UNIVERSITY OF GENOA

Department of Internal Medicine and Medical Specialties (Di.M.I.)

PhD course in Translational Medicine in Oncology and Hematology

XXXI cycle

Curriculum: Genetics Oncology and Molecular Pathology

Academic year 2017/2018

GENOTYPE – PHENOTYPE CORRELATIONS IN CUTANEOUS MELANOMA PATIENTS CARRIER OF THE MITF p.E318K PATHOGENIC VARIANT

PhD candidate

Dr. Giulia Ciccarese

Supervisor

Prof. Paola Ghiorzo

PhD student coordinator

Prof. Alberto Ballestrero

"Research is to see what everybody else has seen and to think what nobody else has thought"

Albert Szent-Gyorgyi

1937 Nobel laureate, Medicine

Index

1. Introduction

1.1 Definition of melanoma

Melanoma is potentially lethal tumor resulting from the malignant transformation of melanocytes, the cells derived from the neural crest that produce melanin. During embryonic life, precursor cells, known as melanoblasts, migrate to the basal cell layer of the epidermis and, less frequently, to the dermis and sebaceous glands. Melanoma can develop from melanocytes located in all these sites or from altered melanocytes called nevus cells in precursor lesion (Langley R 2003). A recent meta-analysis based on 38 observational cohort and case-control studies showed that 29.1% of melanomas likely arose from a pre-existing nevus and 70.9% arose de novo (Pampena R 2017).

1.2 Epidemiology of melanoma

Cutaneous melanoma (CM) is the 12th most common cancer worldwide with an incidence varying among the populations: the highest rates are described in Australia and New Zealand (40.3 per 100,000 and 30.5 per 100,000, respectively) whereas the lowest ones are reported in South-Central and South-Eastern Asia (<0.5 per 100,000) (Dimitriou F 2018).

This variation is due to the different risk factors to which the populations are exposed, as ultraviolet (UV) radiation of varying intensity and genetically determined phenotypic features.

Recent epidemiological studies reported an annual increasing incidence of CM worldwide with 232,000 new cases in 2012 and 351,880 new cases in 2015 with an age-standardized rate of 5 cases per 100,000 persons (Ferlay J 2015). The overall CM incidence is estimated to further increase, mainly due to the lengthening of the human lifetime and the aging of population (Whiteman DC 2016). A recent study on melanoma incidence and mortality in Queensland (Australia) during the years 1995-2014 revealed a stable or falling incidence of both thin (≤ 1 mm) and invasive (> 1 mm) melanoma in people < 60 years. This could be considered as a good marker of the prevention programs that aim to reduce sunburns in early age, one of the strongest risk factors of melanoma (Aitken JF 2018).

CM mortality rates remain stable or decline in both sexes under 40 years of age whereas in males over 60 years the mortality seems to increase; these data suggest that long-running prevention campaigns and early detection strategies can contribute to reduce the CM incidence and mortality rates and that prevention should start in young age (Ghiasvand R 2017).

In Europe, there are sharp differences in melanoma incidence among the countries, possibly related to missed opportunities for early diagnosis and incomplete reporting of CM in Eastern Europe. The estimated age-standardized incidence (measured per 100 000 person-years) varies widely from 19.2 in Switzerland to 2.2 in Greece. Specifically, in 2012, Switzerland registered the highest CM incidence in Europe, followed by the Scandinavian countries and the Netherlands. In the Scandinavian population, the high incidence of CM may be due to a high-risk phenotype (fair skin, hair, and eye colour) in combination to a tanning culture, high levels of UV exposure during (intermittent) holidays and indoor tanning (Forsea AM 2012).

Several studies suggest a significant gender difference in CM incidence and survival worldwide. Higher CM rates have been reported in the male populations compared to the female one.

The anatomical location of CM also varies according to gender. Males tend to present with worse clinical and histological features at primary diagnosis compared to women; moreover, CM in male are more often located on the head, neck and trunk, commonly ulcerated and with a higher Breslow thickness compared to CM in women. The lower mortality rates in females may be due to both biological and behavioural differences in primary (sun behaviour and sun protection) and secondary (nevi controls) prevention compared to the male population (Dimitriou F 2018).

1.3 Classification of melanoma and clinical-histological features

Melanoma can be classified into four main growth patterns, defined by their macroscopic and microscopic features and correlated with their life history: lentigo maligna, superficial spreading, nodular and acral lentiginous melanoma.

- Lentigo maligna (LM) is the least common type of melanoma affecting 4-15% of all melanoma patients. LM is a type of melanoma in situ with a prolonged radial growth phase. The radial growth phase consists of invasion of the papillary dermis by few melanoma cells that have obtained a growth advantage. These cells are able to autonomously proliferate in this location but have not the capacity for aggregative growth. Conversely, the vertical growth phase is characterized by the property of aggregative growth, resulting in the formation of expansive nests or nodules of cells. After a prolonged radial growth phase, LM may turn into invasive melanoma and, once invasive, LM can be lethal. The exact percentage of LM that progress to invasive LM is unknown. LM is a macular, freckle-like lesion characterized by an irregular shape with different shades of colour, including light brown, brown, black and sometimes blue-grey and white, that typically occurs on sun-exposed body regions (especially head and neck, occasionally the hands, arms and legs). LM develops in elderly patients with sun-damaged atrophic skin (the median age at diagnosis is 65 years old). Frequently the lesion is flat and quite large (3 to 6 cm or largers); the nodular portion, if present, may vary from a few millimetres to 1-2 cm in width and indicates invasive melanoma. The histopathology of LM is characterized by an atrophic epidermis with thinning and loss of the rete ridges, containing atypical basal melanocytes. These cells widely vary in size and shape and have hyperchromatic nuclei with different size, as well; they may extend down hair follicles and skin appendages, making the lesion difficult to eradicate by superficial therapies. A mononuclear infiltrate is usually present in the superficial lesions below the proliferated atypical melanocytes. The dermis presents elastotic changes of the connective tissue due to the chronic sun-damage. Diagnosis can be established by biopsy of the darkest portion of the lesion.
- Superficial spreading melanoma is the most common type of cutaneous melanoma representing 70% of all melanomas. It may occur at any site but it frequently develops on the legs of women and on the back of men. The most commonly involved ages are the fourth and

the fifth decades. Various clinical presentations are possible: SSM may present as a deeply pigmented macule or a slightly raised plaque. On a pre-existing nevus, a change consisting in the appearance of a focal area of darkening may be the earliest sign of a SSM. Pigmentation of a SSM can consist of a mixture of colours ranging from dark brown to black and/or from dark blue-grey to pink or grey-white. The absence of pigmentation within a SSM may represent regression. The borders of SSM are usually irregular with indentations, especially associated with enlargement of the lesion. Histopathology of SSM is characterized by a population of melanocytes appearing uniformly atypical: the highly pleomorphism of melanocytes that is typical of LMM is not usually seen in SSM. Biopsy of a slightly elevated hyperpigmented portion of SSM reveals a "pagetoid" distribution (from the bottom to the top) of large melanocytes in the epidermis. The atypical melanocytes of the SSM are large cells that can occur singly or in nests and have a monomorphous appearance. Their cytoplasm contains regularly dispersed, fine granules of melanin that confers to the cells a "dusty appearance".

- Nodular melanoma (NM) is the second most common subtype of melanoma (15-30% of all types). The most considerable feature of nodular melanoma is the fast evolution, happening over several weeks or months, with an apparently lacking radial growth phase. The most commonly affected body sites are the trunk, the head and the neck. NM usually begin de novo in uninvolved skin rather than arising on a pre-existing nevus. It appears as a uniform dark-blue, bluish-red or amelanotic papule or nodule or as a polypoid lesion. Early recognition of NM may be difficult for the frequent lack of the conventional clinical features of melanoma. Indeed, sometimes it can be difficult to distinguish between NM and hemangioma, pyogenic granuloma, blue nevus and pigmented basal cell carcinoma. From histopathological point of view, NM has little tendency for intraepidermal growth: the tumor arises at the dermal-epidermal junction and extends vertically into the dermis; intraepidermal growth is present only in a small group of tumor cells that also invade the underlying dermis. The epidermis

lateral to the areas of this invasion does not contain atypical melanocytes. As in LM and SSM, the tumor may show epithelioid cells, spindle cells, small malignant melanocytes or mixture of all these types.

- Acral lentiginous melanoma (ALM) is infrequent in light-skinned individuals (representing 2-8% of all melanomas) but is the most common melanoma type in dark-skinned individuals representing 60-72% of all melanomas in blacks and 29-46% of all melanomas in Asians. ALM develop on the palms, soles or below the nail plate with the sole being the most common site. However, not all plantar or palmar melanomas are ALM since a minority are SSM or NM. ALM is usually diagnosed in elderly with a median age at onset of 65 years. Clinically, it manifests as a light brown, brown or black flat lesion with variegation in colour and irregular borders. Papules or nodules may be present in the context of the flat lesion. When the colours of the lesion are pink or red, it may be misdiagnosed as a benign lesion (verruca, pyogenic granuloma). ALM is considered an aggressive melanoma with a poor prognosis; however, it may be due to a late diagnosis of an advance disease rather than to an aggressive biologic behaviour of the tumor. Subungueal melanoma is a variant of the ALM subtype that usually involve the first toe or the thumb. An early subungueal melanoma can be recognized as a brown/black discoloration in the nail bed, usually at a proximal location. Hutchinson's sign is the finding of pigmentation of the posterior nail fold and is considered associated with advanced subungueal melanoma. Benign lesions that can mimic a subungueal melanoma are: longitudinal melanonychia, subungueal hematoma, onychomycosis, nevus or pyogenic granuloma. Histopathology of the flat area of ALM shows a basilar proliferation of atypical melanocytes with large nuclei characterized by an atypical chromatin patterns and a cytoplasm filled by melanin granules. In the popular/nodular areas of the lesion, malignant melanocytes have a spindle shape and extend into the dermis.

A rare variant of melanoma is the one arising on a mucosal surface with the most commonly involved mucosae being the nasal and oral cavity, the vulvar or anorectal mucosae. Patients may complain of

bleeding at these sites or may present with a deeply pigmented irregular lesion. Histologically, the intraepithelial growth phase of melanoma of the vulva and conjunctiva are divided into three subtypes: a pagetoid pattern having the typical features of a skin SSM; a lentiginous pattern having some features of LM and ALM; and a mixed pattern characterized by nests of malignant cells with a lentiginous proliferation (Langley R 2003).

Besides the four classic forms of cutaneous melanoma (LM, SSM, NM, ALM) and mucosal melanoma, other unusual variants have been described and classified into four groups corresponding to the architectural patterns, cytologic features, stromal changes and their possible association (for example architectural and cytologic features) (Rongioletti F 2005).

