observed and the patient did not complain for any systemic symptom. Considering the serological positivity, before performing a biopsy, we tested a swab taken from the ulcer with a NAAT for TP and *Herpes simplex virus* (HSV) 1,2; the examination resulted positive for TP and negative for HSV1,2. A diagnosis of primary syphilis was made and a single dose-treatment of intramuscular benzathine penicilline G 2.4 milion units was administered, according to CDC guidelines;[2] the complete resolution of the lesion was observed 3 weeks later (Figure 2). In our opinion this case is interesting for the unusual presentation. The clinical feature is not typical: syphilitic chancre is described as a single indurated eroded nodular lesion,[1] while we observed a wide long-shaped ulcer; moreover, the oral localization is not so frequent, being only 5% of extragenital chancres.[3] The suspect of a syphilitic lesion raised up because of the serological positivity disclosed by routine laboratory examinations and because of the sexual habits of the patient; otherwise we would have firstly considered other diagnosis, mainly a chronic ulcerative herpes simplex, being the patient positive for HIV, or a squamous cell carcinoma. In such cases, direct diagnostic methods for syphilis, as nucleic acid test (NAAT), dark field microscopy (DFM) or direct fluorescent antibody staining for TP (DFA-TP), are mandatory: detecting TP in the lesions allows the correct diagnosis avoiding further invasive investigations. In this regard, NAATs are the most sensitive, the most specific and the earliest tests.[4] In contrast, DFM is highly dependent on the physician's ability, it is influenced by empiric treatments generally self-administered by the patients themselves and it is not useful for extragenital sites: the possible presence of saprophytic treponemas in the mouth and in the anus can cause false positive results.[4] DFA-TP is an immunofluorescence enzyme-based microscopy method whose specificity depends on the type of primary antibody used and upon the concentration of TP in the sample;[4] to the best of our knowledge, up to now, there's no FDA approved DFA-TP diagnostic test. This case confirms the reputation of syphilis as "the great imitator"[5] and stresses the idea of considering syphilis as a possible diagnosis in case of any genital or extragenital ulcerative lesion suddenly spreading in people at risk; in such cases, we believe that a NAAT for TP should be performed in addition to serological tests, especially when DFM is not available or negative and when serology is not yet reactive.

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Different dermoscopic patterns of cutaneous melanoma metastases in the same patient

Dear Editor,

We report the case of a 78-year-old woman that was admitted to our Dermatological Department for multiple and clinically polymorphous macules and nodules located on her right leg (Figures 1-3). The patient referred the amputation of her right toe for acral lentiginous melanoma (Breslow's thickness 3.87 mm, Clark's level IV, 2 mitoses/mm^2 without evidence of regression and ulceration), one year before our clinical examination.

Upon dermoscopic examination, all the lesions, recently arisen between the site of primary melanoma and the draining lymph node basin, showed different features. Those that are red-blue and blue-gray (Figure 1A, C, E, G) varied from a homogeneous bluish pattern mimicking blue nevi (Figure 1B, D) to a saccular one both in macules (Figure 1F) and nodules (Figure 1H). In particular, a brown-grayish macule was observed, with ill-defined and irregularly-shaped bluish-violaceous nests in the upper margin, and with more pronounced and well-defined globules in the nodular lesion (Figure 1H), where they were associated to linear irregular vessels. The patient also presented with brown-colored flats appearing as Clark's nevi (Figure 2A, C), although they did not show regular network or globules, showing polymorphous pattern characterized by central blue-gray dots, melanophages and perilesional erythema (Figure 2B) or fragmentary network, irregular and asymmetrical distributed brown globules and linear irregular vessels (Figure 2D). Rare pink lesions without evidence of pigmentation (Figure 3A, C) showed amelanotic pattern with linear irregular and pin-pointed vessels, erythematous halo in the early macular phase (Figure 3B) and winding vessels at the periphery in the nodular one (Figure 3D). All these macules and nodules were excised to perform histopathological examination that confirmed clinical suspect of in-transit cutaneous malignant melanoma metastases (CMMMs).