According to the architectural pattern, melanoma variants have been classified as follows:

- ✓ polypoid melanoma: an unusual variant of NM with an exophytic pattern of growth; it is constituted by an usually ulcerated nodule connected to the skin by a pedicle; histologically, the nodule is filled with melanoma cells whereas the underlying pedicle is, at least initially, free from them;
- ✓ verrucous melanoma may account for erroneous clinical diagnoses since it can mimic several benign lesion such as papillomatous dermal nevus, seborrhoeic keratosis, papilloma (diagnosed in over 50% of cases); histologically, it is characterized by papillomatous epidermal hyperplasia, hyperkeratosis, parakeratosis and acanthosis; an intraepidermal melanocytic proliferation is indistinguishable from a SSM with adnexal involvement;
- ✓ angiomatoid melanoma refers to metastatic lesions characterized by multiple, cavernous erythrocyte-filled spaces throughout a spindle melanoma cell proliferation mimicking a vascular malignancy; however this term was also used to indicate a primary desmoplastic-amelanotic melanoma with a pattern of microvascular proliferation;
- ✓ angiotropic melanoma is defined by the close apposition to the external surfaces of blood microvessels or lymphatic channels by melanoma cells without evidence of intravasation;

- ✓ primary dermal melanoma is characterized by a dermal/hypodermal nodule composed of atypical melanocytes in the dermis (and/or fat) without any junctional activity or epidermal involvement.

According to the stroma, melanoma variants have been classified as:

- ✓ desmoplastic/neurotropic melanoma: the least rare among the unusual variants of melanoma commonly developing in the sun exposed skin of the head and neck in the sixth or seventh decade; it is locally aggressive and has high rates of local recurrence. Clinically, desmoplastic melanoma melanoma presents as a pigmented macule (with or without a nodular component) or as a pink, firm and sclerotic nodule without any surrounding pigmentation. Histologically, the tumor is composed of spindle cells (who resemble fibroblasts or myofibroblasts), infiltrating the dermis and the hypodermis associated with abundant stromal collagen mimicking a scar or dermatofibroma. A typical patchy lymphoid perivascular infiltrate scattered throughout the tumor is often recognized. Desmoplastic melanoma has typically the propensity to infiltrate the perineurium and endoneurium of the cutaneous nerves. Differential diagnosis includes morpheaform basal cell carcinoma, desmoplastic nevus, sclerosing variants of blue nevus, scar, atypical fibroxanthoma;
- ✓ myxoid melanoma, often confusing with other mucin-containing neoplasms, clinically appears as a pigmented nodule mainly on the extremities and trunk; histologically, spindle- and stellate-shaped atypical cells with prominent nuclei and mitotic figures are embedded in a myxoid stroma;
- ✓ ossifying/chondroid melanoma is characterized by a very unusual histological phenomenon of osteocartilagineous differentiation of the stroma together with proliferation of atypical, pigmented spindle and epithelioid cells.

According to the cytological features, many melanoma variants have been described, including the following:

- ✓ balloon-cell melanoma has a distinctive histological cellular alteration, namely ballooning, in at least 50% of the lesion. The presence of cytological atypia, nuclear pleomorphism, and mitoses allow to distinguish balloon-cell melanoma from the more common balloon-cell nevus;
- ✓ spindle-cell melanoma represents a lesion that is very difficult to diagnose since it may be confused with other spindle-cell neoplasms such as malignant peripheral nerve sheath tumors;
- ✓ signet-ring cell melanoma is characterized by neoplastic melanocytes with abundant clear cytoplasm and large vacuoles compressing the nuclei to the periphery; the signet ring cells are due to the accumulation of cytoplasmic vimentin intermediate filaments;
- ✓ small cell melanoma describes a heterogeneous group of melanomas arising in different settings either in adults, adolescents and children whose common denominator is a population of small cells;
- ✓ animal-type melanoma is a raised blue-black nodule with irregular borders, which may occur at any site, mainly the scalp; histologically, it involves the dermis in its entire thickness and is composed of sheets of pigmented large epithelioid melanocytes containing dusty melanin; This human neoplasm mimics the melanocytic tumors seen in grey horses that are slow-growing and indolent lesions. In humans, the behaviour is unpredictable;
- ✓ amelanotic melanoma may appear as a flat, papular or plaque-like reddish lesion; histologically, such tumors (that often are nodular melanomas) are characterized by nodules and bundles of atypical spindle and epithelioid cells apparently lacking pigmentation. Actually, finely granular melanin in some tumor cells and atypical epidermal melanocytic proliferation can be observed;
- ✓ spitzoid melanoma is a form of melanoma having a cellular composition that resembles the enlarged epithelioid and fusiform cells of Spitz nevus; however, the presence of mitoses and single cell necrosis in the deepest part of the lesion with lack of melanocyte's maturation, the nuclear and nucleolar pleomorphism of the cells, the growth pattern in solid sheets of cells,

the asymmetric distribution of the pigment and an inflammatory infiltrate, allow to obtain the right diagnosis; recently, the use of the term ‘spitzoid melanoma’has been discouraged since it may be confounding suggesting a heterogeneous group of lesions, including Spitz tumor, Spitz-like melanocytic tumor with atypical features and classic melanoma (G. A. Rongioletti F 2015).

Finally, several melanoma variants with combined patterns have been reported:

- ✓ malignant peripheral nerve sheath tumor-like melanoma;
- ✓ malignant blue nevus (to be distinguished from plaque-type blue nevus) (G. A. Rongioletti F 2015);
- ✓ melanoma of the soft parts;
- ✓ nevoid melanoma, which mimics melanocytic nevi;
- ✓ minimal deviation melanoma (characterized by a vertically growing population of cells presenting cytologic features that deviate only minimally from those of nevus cells) (S. B. Rongioletti F 2005).

1.4 Clinical detection of melanoma

Since cutaneous melanoma arises on an easily accessible site, the dermatologists and also the patients have the opportunity to diagnose this tumor at an early and curable time. The early diagnosis is crucial because tumor thickness remains a critical prognostic indicator in primary cutaneous melanoma (Gershenwald JE 2017) (Langley R 2003).

The clinical diagnosis of melanoma relies on a history of sustained change, on assessing the macroscopic morphologic features of the lesions and it is mainly related to the overall degree of order and symmetry of the lesion. The macroscopic morphologic features used in assessing melanocytic lesions include size, colour, borders, surface topography on tangential lighting and symmetry. The *ABCD* rule can be applied for diagnosis of all types of melanoma, except for NM:

A means asymmetry; *B* means irregular, notched borders; *C* means colour that in melanoma is not uniform but may display all shades of brown, black, grey, pink, white, blue; *D* means diameter >6 mm.

Melanoma has an initial growth in the skin and mucosa that is common to all the subtypes of the disease, except for NM (radial growth phase). This phase is characterized by a flat or slightly popular surface and horizontally extended growth. Clinically, in this phase melanoma is an asymmetric lesion with irregular borders and variation of colour pattern: the presence of blue-black, red and white colours is particularly suggestive for melanoma; focal black areas, especially if newly developed, are also suspicious; focal white, grey and pink areas correspond to regression. The most suspicious sign suggesting melanoma is a lesion that persistently changes in size and colour. Other findings, as elevation, ulceration and bleeding, generally indicate a more advanced primary melanoma (these changes typically occur over the course of weeks/months).

Non invasive clinical techniques for early diagnosis of melanoma include: manual dermoscopy, computerized digital imaging (computerized dermoscopy), ultrasonography and confocal scanning laser microscope.

The term 'dermoscopy' was first used by Friedman and colleagues in 1991 to indicate a diagnostic technique that links clinical dermatology and dermatopathology by enabling the visualisation of morphological features not seen by the naked eye. Dermoscopy consisting in the examination of a pigmented lesion with a lens or handheld device (dermatoscope) that magnifies the skin in such a way that colour and structures in the epidermis, dermo-epidermal junction and papillary dermis become visible (Soyer HP 2012). It therefore represents valuable tool largely used by dermatologists for early diagnosis of melanoma and for facilitating the differentiation of other benign and malignant pigmented skin lesions (S. H. Argenziano G 2001). The dermoscopic patterns of acquired melanocytic lesions of the entire skin surface (except for the acral sites) are classified as: reticular, globular, homogenous, multicomponent, reticular-globular, reticular-homogenous, globular-homogenous and unspecific pattern. This latter was defined as a pattern

lacking specific features related with a melanocytic or non melanocytic lesions. Conversely, the following dermoscopic patterns were considered to assess acral melanocytic lesions: parallel furrow, parallel ridge, lattice-like, fibrillary (S. H. Argenziano G 2003) (Z. I. Argenziano G 2007). The histological features and the diagnostic significance of the main dermoscopic patterns are summarized in Table 1 (Langley R 2003).

Surface dermoscopic criteria	**histologic features**	**diagnostic significance**
Pigment network	pigmented rete ridges	melanocytic lesion
regular pigment network	regularly distributed rete	benign melanocytic lesion
irregular pigment network	irregularly distributed rete	dysplastic nevi or melanoma
broadened pigment network	broad rete ridges with increased number of atypical melanocytes	early melanoma
black dots	collection of pigmented cells in the cornified layer	melanoma
brown globules	pigmented nests in the papillary dermis and dermal-epidermal junction	melanocytic nevus (if regular); melanoma (if irregular)
blue-grey veil	areas of regression	fibrosis, presence od melanophages in melanoma

Table 1. Correlation between dermoscopic and histologic features of pigmented skin lesions.

Results of the 2001 Consensus Net Meeting on Dermoscopy (S. H. Argenziano G 2003) showed that the following three criteria were mainly important in distinguishing melanoma from other benign pigmented lesions: dermoscopic asymmetry of colour and structure; atypical pigment network and blue-white structures. Statystical analysis showed that the presence of two of these three criteria indicates a high likelihood of melanoma (Soyer HP 2012).

1.5 Histological diagnosis of melanoma

Excisional biopsy and histological examination are essential for a reliable diagnosis of melanoma. The histological diagnosis is based on the assessment of a series of diagnostic findings, especially architectural findings. Cytological atypia is considered necessary for a melanoma diagnosis: specifically, fully evolved atypia refers to cellular and nuclear enlargement, variations in nuclear size and shape, nucleus hyperchromasia with irregular chromatin distribution and enlargement of nucleoli. In melanoma, large epitheliod cells are characterized by a granular or "dusty" cytoplasm whereas spindle cells have less cytoplasm with high nucleus-to-cytoplasm ratios. The architectural findings suggestive of melanoma include: large size (>5 mm), asymmetry and, importantly, the pagetoid spread (intra-epidermal upward) of melanocytes. In the dermal component, areas of regression and nodule formation are possible (Urso C 2005). Noteworthy, the upward migration of cells is possible also in acral nevi of children and in Spitz nevi but in the benign lesions, the migratory cells have benign cytologic features.

The antisera used in immunohistochemistry for the routine evaluation of paraffin embedded specimens include S-100 protein and HMB-45. S-100 protein is expressed by virtually all malignant melanomas and melanocytic nevi but also by a variety of other tumors whereas HMB-45 is a monoclonal antibody with high specificity for malignant melanoma (Langley R 2003).

1.6 Staging system of melanoma

Accurate documentation of the extent of melanoma is crucial for determining the best treatment and for assessing prognosis. The American Joint committee on cancer (AJCC) has recently approved a new melanoma staging system (Gershenwald JE 2017). Based on analyses of a large database comprising more than 46,000 patients from 10 centers worldwide with stages I, II and III melanoma diagnosed since 1998, the AJCC Melanoma Expert Panel introduced several important changes to the Tumor, Nodes, Metastasis (TNM) classification and stage grouping criteria of the existing seventh edition AJCC stage IV database (Gershenwald JE 2017). The definition of primary tumor, regional

lymph nodes and distant metastasis according to the new staging system are summarized in Tables 2-4. The clinical stage based on the TNM system is described in Table 5 (Gershenwald JE 2017).

T CATEGORY	THICKNESS	ULCERATION STATUS
TX: Primary tumor thickness cannot be assessed (eg, diagnosis by curettage)	Not applicable	Not applicable
T0: No evidence of primary tumor (eg, unknown primary or completely regressed melanoma)	Not applicable	Not applicable
Tis (melanoma in situ)	Not applicable	Not applicable
T1	≤1.0 mm	Unknown or unspecified
T1a	<0.8 mm	Without ulceration
T1b	<0.8 mm	With ulceration
	0.8–1.0 mm	With or without ulceration
T2	>1.0–2.0 mm	Unknown or unspecified
T2a	>1.0–2.0 mm	Without ulceration
T2b	>1.0–2.0 mm	With ulceration
T3	>2.0–4.0 mm	Unknown or unspecified
T3a	>2.0–4.0 mm	Without ulceration
T3b	>2.0–4.0 mm	With ulceration
T4	>4.0 mm	Unknown or unspecified
T4a	>4.0 mm	Without ulceration
T4b	>4.0 mm	With ulceration

Table 2. Definition of primary tumor (T).

	EXTENT OF REGIONAL LYMPH NODE AND/OR LYMPHATIC METASTASIS	
N CATEGORY	NO. OF TUMOR-INVOLVED REGIONAL LYMPH NODES	PRESENCE OF IN-TRANSIT,SATELLITE, AND/OR MICROSATELLITE METASTASES
NX	Regional nodes not assessed (eg, sentinel lymph node [SLN] biopsy not performed, regional nodes previously removed for another reason); Exception: pathological N category is not required for T1 melanomas, use clinical N information	No
N0	No regional metastases detected	No
N1	One tumor-involved node or any number of in-transit, satellite, and/or microsatellite metastases with no tumor-involved nodes	
N1a	One clinically occult (ie, detected by SLN biopsy)	No
N1b	One clinically detected	No
N1c	No regional lymph node disease	Yes
N2	Two or 3 tumor-involved nodes or any number of in-transit, satellite, and/or micro-satellite metastases with one tumor-involved node	
N2a	Two or 3 clinically occult (ie, detected by SLN biopsy)	No
N2b	Two or 3, at least one of which was clinically detected	No
N2c	One clinically occult or clinically detected	Yes
N3	Four or more tumor-involved nodes or any number of in-transit, satellite, and/or microsatellite metastases with 2 or more tumor-involved nodes, or any number of matted nodes without or with in-transit, satellite, and/or microsatellite metastases	
N3a	Four or more clinically occult (ie, detected by SLN biopsy)	No
N3b	Four or more, at least one of which was clinically detected, or the presence of any number of matted nodes	No
N3c	Two or more clinically occult or clinically detected and/or presence of any number of matted nodes	Yes

Table 3. Definition of Regional Lymph Node (N).

	M CRITERIA	
M CATEGORY[b]	**ANATOMIC SITE**	**LDH LEVEL**
M0	No evidence of distant metastasis	Not applicable
M1	Evidence of distant metastasis	See below
M1a	Distant metastasis to skin, soft tissue including muscle, and/or nonregional lymph node	Not recorded or unspecified
M1a(0)		Not elevated
M1a(1)		Elevated
M1b	Distant metastasis to lung with or without M1a sites of disease	Not recorded or unspecified
M1b(0)		Not elevated
M1b(1)		Elevated
M1c	Distant metastasis to non-CNS visceral sites with or without M1a or M1b sites of disease	Not recorded or unspecified
M1c(0)		Not elevated
M1c(1)		Elevated
M1d	Distant metastasis to CNS with or without M1a, M1b, or M1c sites of disease	Not recorded or unspecified
M1d(0)		Not elevated
M1d(1)		Elevated

CNS indicates central nervous system; LDH, lactate dehydrogenase.

Table 4. Definition of Distant Metastasis (M).

WHEN T IS…	AND N IS…	AND M IS…	THEN THE CLINICAL STAGE GROUP IS…
Tis	N0	M0	0
T1a	N0	M0	IA
T1b	N0	M0	IB
T2a	N0	M0	IB
T2b	N0	M0	IIA
T3a	N0	M0	IIA
T3b	N0	M0	IIB
T4a	N0	M0	IIB
T4b	N0	M0	IIC
Any T, Tis	≥N1	M0	III
Any T	Any N	M1	IV

Table 5. Clinical stage of melanoma based on TNM classification.

1.7 Evolution of melanoma

Approximately 15-36% of patients with stages I and II melanoma experience recurrence or metastasis during their clinical course. Melanoma typically recurs or metastasize in a stepwise manner consisting of: local recurrences, regional mestases and distant metastases.

Local recurrence consists in any recurrence in close proximity to the surgical scar for primary cutaneous melanoma. The reported frequency of local recurrence is approximately 3 percent and is related to tumor thickness with increased rates in patients with thicker primary lesion.

In-transit metastases are defined as small tumor emboli within the dermal and subdermal lymphatics between the primary tumor and the regional lymph node basin.

The presence of regional nodal disease is highly predictive of visceral metastases. The risk of regional node metastasis varies by primary tumor thickness: patients with melanomas that are <0.8 mm, 0.8 to 1.5 mm, 1.5 to 4 mm and >4 mm have nodal metastases at 3 years in 2-3%, 25%, 57% and 62%, respectively.

Melanoma can metastasize to any organ and has a certain pattern of metastases that make clinician able to manage the disease; indeed, the tumor spreads most frequently to the non-visceral sites: skin, subcutaneous tissue and distant lymph nodes (42-57% of cases). Visceral metastases to the lungs, liver, brain, bone and intestine are the next common sites.

The incidence of late recurrences of melanoma (10 years or more years after initial diagnosis and treatment) is approximately 0.93 to 6.7% (Langley R 2003). A large series of more than 7000 patients with melanoma includes 168 patients with late recurrences: the thickness of the primary tumor ranges between 0.34 and 6.3 mm (mean thickness 1.6 mm) and the mean time to recurrence was 14 years for patients having recurrences after 10 years (Crowley NJ 1990).

A recent study investigated the prognostic risk factors for first recurrence in patients with localized stages I-II cutaneous melanoma and found that tumour thickness was the predominant risk factor followed by presence of ulceration, Clark's level of invasion and histogenetic type (Lyth J 2017).

1.8 Risk factor for cutaneous melanoma

The development of CM is due to multiple different causal pathways and reflects the interaction between environmental and host related risk factors.

1.8.1 Solar ultraviolet radiation

Exposure to ultraviolet radiation (UV), both natural (sun exposure) and artificial (indoor tanning with sunbed), is the major known environmental risk factor for CM development.

Sun exposure is absolutely the major environmental cause of CM.

Knowledge of the association between sun exposure and CM comes mainly from case-control and cohort epidemiological studies. Measurements of individual sun exposure are commonly classified as intermittent (short, intense sun exposure through activities such as sunbathing, outdoor recreations and holidays in sunny climates of a person who works indoor all year round, as an office worker), chronic (more continuous, primarily occupational exposure, as a farmer or a fisherman), and total sun exposure (the sum of intermittent and chronic exposure).

There is strong evidence that an intermittent sun exposure increases the risk of CM. Chronic sun exposure shows no association, or a weak inverse association with CM risk. Total lifetime sun exposure is positively associated with CM risk, but the relationship is weaker than that for intermittent sun exposure. A typical marker of an intermittent sun exposure is sunburn.

The weak association with chronic sun exposure may be due to its promotion of epithelial thickening and this together with a tanning effect may offer a modest protection against later exposure to solar radiation. (Gandini S 2005)

A recent analysis of two case-control studies found no association between occupational exposure and melanoma risk and no indication of confounding by recreational exposure. (Vuong K 2013)

Importantly, the presence of actinic keratoses, as markers of high cumulative sun exposure, is consistently positively associated with risk of CM. (Z. M. Olsen CM 2011)

Melanoma risk depend not only from the type of sun exposure (intermittent, chronic or total), but also by body site, age, and phenotype of the host. CM arising on the head and neck have been associated

to chronic sun exposure and older age at diagnosis; CM of the trunk and limbs to younger ages and intermittent exposure. Sun exposure can cause melanoma on all body sites, but the risk is higher for usually sun-exposed sites than occasionally exposed sites. For sunburn, strong positive associations have been found at all body sites. (Caini S 2009)

Sun exposure and sunburns in early-life, as in childhood, is probably an important risk factor. Migrant studies show that children who migrate to a sunnier country from a less sunny country before the age of 10 adopt the incidence rates of the new country (Levine H 2013). Berwick et al. found that higher UVB dose in early life (age 10) was associated with poorer survival from melanoma (R. A. Berwick M 2014). However, meta-analyses studies have described increased risk of CM with increasing number of sunburns during all life periods (childhood, adolescence, adulthood), without significant differences between sunburns in childhood and adulthood (Gandini S 2005).

The UV radiations are subdivided by wavelength into UVA (320–400 nm), UVB (280–320 nm) and UVC (100–280 nm). UVC is blocked by stratospheric ozone and does not reach surface of the earth whereas UVB and UVA are responsible for the UV-induced skin damage. Due to different electrophysical properties, UVB causes direct DNA damage, whereas UVA causes indirect DNA damage through generation of reactive oxygen species (D'Orazio J 2013).

1.8.2 Artificial UV radiation

Several studies have investigated the relationship between indoor tanning and CM and found a causal positive association, especially when first use was before 35 years of age. Furthermore, the risk is increased with the number of sessions.