Since the diagnosis of acral lentiginous melanoma is often delayed, this tumor had a poor prognosis.[1, 2] In particular, it was demonstrated that high Breslow thickness and mitotic rate, primary tumors on the extremity, age older than 50 years, ulceration, vascular invasion an positive lymph nodes significantly increase the risk of in-transit CMMMs.[3, 4]

In-transit metastasis of malignant melanoma, defined as the proliferation of melanoma cells between the primary tumor and the

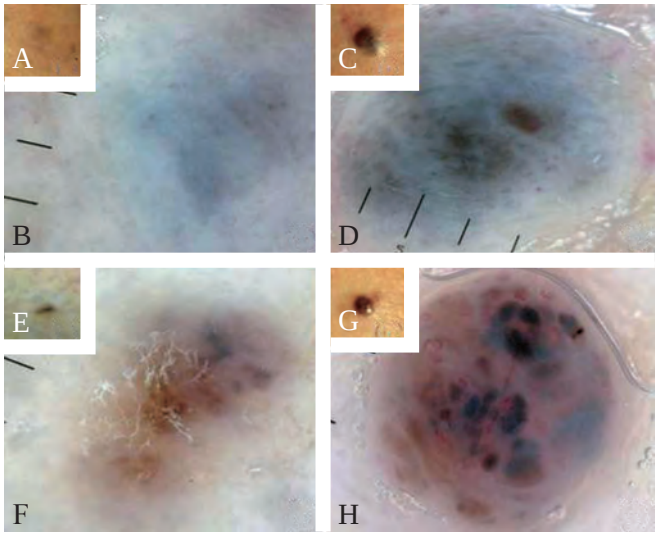

Figure 1.—Macular (A) and nodular (C) bluish lesions showing a blue homogeneous pattern (B) (dermoscopy ×10). Brown macule (E) characterized by a light brown-grayish color and by the presence of blue-violaceous ill-defined nests located at the top (F). Blue-violaceous nodule (G) with the typical saccular pattern where linear irregular vessels are observed between blue well-defined "sacculi" (H) (dermoscopy ×10).

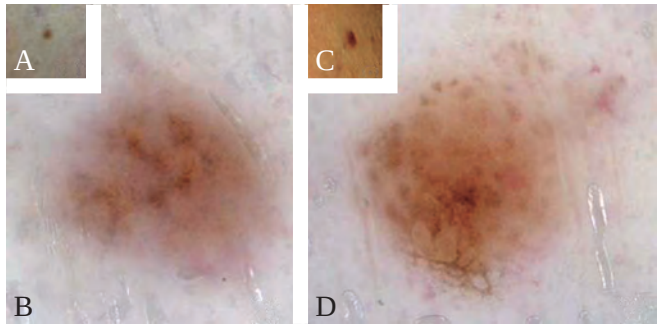

Figure 2.—Colored flats mimicking Clark's nevi (A, C). Macular lesions showing central blue-gray dots, melanophages and perilesional erythema (B) or fragmentary network, irregular and asymmetrical distributed brown globules and linear irregular vessels (D) (dermoscopy ×10).

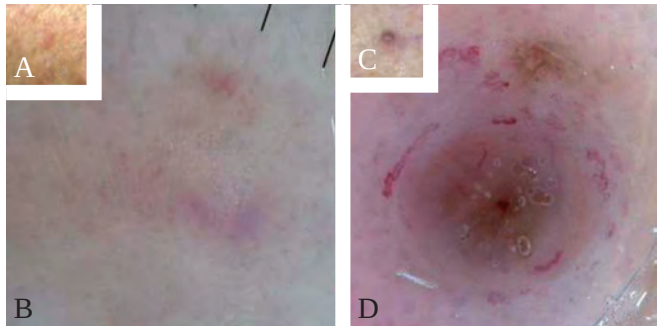

Figure 3.—Pink-red macule (A) and nodule (C). Amelanotic pattern presenting with unspecific dermoscopic features and linear irregular and pin-pointed vessels associated to erythematous halo (B) and winding vessels located at the periphery in the nodular lesion (D) (dermoscopy ×10).