Indoor tanning has become an important source of UV exposure in many countries. With sunbeds, up to 95–100 % of the body is exposed to UV radiations compared to 15–50 % during outdoor activities. (Boniol M, Cutaneous melanoma attributable to sunbed use: systematic review and meta-analysis 2012) (Boniol M, Correction. Cutaneous melanoma attributable to sunbed use: systematic review and meta-analysis 2012)

Measurements of modern sunbeds show UV irradiance higher than midday summer sun in Southern Europe and Australia and often exceeding the limits allowed by safety standards/regulations (Hornung RL 2003).

The irradiance from modern sunbeds is mainly in the UVA range with a fraction of UVB, and this is alarming in light of the increased focus on UVA as a carcinogen (Dimitriou F 2018).

Since Denmark has the highest prevalence of sunbed use reported and one of the highest incidences of skin cancer worldwide, in 2007 an ongoing long-term antisunbed campaign was launched. Sunbed use was evaluated by annual cross-sectional surveys and skin cancer incidence was modelled in the Prevent programme, using population projections, historic cancer incidence, sunbed use exposure and relative risk of sunbed use on melanoma. The authors showed the value of prevention and of long-term planning in prevention campaigning: sunbed use was reduced significantly during 2007-2015 and, consequently, significantly fewer skin cancer cases are anticipated during 2007-2040 (Køster B 2018).

The World Health Organization declared that persons who should never use sunbeds are: people under 18s; people who have very fair skin and burn easily or tan poorly; people with a lot of freckles or moles; people who have had skin cancer or have a family history of the disease; people using medication that could make their skin more sensitive to UV; people who already have extensive "sunlight" damage (WHO s.d.).

Following the precedent set by Brazil, which passed a resolution in 2009 banning the use of commercial tanning beds for cosmetic purposes, Australia is the second country in the world to implement bans (Craig A. Sinclair 2014) . Nowadays, in many other countries in the world, including in Europe Italy, France and Switzerland, the use of sunbed is forbidden for minors and pregnant women.

1.8.3 Other environmental factors

Several studies have reported that exposure to polycyclic aromatic hydrocarbons, benzene or other chemical products used in the printing industry are associated with the development of melanoma

(Linet M 1995). Chemical, electrical/electronic workers and workers in occupations exposed to ionizing radiation (medical X-ray workers, radiology technologists, dental workers) seem to be at increased risk of melanoma, as well (Nelemans PJ 1993) (Sigurdson AJ 2003).

Studies among airline pilots and cabin crews who might presumably be routinely exposed to cosmic radiation during high altitude flight revealed opposite results about their increased risk of developing CM compared to the population not exposed to cosmic radiation (A. R. Pukkala E 2002) (A. A. Pukkala E 1995). However, it should be noted that previously cited occupational studies are based only on employment records and are not adjusted for the major known risk factors for CM as phenotypic characteristics, nevi count and UV radiation exposure.

Several studies suggested an increased CM risk in individuals exposed to organochlorine compounds as polychlorinated biphenyls (PBCs), contained in some insecticides, and some chlorine-based pesticides. Even if most countries banned the production and use of PBCs when the potential adverse effects of PCBs became clear (USA, Canada and Australia in the 1970s, European Union in the 1980s), PCBs are extremely persistent organic pollutants and survive in the environment for many years. The carcinogenic mechanisms of PBCs include formation of reactive oxygen species, endocrine disruption and immune compromise. Human exposure to PBCs in developed countries is through dietary intake of fish and animal products (B. D. Berwick M 2016).

1.8.4 Host-related factors

Host-related factors can greatly modify an individual's response to UV radiations exposure, the principal environmental risk factor for CM. Host related factors are: nevi; skin, hair and eye colors; ability to tan and propensity to burn.

1.8.4.1 Nevi

Nevi are benign aggregations of melanocytes. The number of nevi have been considered in many studies the most important risk factor for CM, with increased number of nevi associated with increased risk of the disease (Bataille V 2008) (Markovic SN 2007) (B. D. Berwick M 2016).

A meta-analysis showed that individuals with more than 100 normal nevi are at almost seven times greater CM risk than individuals with few nevi (≤15) (Gandini S 2005); the increase in risk of CM is considered proportional to the number of nevi (Markovic SN 2007). The size of nevi also increases the risk of melanoma, especially for nevi greater than 2.0 mm in diameter.

Dysplastic or atypical nevi are also associated with an increased risk of melanoma and the increase in risk is proportional to the number of dysplastic nevi (DN). Indeed, individuals with atypical mole syndrome also known as dysplastic nevus syndrome (characterized by at least two atypical nevi, >100 normal nevi and nevi on unusual body sites as scalp, soles, buttocks, breast) especially with a family history positive for melanoma, are at high risk of developing melanoma (B. D. Berwick M 2016).

An interaction between sun exposure and nevi has been described in studies which found that intense and intermittent sun exposure during childhood and adolescence, especially if associated with sunburns, is related with an increased number of nevi and with the development of atypical nevi, suggesting a link between sun exposure and melanoma (Garbe C 1994) (Harrison S 1994).

Whiteman DC et al. proposed two pathways for melanoma-genesis:

1) one pathway associated with melanocyte proliferation: individuals with a propensity for melanocytic instability that are at risk of developing melanoma, especially on body sites not chronically exposed to sunlight (such as the trunk) through a proliferative melanocytic pathway, and characterized by atypical nevi and/or high numbers of nevi;
2) another pathway associated with chronic sun exposure: individuals prone to melanoma especially on body sites regularly exposed to the sun, like the face or hairless scalp, due to chronic sun exposure (Whiteman D 2003).

1.8.4.2 Pigmentation features

An inverse relationship exists between melanoma risk and degree of skin pigmentation. Fair-skinned (Fitzpatrick phototype I-II) individuals have risk for CM very much higher than dark-skinned individuals. Vice versa, individuals of non-European descent, who are commonly darker-skinned,

have an up to 10–20-fold less risk for CM than individuals of European descent, who are lighter-skinned (Bataille V 2008).

The skin reaction to the sun is also a predictor of CM risk. Skin that easily develops freckles has a tendency to burn or an inability to tan and therefore an increased propensity for the developing CM (Bressac-de-Paillerets B 2002).

Hair and eye colors seem to be related to CM risk. Specifically, red/blonde hair and blue/green eyes confers higher CM risk compared to non red/blond hair and non blue/green eyes (B. D. Berwick M 2016).

1.8.4.3 Germline Genetic Factors

Although the etiology of CM involves host characteristics and environmental risk factors, the main risk factor is a positive family history (Pho L 2006) (Mayer JE 2014). About 10% of people with CM report having a first/second-degree relative with the same disease: this might be due to the sharing of genetic risk factors or of environmental risk factors, or both, between relatives. A meta-analysis of 22 studies estimated that the relative risk of melanoma in individuals with one or more affected first-degree relatives to be 2.06 (95% CI 1.72-2.45) (NK 2003) (C. H. Olsen CM 2010).

About 45% of these familial melanomas (melanomas occurring in at least two first/second degree relatives of the same branch of a family) have been attributed to inheritance of a mutation in a highly penetrant predisposition gene (Goldstein 2007). The 55% “missing inheritance” might be due to the inheritance of lower-penetrance predisposition genes and with inheritance of polymorphisms and/or shared environmental exposures that predispose to CM, determining a familial pattern of CM inheritance (L. O. Leachman SA 2017).

The concept of “penetrance” refers to the proportion of individuals with a mutated genotype that express the phenotype. If all carriers of the mutated genotype express the phenotype, the penetrance is complete whereas the penetrance is incomplete or reduced if some of the mutated individuals do not express the phenotype (Jameson JL 2005).

1.8.4.4 High risk (and high penetrance) genes mutations

The most significant high-risk melanoma susceptibility gene is cyclin-dependent kinase (CDK) inhibitor 2A (CDKN2A), identified in approximately 40% of melanoma prone families. Mutations in CDK4, another high-risk melanoma susceptibility gene, are rare (Thompson JF 2005) (Goldstein 2007) (Puntervoll HE, Melanoma prone families with CDK4 germline mutation: phenotypic profile and associations with MC1R variants 2013) (Bruno W 2016).

- CDKN2A is a tumor suppressor gene involved in cell cycle control, tumor suppression and melanocyte senescence. It was identified in 1994 as the first high-penetrance melanoma susceptibility gene (Kamb A 1994). The CDKN2A gene on chromosome 9p21 consists of four exons that encode two unrelated proteins in different reading frames arising from alternatively spliced transcripts: p16 (p16INK4A) and p14 (p14ARF). The main tumour suppressor activity of p16INK4A is through inhibition of cyclin-dependent kinases 4 and 6 (CDK4 and CDK6), thus maintaining retinoblastoma protein (RB) in a hypophosphorylated state to prevent cell cycle S-phase entry. p14ARF is a positive regulator of the tumor suppressor gene p53, and therefore a loss of p14ARF allows for accumulation of DNA damage as cells escape the senescence barrier. Autosomal dominant inheritance of germline CDKN2A mutations has been implicated in approximately 20–40% of familial melanoma but the mutation frequency varies between different geographical regions (Goldstein AM 2006).

  Geographically linked founder mutations have been documented, with some occurring as a single predominant mutation based on common ancestry. CDKN2A founder mutations have been found in Sweden and the Netherlands (p.Arg112dup and p16-Leiden, respectively) originating in northern Europe 2000 years ago. In Europe, G101W occurs as a founder mutation in France, Italy and Spain (Goldstein AM 2006). A number of common mutations are shared between Australia and the UK, including M53I, IVS2-105A/G, R24P and L32P, reflecting a shared ancestry from British colonisation of Australia in the late 18th century. Differences in mutation penetrance between regions may reflect a combination of genetics

and environment factors, where family members are predicted to share the same UV exposures as well as a number of other heritable genetic modifiers (Goldstein AM 2006). Considering globally all CM cases in the population, independently from inheritance, only 1-4% of cases carry a germline CDKN2A mutation (Bruno W 2016). The prevalence is much higher when a strong family history of melanoma or multiple primary tumors are present (Harland M 2014) (Bruno W 2016) (M. K. Cust AE 2018). Carriers of a CDKN2A mutation have a substantial lifetime risk of CM: population-based estimates indicate that around 30-50% of the CDKN2A mutation carriers will develop melanoma by age 80 years (H. M. Cust AE 2011). However, the differences in CM incidence and prevalence of CDKN2A mutations among countries are such that there is no single guideline for genetic testing that could be applied worldwide (Bishop DT 2002) (Bruno W 2016). In low melanoma incidence countries, as France and Italy, Leachman et al. proposed that 2 cancer events, including CM and/or pancreatic cancer, either in the patient or in a family member, are criteria enough to identify the patients which would benefit from a genetic counselling and testing (C. J. Leachman SA 2009). The validity of these criteria has been recently confirmed in an Italian study by Bruno W et al. who suggested that Italian patients who developed 2 melanomas, even in situ, should be referred for genetic counselling, even in the absence of family history (Bruno W 2016). Indeed, they found CDKN2A germline mutations in 19% of patients with multiple primary melanomas (MPM) versus 4.4% of patients with single primary melanoma; in familial MPM cases the mutation rate varied from 36.6% to 58.8%, whereas in sporadic MPM cases it varied from 8.2% to 17.6% in patients with 2 and 3 or more melanomas, respectively. The authors explained that the CDKN2A mutation prevalence in the population of the Liguria region might be influenced by the founder effect (Ghiorzo P1 1999) (Ciotti P 2000) but this high prevalence in a defined area could not imply a national predictive value (Mantelli M 2002).

- CDK4 was the second high risk melanoma susceptibility gene, identified in 1996 (Zuo L 2015). CDK4 is an oncogene located within the 12q14 chromosomal region and encodes a

protein that controls cell cycle progression through the G1 phase. To date mutations in this gene have been described in less than 20 melanoma-prone families worldwide and in all of them the mutation affects the same amino acid (Arginine 42) (Puntervoll HE, Melanoma prone families with CDK4 germline mutation: phenotypic profile and associations with MC1R variants 2013) (Read J 2016). This amino acid is located in the p16INK4A binding domain of the CDK4 protein: when CDK4 is mutated, p16INK4A cannot inhibit the CDK4 kinase activity resulting in the progression of the cell cycle (Potrony M 2015).

- Another high risk melanoma susceptibility gene is the breast cancer 1 (BRCA1) associated protein 1 (BAP1) gene. Germline mutations of BAP1 have been associated with a cancer syndrome characterized by the presence of several types of tumor: CM, uveal melanoma, mesothelioma, renal cell carcinoma (RCC), atypical Spitz tumors, atypical intradermal tumors (MBAITs) and basal cell carcinomas (Aoude LG 2013) (Carbone M 2012) (Walpole S 2018). BAP1 has been categorized as a tumor suppressor but it is still not known if it has also other activities. BAP1 has roles in numerous cellular processes, including DNA damage response, cell cycle regulation, cell growth, metabolism and regulation of inflammatory responses. A very recent review of the BAP1 tumor predisposition syndrome by Walpole and colleagues found 181 families carried 140 unique BAP1 germline variants (Walpole S 2018).
- Protection of Telomeres 1 (POT1) is a component of the telomeric shelterin complex that directly binds with high specificity to single-stranded telomeric repeats. It prevents inappropriate processing of exposed chromosome ends by DNA damage response pathways and regulates telomerase function playing a critical role in maintaining telomere integrity and regulating telomere length (Shi J 2014).
- Horn and colleagues identified a germline mutation in the promoter of telomerase reverse transcriptase (TERT) in a melanoma prone family using multipoint linkage analyses and target enriched high-throughput sequencing. TERT is located in 5p15 and encodes the catalytic

subunit of the telomerase, which is the ribonucleoprotein complex that maintains telomere length (Horn S 2013).

1.8.4.5 Intermediate Risk (and medium penetrance) Gene Variants

- The melanocortin-1 receptor (MC1R) gene, encoding the melanocyte stimulating hormone (MSH) receptor, was identified as the first low/medium penetrance gene related to melanoma risk. It is the main gene that determines the skin and hair colours, although it can act via pigmentary and non-pigmentary pathways to influence melanoma development. There are many common MC1R variants, but only six of them are referred as "red hair colour (RHC) phenotype" or "R" variants: these RHC variants are associated with red hair, fair skin, freckling, poor sun sensitivity and with a greater-than-twofold increased melanoma risk. The other MC1R variants (usually referred as "r" or "non-RHC") have a relatively weak association with red hair color phenotype and also a weaker association with melanoma risk (Raimondi S 2008) (Williams PF 2011) (Tagliabue E 2018). Although each variant individually is associated with a small increase in risk of melanoma, some people carry more than one variant and the combined effect can be large (e.g., more than fourfold increased risk of melanoma for people carrying 2 "R" alleles compared to wild-type alleles) (G. C. Cust AE 2012).
- More recently, in 2011, the p.E318K variant of the microphthalmia-associated transcription factor (MITF), was identified as an intermediate-penetrance melanoma susceptibility gene in individuals affected by melanoma and kidney cancer through a candidate gene approach and whole-genome sequencing of melanoma-prone families (Bertolotto C 2011) (Yokoyama S 2011). MITF encodes a member of the Myc supergene family of basic helix–loop–helix zipper transcription factors. It regulates several genes functions of the melanocytes: development, differentiation, survival, cell cycle regulation, and pigment production and is also critical in controlling proliferation, migration and invasion of melanoma cells (Koludrovic D 2013). In addition, MITF acts not only as a

master transcription factor, involved in cell cycle regulation, but also as a transcriptional repressor (Soura E 2016) (Figure 1).

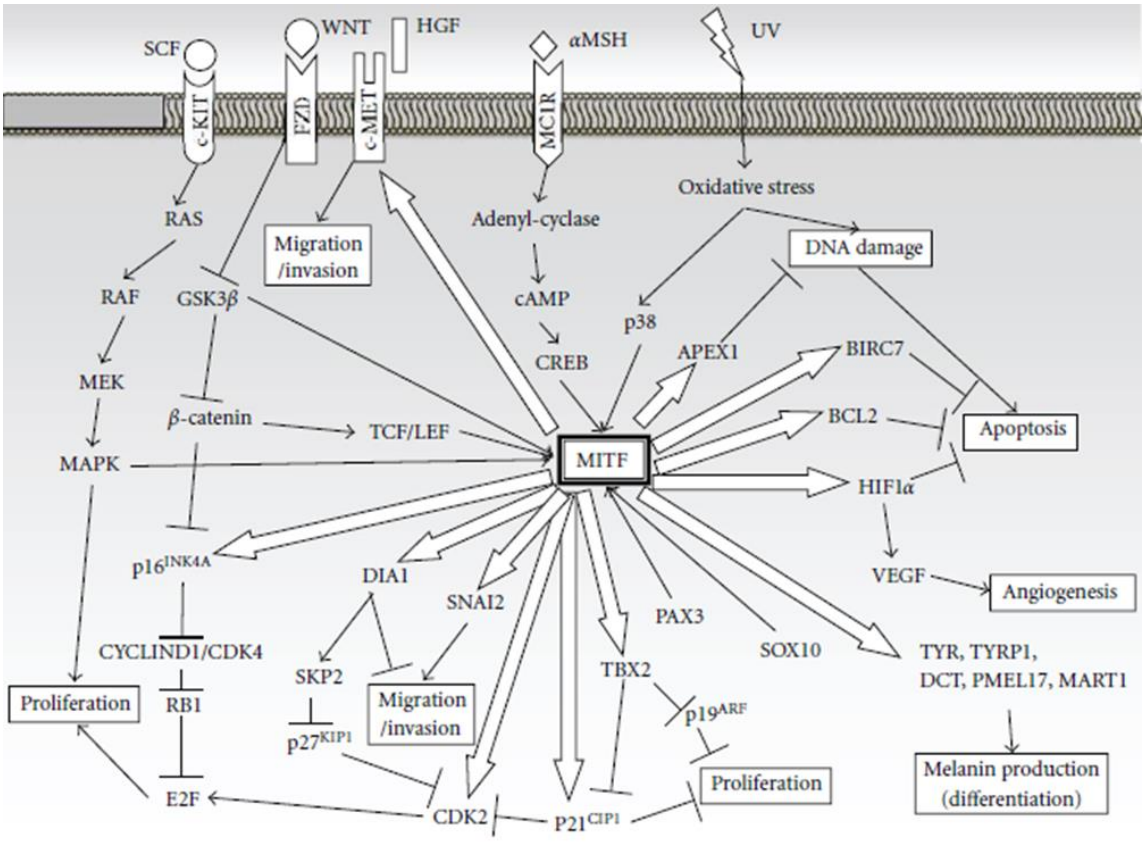


Figure 1. MITF is a protein involved in many biological processes including the development and differentiation of melanocytes to define the skin pigmentation.

Specifically, the M-isoform of MITF, expressed in melanocytes, regulates expression of a large set of genes promoting proliferation and invasion controlling the expression of MET5 and CDKN2A/p16INK4A5, which have key roles in melanoma development (Bertolotto C 2011) (Yokoyama S 2011) (Figure 2).

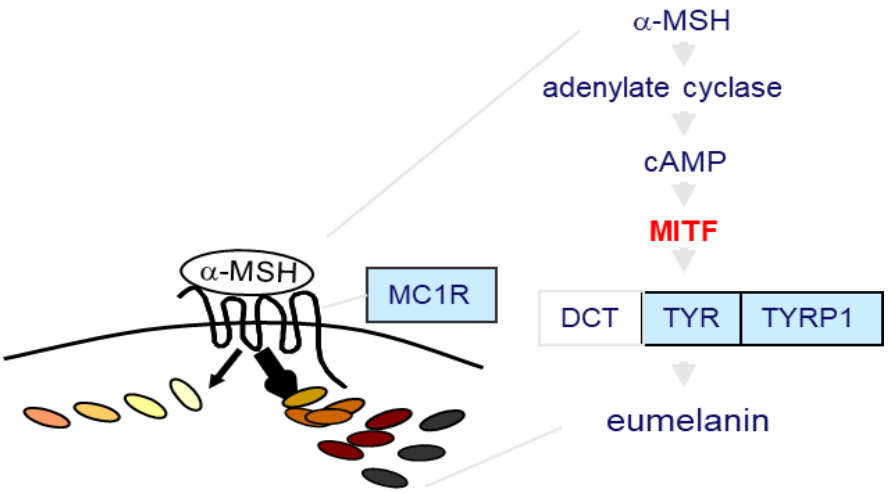


Figure 2. UV exposure of the epidermis promotes α-MSH release from pituitary gland, which then binds to the melanocortin-1 receptor (MC1R), a transmembrane G-protein-coupled receptor located on the cell membrane of melanocytes. This binding culminates in the activation of the M-isoform of MITF (MITF-M), expressed in melanocytes through a cAMP mediated signaling pathway. The activation of MITF, in turn, induces the transcription of pigmentation-related genes, which produce eumelanin that protects cells from UV damage.

The A-isoform of MITF (expressed in kidney) plays some roles in renal cell transformation that have yet to be elucidated (Bertolotto C 2011).

The p.E318K variant alters the SUMOylation of MITF thus impairing MITF inhibitory activity (M 2012) (Bertolotto C 2011) (Figure 3).