TABLE I.—*Dermoscopic patterns suggestive for CMMMs.*[5]

Patterns	Description
Blue homogeneous	Diffuse blue structureless pigmentation
Vascular (or amelanotic)	Polymorphic angiectatic blood vessel and/or aneurysm of the vessels, in particular winding vessels and area of polymorphic and/or horizontally capillary prominence especially at the border of each lesion
Saccular	Round or ovoid junctional nests of atypical proliferating melanocytes appearing red-blue, red brown or blue-gray-colored
Other features	
Perilesional erythema	Dilatated capillaries
Pigmentary halo and peripheral grayish patches	Presence of melanin in the reticular dermis
Gray spots and streaks	Intravascular melanoma cell infarcts

draining lymph node basin due to intralymphatic tumor dissemination, may affect from 2.5% to 23% of melanoma patients.[3, 4]

Our patient had previous excision of an acral lentiginous melanoma characterized by the presence of other three of these factors (high Breslow thickness and mitotic rate, age older than 50 years) and developed these recurrence subtypes in the first years of follow-up.

CMMMs are usually small and un-coalesced, clinically homogenous or non-homogenous, of different colors — from violaceous blue to reddish pink, mimicking common and blue nevi —, hemangioma but also primary cutaneous melanoma. It is possible to observe only one solitary or more frequently multiple lesions. Since clinical guidelines are not always reliable and subsequently these in-transit metastases are often misdiagnosed, dermoscopy could help dermatologists to early detect them, above all in high risk patients. As reported in the literature (Table I), some dermoscopic features such as blue homogeneous, vascular and saccular patterns associated with peripheral grey spots, perilesional erythema or pigmentary halo are strong indicators of CMMMs.[5]

Our patient presented clinically and also dermoscopically heterogeneous CMMMs, showing at the same time all the dermoscopic patterns previously described in the literature. In conclusion, since CMMMs are very difficult to diagnose, it is crucial to know their different dermoscopic features, which could all be found in the same patient — as is our case — in order to allow their excision and to avoid their spread.

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Hereditary punctate keratoderma: clinical, pathology, treatment and follow-up

Dear Editor,

First described by Brown in 1971, punctate keratoderma (PK) [1] is a rare disorder, characterized by keratotic lesions on the palmo-plantar regions. Two types of PK have been described: the idiopathic and the hereditary variant.[2] The idiopathic PK has been more commonly reported in the literature, often in association with neoplastic and non-neoplastic disorders.[2-5] Contrariwise, few documented cases of hereditary PK have been reported in the literature supported by clinical and pathological pictures.[2]

A 66-year-old Caucasian man presented to our Institute with yellowish and asymptomatic keratotic papules, which had appeared gradually over the past 9 years on both palms without any involvement of the soles (Figure 1A, B). His 45-year-old daughter also presented small, yellowish/whitish, asymptomatic, keratotic papules on her palms, that had appeared over the last 6 years

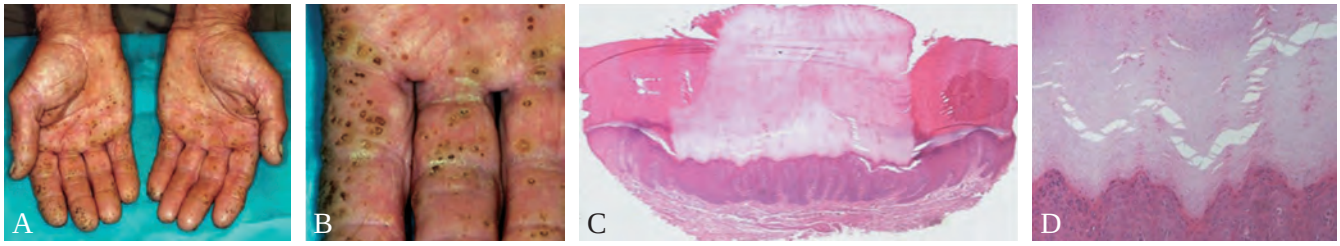

Figure 1.—A) Yellowish keratotic papules on father's palms; B) yellowish keratotic papules of the palms (close view); C) acanthosis, hypergranulosis and orthokeratosis with a distinct thick column of parakeratosis (H&E 10x); D) detail of the distinct thick column of parakeratosis (H&E 30x).