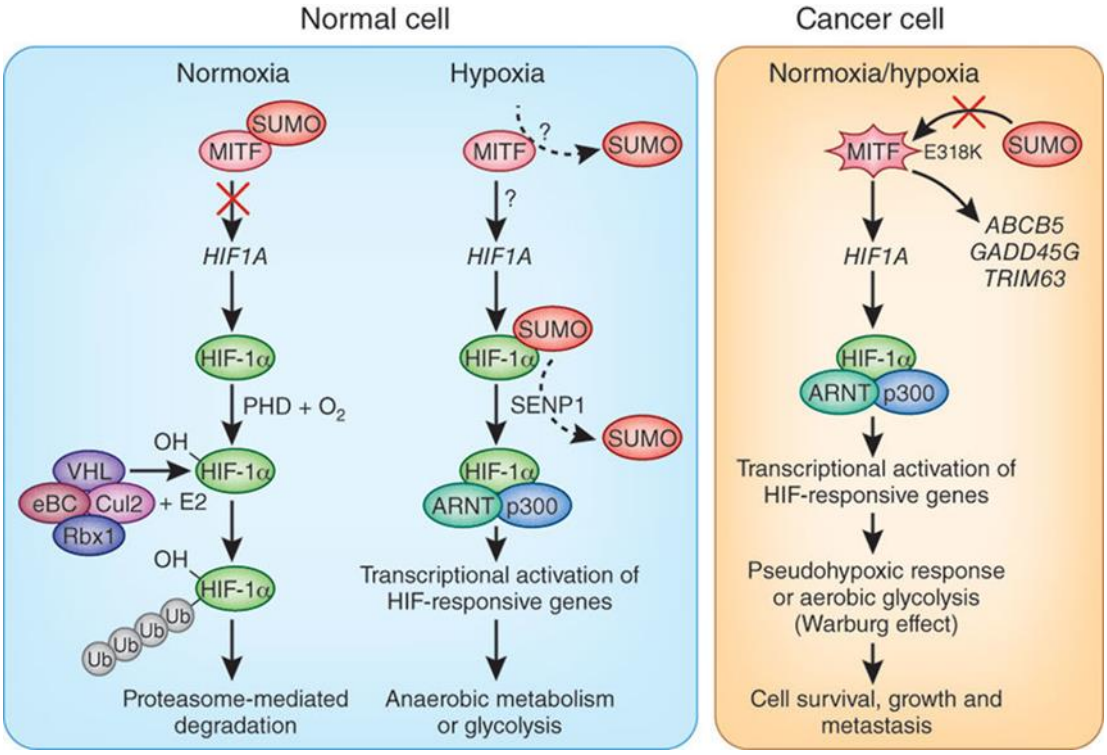


Figure 3. Novel E318K substitution in MITF occurs in SUMO consensus site. Mutation disrupts sumoylation, loss of sumoylation enhances transcription of some but not all MITF responsive genes

The patients carrier of p.E318K variant of MITF have a more than fivefold increased risk of developing melanoma (both familial and sporadic), renal cell carcinoma (RCC) or both cancers than non-carriers. The p.E318K variant has also been associated with increased nevus count, non-blue eye color and developing of multiple primary melanoma (Bertolotto C 2011) (Yokoyama S 2011). Interestingly, Berwick et al. reported that variations in MC1R and MITF are more strongly associated with melanoma in people with darker phenotypic traits than those with fairer complexions and that risk of melanoma among carriers with "low-risk" phenotypes was as great or greater than among those with at-risk phenotypes (M. J. Berwick M 2014).

1.8.4.6 Low risk (and low penetrance) Gene Variants

Since 2008, genome-wide association studies (GWAS) improved the knowledge of melanoma genetics allowing the discovery of low-penetrance melanoma susceptibility genes on the basis of large, often unselected, case-control studies (Barrett JH 2011) (D. F. Bishop DT 2009) (Manolio TA 2009).

GWAS have identified several variants in genes related to pigmentation and associated with melanoma risk: MC1R, TYR, ASIP, SLC45A2, IRF4, and TYRP1. Risk variants for melanoma also lie in or near MTAP, PLA2G6, and IRF4, TERT/CLPTM1L, loci that are associated with nevus count variation. GWAS identified also susceptibility genes that do not act via pigmentation pathways but are involved in other cellular processes as DNA repair and cell cycle control, including genes in or near ATM, CASP8, CCND1, MX2 (Amos CI 2011) (Figure 4). Each of these low-penetrance variant increases only slightly the risk of melanoma; however, carrying several variants together can significantly increase this risk, which can be further modified by environmental factors, for example by UV exposure (B. D. Berwick M 2016).

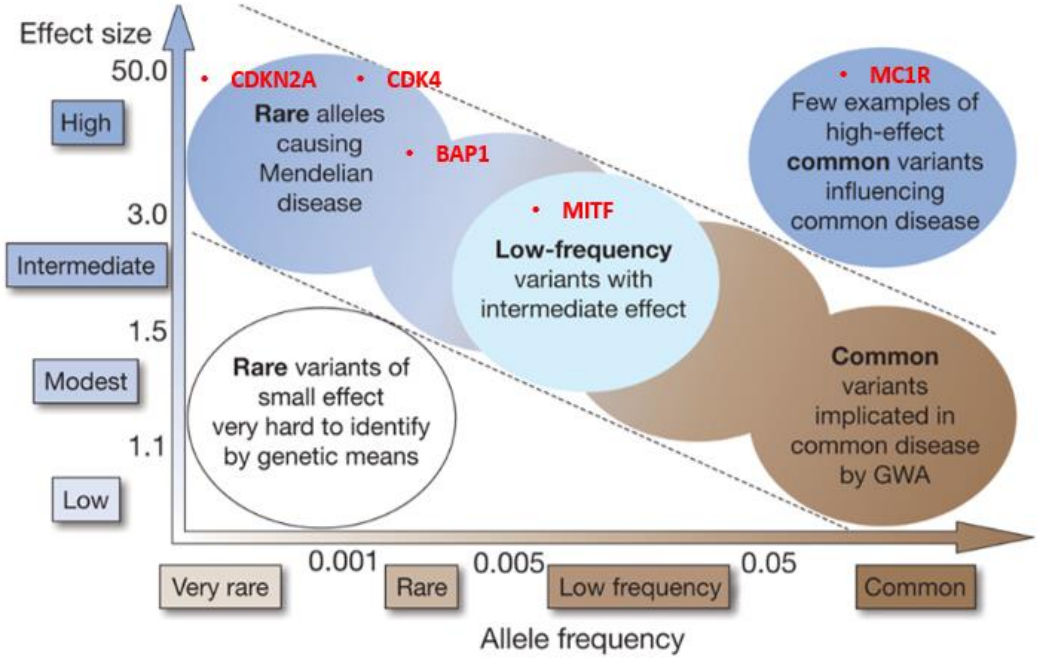


Figure 4. Feasibility of identifying genetic variants by risk allele frequency and strength of genetic effect (odds ratio) modified from Manolio TA et al. 2009 (Manolio TA 2009)

1.9 Somatic genetic features of melanoma

Genetic analyses in melanoma are based also on tumor, or somatic, alterations. Melanoma is a heterogeneous disease with a variety of histologic subtypes, as previously described.

- NRAS and BRAF mutations, mutually exclusive of each other, are found, respectively, in 10–30 and 25–60 % of primary melanomas. BRAF-mutant melanomas are associated with young age at diagnosis, intermittently sun-exposed sites (for example the trunk), SSM subtype, absence of solar elastosis and presence of mitoses. BRAF-mutant melanomas are more common in patients with high number of nevi and with the presence of nevi adjacent to the melanomas. Indeed, approximately 70% of nevi contain BRAF mutations. NRAS-mutant melanomas are associated with older age at diagnosis than BRAF-mutant melanomas and are not associated with a specific anatomic region; moreover, NRAS-positive melanomas are frequently of the nodular subtype and show increased Breslow thickness than BRAF-melanomas and presence of mitoses (B. D. Berwick M 2016).
- RAC1-mutant melanomas are more common in older men on the head and neck, while TERT promoter mutations in melanomas are associated with older age, increased Breslow thickness, nodular subtype and ulceration (B. D. Berwick M 2016).
- Less frequently, melanomas contain KIT mutations, particularly mucosal melanoma or melanomas of acral or sun-damaged sites. GNAQ and GNA11 mutations were discovered in uveal and central nervous system melanomas. In addition, frequently, melanomas also contain PTEN, CDKN2A, CDK4, and CCND1 copy number alterations. Newer sequencing methods for tumors have allowed studies to identify several additional somatic mutations in melanoma as NF1 and RAC1 mutations and BRAF gene fusions. It was also discovered that 30–40 % of melanomas harbour mutations in the promoter region of the telomerase reverse transcriptase (TERT) gene, and these mutations occur more frequently in BRAF-mutant melanomas (B. D. Berwick M 2016).

1.10 Melanoma survival

Among ecological studies investigating mortality and survival after melanoma diagnosis, there are conflicting results. Indeed, Lachiewicz et al. have found no association between latitude or other measures of UV exposure and mortality from melanoma (Lachiewicz AM 2008) while other studies have reported a positive association between increasing latitude (and so decreasing UV) and increasing melanoma mortality rates (Shipman AR 2011). Also the analytic studies evaluating mortality in relationship to solar exposure prior to melanoma diagnosis have mixed results. Berwick et al. showed an inverse association between solar exposure and melanoma mortality: this finding might indicate a beneficial effect of sun exposure in relationship to survival with melanoma mediated by vitamin D produced by sun exposure (A. B.-P. Berwick M 2005). Another study from the UK measured serum vitamin D at diagnosis and found that those with the highest level of serum vitamin D had the best survival (Newton-Bishop JA 2009).

A recent Swedish study showed that CDKN2A germline mutatione positive patients with familial melanoma had worse prognosis in terms of survival than CDKN2A germline mutatione negative patients and untested patients with sporadic melanoma (Helgadottir H 2016). Similarly, CDKN2A mutations were associated with worse survival in another study involving Swedish individuals affected by multiple primary melanomas (T. R. Helgadottir H 2017).

We therefore investigated whether poor survival associated with CDKN2A germline mutations was confirmed also in a high mutation prevalence cohort of Italian patients with melanoma and we found no difference in overall survival or melanoma-specific survival between CDKN2A mutated and CDKN2A not-mutated patients. To explain this finding we hypothesized that the intensive clinical surveillance of our CDKN2A mutated patient might have modulated survival, possibly by counteracting negative effects on survival owing to the mutation itself. However, our CDKN2A mutated patients were more likely to develop multiple melanomas and to undergo surgical excision of dysplastic nevi than not mutated patients (Dalmasso B 2018).

2. Aims of the research

Due to the above-mentioned link between p.E318k MITF germline variant and melanoma and kidney cancer susceptibility, current research is focusing on the relationship between this variant and the clinical-phenotypical features of the carrier patients (M. J. Berwick M 2014) (Bassoli S 2018) (Ghiorzo P 2013) (P.-B. J. Potrony M 2016) (Soura E 2016) (Stoehr CG 2016) (Sturm RA 2014).

However, data on the dermoscopic features of nevi and melanomas in these patients is still limited (Bassoli S 2018) (P.-B. J. Potrony M 2016) (Sturm RA 2014) and predisposition to other cancers needs to be further investigated.

The aim of the present work was to retrospectively study the genotype-phenotype correlations in melanoma patients carrier of the p.E318K MITF germline variant (called MITF+ patients), compared with non-carrier melanoma patients (MITF-).

Among the phenotypic features that we analysed, dermoscopic findings of histopathologically diagnosed dysplastic nevi (DN) and cutaneous melanomas in MITF+ and MITF- patients were included.

3. Patients and methods

3.1 Selection of patients

Between 2000 and 2017 a consecutive series of 1386 patients were recruited at the Genetics of Rare Cancers Unit, Department of Internal Medicine and Medical Specialties (Di.M.I.), University of Genoa. This cohort included probands of melanoma-prone families and apparently sporadic patients diagnosed with multiple primary melanomas who underwent genetic testing for diagnostic or research purposes. Also sporadic patients with melanoma, tested for research purposes only, were included. All patients, except one already characterized for germline status, were subjected to genetic testing for CDKN2A, CDK4, MC1R and MITF germline variants in our laboratory. For all patients, we collected and stored clinical and pathological information. In addition, when available, we collected dermoscopic images of the histopathologically diagnosed DN and cutaneous melanomas. Indeed, DN cannot be defined by clinical features but precise histological criteria must be satysfied: the diagnosis requires both of the 2 major criteria (proliferation of atypical melanocytes extending beyond the dermal component; atypical melanocytes arranged in a lentiginous/epithelioid-cell pattern) and at least 2 minor criteria (lamellar/eosinophilic fibrosis; neovascularization; inflammatory response; fusion of rete ridges) (Rosendahl CO 2015).

For 667 of the patients included in this study, molecular and, partly, clinical informations have been previously described (Ghiorzo P 2013).

All recruited patients signed a written informed consent according to the protocol approved by the local ethics committee.

From the melanoma cohort, we excluded all patients lacking information on germline status and stage and those with ocular and mucosal melanomas. In addition, to avoid confounding effects by CDKN2A and CDK4, patients with concurrent CDKN2A and CDK4 pathogenic variants were also excluded from this study. Subsequently, we gathered MITF+ patients and MITF- patients into two separate study groups. Patients selection workflow is detailed in Figure 5.

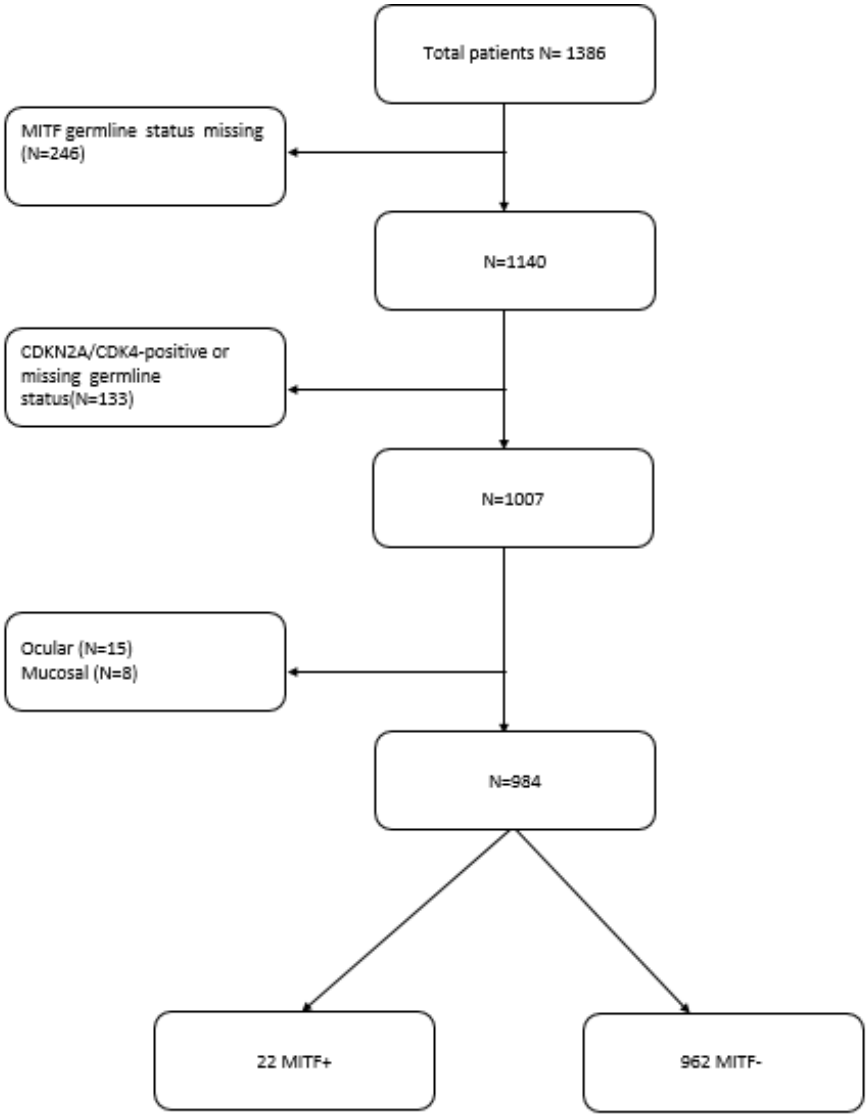


Figure 5. Patients selection workflow.

3.2 Collection of clinical, pathological and dermoscopic data

Clinical informations were collected through a questionnaire, administered by a trained interviewer, and included phenotype, as well as personal and family history of melanomas and other tumors, as previously described (Bruno W 2016) (B. L. Ghiorzo P 2012) (Stratigos AJ 2018). Either clinical records or local cancer registry data were used to collect pathological information, including tumor histological type and staging according to the American Joint Committee on Cancer (AJCC)'s TNM staging system (Gershenwald JE 2017). For both MITF+ and MITF- patients, the following

phenotypical and clinico-pathological features were studied: phototype, freckles, hair and eye color, total number of nevi with diameter >2 mm, number of histopathologically diagnosed DN and number of histopathologically diagnosed cutaneous melanomas; concerning the features of the first diagnosed melanoma, we analyzed age at diagnosis, anatomical site, histotype, Breslow thickness (mm), sentinel lymph node and stage.

Dermoscopic images of those skin lesions that appeared clinically suggestive of melanomas were collected through the FotoFinder dermoscope Medicam 1000 (FotoFinder Systems GmbH, Bavaria, Germany) either during dermatologic visits performed for screening (first visit) or during follow-up visits at the Dermatologic Clinic of the San Martino Hospital (Genoa, Italy) and at the Dermatologic outpatient clinic, Division of Oncology, Centro di Riferimento Oncologico (CRO), Aviano National Cancer Institute (Aviano, Italy) and revised by two dermatologists (G.C. and M.A.P.).

The analysis of global dermoscopic pattern was retrospectively performed on all available dermoscopic images according to the dermoscopic classification of acquired melanocytic nevi, as follows: reticular, globular, homogenous, multicomponent, reticular-globular, reticular-homogenous, globular-homogenous and unspecific pattern. This latter was defined as a pattern lacking specific features related with a melanocytic or non melanocytic lesions. Conversely, the following dermoscopic patterns were considered to assess acral melanocytic lesions: parallel furrow, parallel ridge, lattice-like, fibrillary (S. H. Argenziano G 2003) (Z. I. Argenziano G 2007).

3.3 Molecular Analysis

All patients provided a blood sample from which we extracted genomic DNA. Purified DNA samples were then amplified by conventional polymerase chain reaction (PCR) and analyzed by Sanger sequencing to assess the germline status of CDKN2A, CDK4, MC1R and MITF. Samples processing and analysis were performed as previously described (B. L. Ghiorzo P 2012).

3.4 Statistical analysis

To assess the difference of a numerical variable between the two study groups of MITF+ and MITF- patients (age at diagnosis, Breslow thickness, number of melanomas diagnosed, total number of nevi, number of dysplastic nevi) we used the Mann-Whitney U test.

To assess the association between MITF+ germline status and a categorical variable (hair and eye color, familial status, sentinel node status, familiarity for pancreatic and kidney cancer, site of first melanoma, histotype of first melanoma, dermoscopic pattern of DN and melanomas grouped together, MC1R germline status, histologically diagnosed melanomas and DN analyzed as a dichotomous variable), we used the Fisher's exact test.

Kruskal-Wallis test was used to analyze the association between MITF germline status and an ordinal variable (phototype, freckles, tumor stage, number of nevi grouped in three categories).

4. Results

After excluding 246 patients with missing information on MITF mutational status, 134 patients either positive for CDKN2A/CDK4 pathogenic variants or with missing information on CDKN2A/CDK4 germline status, and 23 patients affected by ocular or mucosal melanomas, our study cohort comprised 984 cutaneous melanoma patients, 22 MITF+ and 962 MITF- (Figure 2). Of the 22 MITF+ patients, 5 had a positive family history of melanoma whereas the remaining 17 were apparently sporadic cases. Overall, 6 patients, all apparently sporadic cases, developed multiple melanomas. Even though the overall prevalence of the MITF p.E318K variant was 2.2% (22 of 984), MITF p.E318K was more common among multiple primary melanoma patients (MPM) (5% as compared to 2% in single melanoma patients). All MPM MITF+ patients were sporadic, whereas among single melanoma patients MITF p.E318K rate was similar in familial and sporadic subgroups, as shown in Table 5. The distribution of MC1R variants did not significantly differ between the two study groups (p=0.45) (Table 6). In the MITF+ group, two patients had amelanotic/hypomelanotic melanomas; both patients carried one red-hair-color (RHC) MC1R variant (R169W, R142H).

		***MITF+* (N)**	**%**	***MITF-* (N)**	**%**	
SPM	**Fam**	5	1	114	13	
	Spo	11	1	742	85	
	Total	16	2	856	98	872
MPM	**Fam**	0	0	16	15	
	Spo	6	5	90	80	
	Total	6	5	106	95	112
		22		962		984

Table 6. Rate of MITF+ patients among single and multiple primary melanoma cases and among sporadic and familiar cases. Abbreviations. SPM= single primary melanoma. MPM= multiple primary melanoma. N= number of patients. %= percentage of patients. Fam= familial. Spo=sporadic.

4.1 Clinico-pathological features

The two study groups displayed significant differences with regards to: total number of nevi, number of histopathologically diagnosed DN and melanomas, histotype of first melanoma and family history of kidney cancer (Table 2). More specifically, MITF+ patients had, in median, a higher total number

of nevi (Figure 6) compared to MITF- patients: 28% of MITF+ patients had more than 50 nevi, compared to 19% of MITF- patients (p= 0.04).