(Figure 2A). Clinical examination did not highlight any further alterations and the familial and personal medical history was negative for malignancies, as well as for internal and skin diseases in both patients. A punch biopsy of the palm of each patient was performed. The pathological examination revealed in skin specimens acanthosis, hypergranulosis and orthokeratosis, with a distinct thick column of parakeratosis in the central part of lesions (Figures 1C, D, 2B). Dyskeratosis, vacuolated cells and atypical cells were not observed. Therefore, a final diagnosis of PK was made. The father received acitetrin and an improvement of the lesions was observed. However, the lesions arose again after stopping the treatment. Contrariwise, his daughter refused any therapy. After a follow-up of 8 years, they both continue to perform clinical control in our Department and routine systemic examinations.

Inherited PK is an autosomal dominant disease, usually not associated with internal or malignant disorders. For this reason, inherited PK it has been reported as the benign type of PK.[2]

PK may be mistaken for several diseases, including viral warts, nevoid basal cell carcinoma, pitted keratolysis, arsenical keratosis, and palmoplantar porokeratosis (PP).[3] However, it is important to highlight that PK and PP are different skin diseases.[2, 5] Indeed, PK clinically lacks the typical centrifugally expanding circles of PP. In addition, PK lesions do not form plaques. Pathologically, only PP shows, beneath the parakeratotic column, dyskeratosis and vacuolated keratinocytes in the epidermis. Furthermore, it has been reported that (under electron microscopic examination) the epidermis of PK, under the parakeratotic column, does not show dyskeratosis, vacuolar degeneration or exaggerated clumping of tonofilaments, that contrariwise have been highlighted in PP.[5]

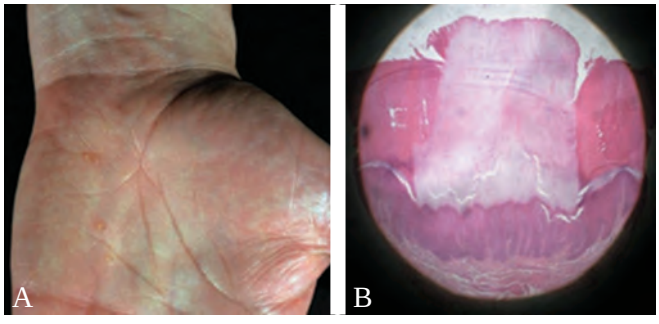

Figure 2.—A) Yellowish keratotic papules on daughter's palms; B) acanthosis, hypergranulosis and orthokeratosis with a distinct thick column of parakeratosis (H&E 10x).

In acquired PK, the incidence of malignancies, as well as other internal disease, remains inexplicably high. However, based on the long follow-up (14 years), both patients did not show any internal disorders or malignancies associated to PK.

On the one hand, topical treatments of PK, including urea, salicylic acid, vitamin A, retinoids, and tacalcitol, usually show poor results.[2] On the other hand, systemic therapy with oral retinoids could lead to a good improvement of lesions.[2] However, recurrence after stopping therapy is common,[2] as reported in our case.

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Dissecting cellulitis: responding to topical steroid and oral clindamycin

Dear Editor,

Dissecting cellulitis of the scalp (DCS) is a rare disease, characterized by painful deep nodules and sinus tracts, discharging purulent secretions, principally localized at vertex and occiput. The development of chronic inflammation, caused by the primitive occlusion of the follicles, can lead to a patchy alopecia.[1]

The incidence of DCS is still not completely known and it is underestimated and often confused with other entities. DCS can appear as a single nosological entity or as a part of a complex of disease.[1, 2] When it is combined with acne conglobate and hidradenitis suppurativa DCS corresponds to the follicular occlusion triad; the additional presence of pilonidal cysts defines the follicular occlusion tetrad.[2]

DCS affects almost exclusively young black males. Only 10% of cases is detected in white males,[1] and it may occasionally also involve young females.[3]

Here we report four consecutive cases of DCS affecting two white males and two white females, responding to systemic antibiotics and topical corticosteroid.

Patient 1 is a 26-year-old man who presented with a two-year history of multiple, painful, firm nodules, principally on the vertex. He also had flecked scarring alopecia, covering the nodules (Figure 1A). He was a hard smoker, but his medical past history was unremarkable. Blood routine test were negative. After a treatment with clindamycin 300mg twice a day for a total of 30 days and 0.05% clobetasol propionate topical foam once a day, we observed a reduction of the number and the texture of nodules (Figure 1B). The patient is currently still in treatment and is being evaluated every 3 weeks at our Institute.

Patient 2 is an 18-year-old woman with a 10-month history of disseminated follicular pustules and nodules in the vertex region, who was admitted to our Institute (Figure 1D). She started clindamycin 300 mg twice a day for a total of 30 days and 0.05% clobetasol propionate topical foam once a day. The first follow-up was done after one month. Our clinical examination revealed an important improvement of the number of nodules and no follicular pustules were detected (Figure 1E). The patient is currently still in treatment and is being evaluated every 3 weeks at our Institute.

Patient 3 is a 28-year-old man who presented with a three-year history of patchy alopecia, covering occipital draining sinuses

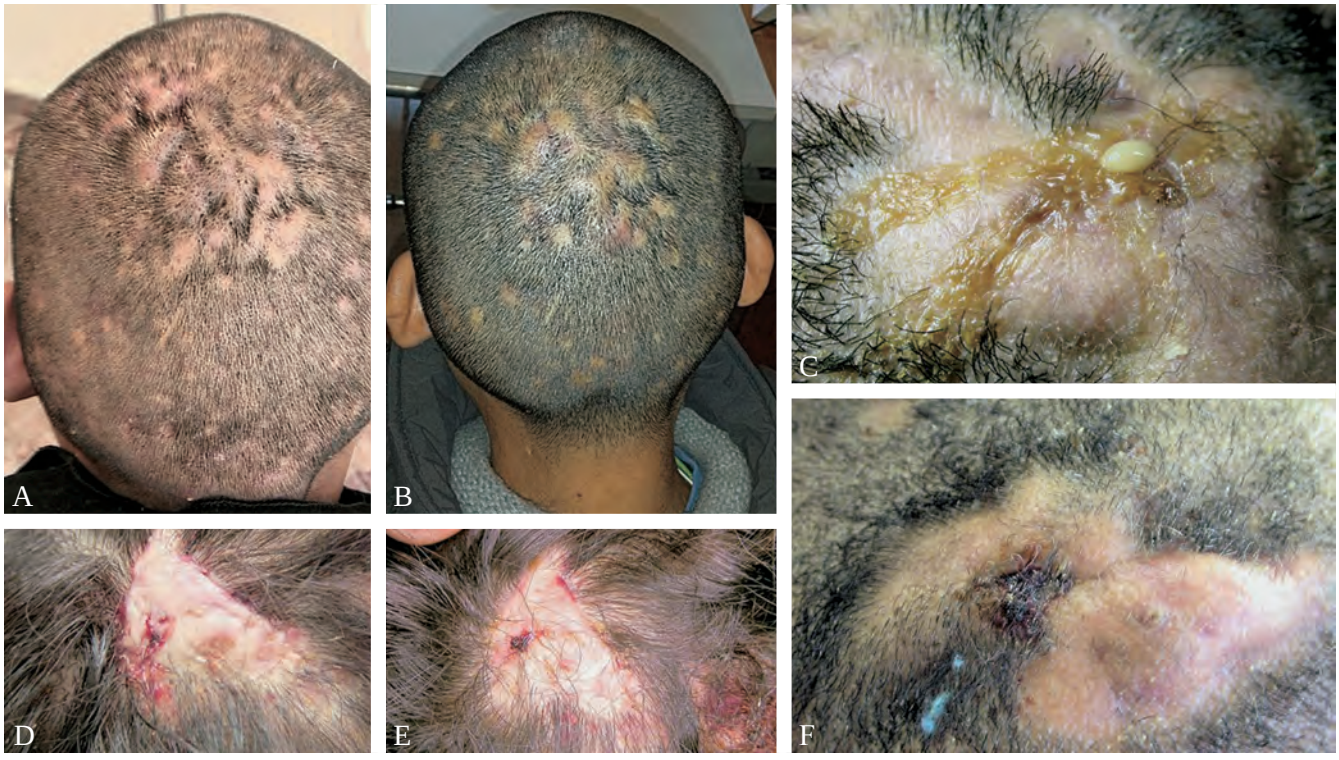

Figure 1.—Clinical pictures taken before and after therapy: A, B) patient 1; C, F) patient 2; D, E) patient 3.

(Figure 1C). His medical history was positive for acne conglobate. Routine laboratory tests were within normal ranges. Cultures of draining purulent lesions were negative for fungi and positive for *S. aureus*. After a treatment with clindamycin 300 mg twice a day for a total of 30 days and 0.05% clobetasol propionate topical foam once a day, there was a remarkable reduction of drainage of the occipital lesions (Figure 1F). The patient is currently still in treatment and is being followed at our Institute.

Patient 4 is a 33-year-old woman with a 15-month history of several, painful, abscesses on the vertex and the occipital area of the scalp was admitted to our Institute. Her medical history was positive for ulcerative rectocolitis. Routine laboratory tests were within normal ranges. Culture of aspirated abscess material for bacteria and fungi was negative. The therapeutic scheme followed by the patient was initially clindamycin 300 mg twice a day for a total of 30 days and 0.05% clobetasol propionate topical foam once a day. At first follow-up, she reported a notable improvement of the pain. Our physical examination revealed a decrease of the number of the abscesses and no suppuration was detected. Unfortunately, the patient was lost at follow-up.

DCS was first described by Spitzer in 1903 and later Hoffman coined the term "*perifolliculitis capitis abscedens et suffodiens*" in 1907.[1]

Clinically, DCS shows deep, painful dermal nodules, sinus tracts and abscesses, involving the scalp. Then, nodules become fluctuating, discharging pus., Due to the granulomatous inflammatory response, patchy alopecia can develop. S. aureus, anaerobic bacteria, and trauma, have been evaluated as further pathophysiological correlations.[1, 2] Furthermore, DCS may be associated with musculoskeletal disorders, keratitis-ichthyosis-deafness syndrome and pyoderma vegetans,[1] showing a wide range of associated disorders.

Even though the etiology of DCS is still not completed known, a pivotal role is played by the primary follicular hyperkeratosis, resulting in accumulation of follicular materials.[1, 4] In addition, a hypersensitivity to Propionibacterium acne is reported, leading to a chronic inflammatory response.[1-3] The colonization by several bacteria is subsequent to the follicular infundibula hyperkeratinisation.[1]

Histopathologically, we can differentiate two different stages.[4] Early we can detect a follicular and perifollicular infiltration of neutrophils. Later we can find a granulomatous reaction, caused by the discharge of follicular material in dermis, leading to follicular destruction and, clinically, to scarring patchy alopecia.

DCS represents a challenge for the dermatologist, because there is not standardized therapy and often a combined surgical and medical approach is necessary. Broad spectrum antibiotics, such as erythromycin or tetracycline for 4-6 weeks, are used to extinguish the inflammatory flares. However, systemic or intralesional steroid have been employed to reduce the acute inflammation.[1, 5] Isotretinoin can represent a valid treatment, determining a long-lasting remission. Isotretinoin leads to apoptosis and cell cycle arrest, particularly in the sebaceous gland, followed by the skin adopting a wound-healing-like pattern of gene expression, with subsequent repair and remodeling. After 8 weeks of treatment, the genes involved in the metabolism of steroids were down-regulated, while those that encode structural proteins such as collagens and fibronectin were up-regulated. Clinical experience suggests it may be dose-dependent with reduced scarring and hypertrophic scarring.[5] Infliximab and adalimumab are currently employed with success.[5] Regarding the surgical approach, different procedures are reported in literature, with variable results. Carbon dioxide laser, skin grafting scalp extirpation, and marsupialization of cyst wall are singularly attempted in the pre-biological era as relief in cases not responsive to medical therapy.[5]

In conclusion, DCS is a very uncomfortable disease, which often provokes an important reduction of the quality of life of patients. Although DCS represents a rare nosological entity, it is important for the clinicians to detect it during the early stages, when the typical patchy alopecia is non-scarring yet. Hence, a much-deepened physical examination is required, especially in females, in which this disease is rarer than males.

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