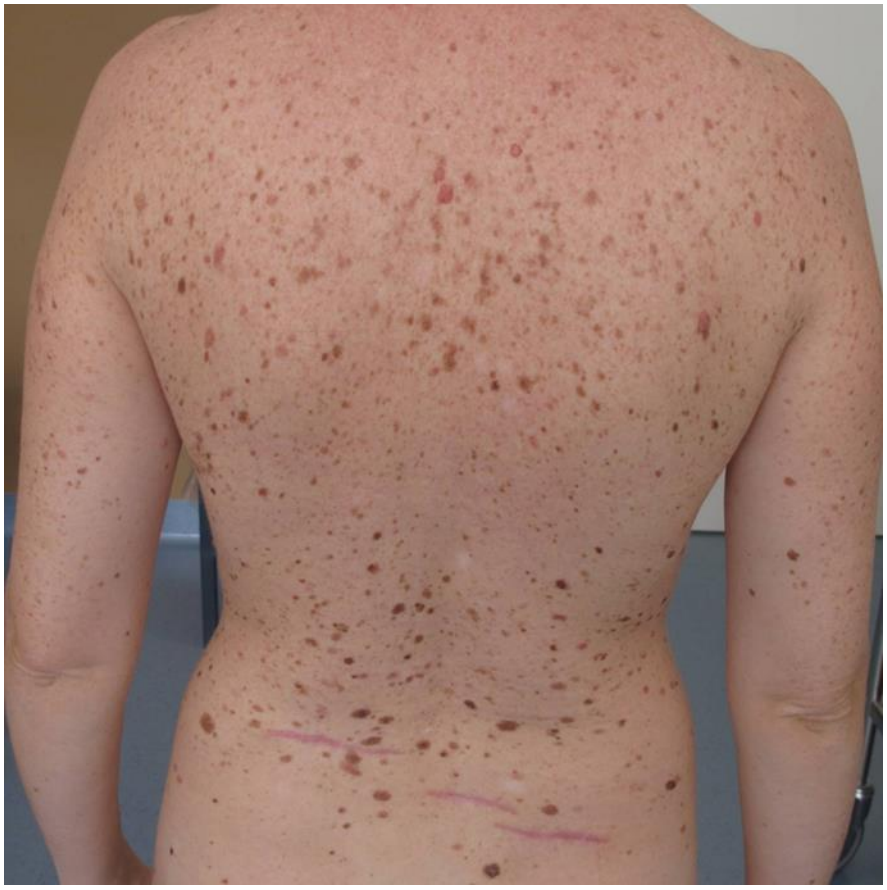


Figure 6. Clinical image of the back of a 33-year-old female MITF+ CDKN2A- patient at a follow-up visit showing that she had a high number of melanocytic nevi (> 100). The patient had a negative family history for melanoma and other cancers; at the time of the visit she has already removed 10 melanomas and 11 dysplastic nevi.

Patients with at least one histologically diagnosed DN were more frequent in the MITF+ group, (50% vs 10% in the MITF- group, p <.001) with a higher median DN removal compared to MITF- (median=0.5, IQR=0-1 and median=0, IQR=0-0, respectively; p <0.001).

Concerning the histotype of first diagnosed melanoma, MITF+ patients showed a higher rate of nodular melanomas than MITF- patients (32% and 16%, respectively, p= 0.04).

Patients with MPM were more frequently MITF+ (27% compared to 11% of MITF- patients, p= 0.03). A positive family history for kidney cancer was more frequent among MITF+ patients (18% versus 4% of MITF- patients; p= 0.01).

We also compared phenotypical features between the MITF+ and MITF- patients, and no significant differences were found as regards as phototype, hair and eyes color, freckles, age at first melanoma diagnosis, anatomical site, Breslow thickness, sentinel lymph node, stage of first melanoma and family history of melanoma or pancreatic cancer (Table 7).

Features	**N**		***MITF+*** **N (%)**	***MITF-*** **N (%)**	**OR**	**Lower CI**	**Upper CI**	**p-value**
Phototype	927	I	0 (0)	52 (6)				0.27
		II	15 (75)	478 (53)				
		III	5 (25)	353 (39)				
		IV	0 (0)	24 (3)				
Freckles	349	None	8 (4)	77 (23)				0.37
		Rare	4 (2)	126 (38)				
		Few	7 (35)	89 (27)				
		Many	1 (5)	37 (11)				
Hair color	932	albino	0 (0)	1 (0)				0.06
		red	1 (5)	49 (5)				
		blond	9 (43)	201 (22)				
		blond_red	0 (0)	9 (1)				
		brown	7 (33)	565 (62)				
		black	4 (19)	86 (9)				
Eye color	931	light blue	7 (37)	214 (23)				0.50
		blue	1 (5)	38 (4)				
		green	2 (11)	98 (11)				
		grey	0 (0)	21 (2)				

		light brown	1 (5)	206 (23)				
		dark brown	8 (42)	324 (36)				
		black	0 (0)	5 (1)				
		hazel	0 (0)	6 (1)				
Number of nevi	492	<10	3 (17)	200 (42)				**0.04**
		10-50	10 (55)	185 (39)				
		>50	5 (28)	89 (19)				
Histologically diagnosed Dysplastic nevi	866	median (IQR)	0 (0-1)	0 (0-0)				**<0.001**
		0	10 (50)	769 (90)	0.1	0.04	0.28	**<0.001**
		1+	10 (50)	77 (10)				
Familial	984	Spo	17 (77)	833 (87)	0.53	0.18	1.86	0.21
		Fam	5 (23)	129 (13)				
Age at first melanoma	858	median (IQR)	44 (32.25-58.75)	49 (38.99-61.71)				0.20
N. of melanomas removed	983	median (IQR)	1 (1-1.75)	1 (1-1)				**0.02**
		1	16 (73)	855 (89)	0.33	0.12	1.06	**0.03**
		2+	6 (27)	106 (11)				

Breslow mm	930	median (IQR)	1 (0.6-2.025)	1 (0.35-1.765)				0.22
Sentinel node	337	Neg	6 (27)	266 (28)	0.71	0.12	7.37	0.65
		Pos	2 (9)	63 (7)				
Stage	771	IS	2 (17)	122 (16)				0.65
		I	8 (67)	473 (62)				
		II	2 (17)	75 (10)				
		III	0 (0)	56 (7)				
		IV	0 (0)	33 (4)				
Pancreatic cancer in family	972	No	19 (86)	901 (94)	0.35	0.1	1.88	0.11
		Yes	3 (14)	49 (5)				
Kidney cancer in family	971	No	18 (82)	910 (95)	0.19	0.06	0.82	**0.01**
		Yes	4 (18)	39 (5)				
Site of first melanoma	943	head and neck	1 (5)	74 (8)				0.27
		trunk	8 (40)	464 (50)				
		arms	6 (30)	125 (14)				
		legs	5 (25)	260 (28)				
Histotype of first melanoma	722	Acral	2 (9)	11 (2)				**0.04**

		Lentigo maligna	0 (0)	36 (5)				
		Nodular	7 (32)	132 (19)				
		SSM	13 (59)	454 (64)				
		other	0 (0)	67 (10)				
MC1R	576	-/-	6 (30)	165 (30)				0.45
		r/-	4 (20)	158 (28)				
		r/r	2 (10)	24 (4)				
		R/-	6 (30)	111 (21)				
		R/r	1 (5)	74 (13)				
		R/R	1 (5)	24 (4)				

Table 7. Clinical, pathological and molecular characteristics of the study groups.

Abbreviations. N= number of patients, %= percentage of patients, OR=Odds Ratio, Lower CI= lower confidence interval limit, Upper CI= upper confidence interval limit, IQR= inter-quartile range, Spo= sporadic, Fam= familial, Neg= negative, Pos= positive, SSM= superficial spreading melanoma. IS= in situ melanoma, R= MC1R red hair color variant, r= MC1R non-red hair color variant.

4.2 Dermoscopic features

The dermoscopic patterns of 23 lesions (including DN and melanomas) belonging to four MITF+ patients were compared with those of 47 lesions (DN and melanomas) belonging to 37 MITF- patients (Table 8).

	MITF+		***MITF-***		**p-value**
Dermoscopic pattern	**N**	**%**	**N**	**%**	
Unspecific	6	26	4	8	**<0.001**
Reticular	1	4	1	2	
Globular	0	0	3	6	
Homogeneous	2	9	0	0	
Globular-homogenous	5	22	2	5	
Reticular-homogenous	6	26	3	6	
Reticular-globular	0	0	12	26	
Multicomponent	2	9	22	47	
Parallel ridges (or other patterns typical of acral melanoma)	1	4	0	0	
Total	23	100	47	100	

Table 8. Dermoscopic patterns of MITF+ and MITF- dysplastic nevi and cutaneous melanomas.

Abbreviations. N= number of dysplastic nevi/melanomas; %= percentage of dysplastic nevi/melanomas.

When evaluating melanoma dermoscopies only, nine lesions belonging to three MITF+ were compared with those of 23 lesions belonging to 22 MITF- (Table 9).

	MITF+		***MITF-***	
Dermoscopic pattern	**N**	**%**	**N**	**%**
Unspecific	4	45	3	13
Reticular	1	11	0	0
Globular	0	0	2	9
Homogeneous	0	0	0	0
Globular-homogenous	1	11	1	4
Reticular-homogenous	0	0	0	0
Reticular-globular	0	0	2	9
Multicomponent	2	22	15	65
Parallel ridges (or other patterns typical of acral melanoma)	1	11	0	0
Total	9	100	23	100

Table 9. Dermoscopic patterns of MITF+ and MITF- cutaneous melanomas.

Abbreviations. N= number of melanomas; %= percentage of melanomas

Of the 23 dermoscopic images from the four MITF+ patients, seven melanomas (four with unspecific, two with multicomponent and one with globular-homogenous pattern) and ten DN (two with homogenous, four with reticular-homogenous and four with globular-homogenous pattern) belonged to one single patients. This patient actually developed 10 melanomas, only 7 of which had dermoscopic images.

When we analysed the global patterns of DN and melanomas, grouped together as a single variable, the unspecific, globular-homogenous and reticular-homogenous patterns were more frequent in MITF+ compared to MITF- patients; conversely, the multicomponent pattern was more common in MITF- than in MITF+ patients, as shown in Table 8 ($p < 0.001$).

We could not perform the same analysis only including melanoma dermoscopies because of small sample size. However, as regard to melanomas, the frequency of global dermoscopic patterns among the two study groups is reported in Table 9. The unspecific pattern (Figure 7 A, B) was found more frequently in melanomas of MITF+ (44% of the lesions) than in those of MITF- patients (14% of the lesions), while the multicomponent pattern (Figure 7 C, D) was seen more frequently among melanomas of MITF- (65% of the lesions) than those of MITF+ patients (22%).

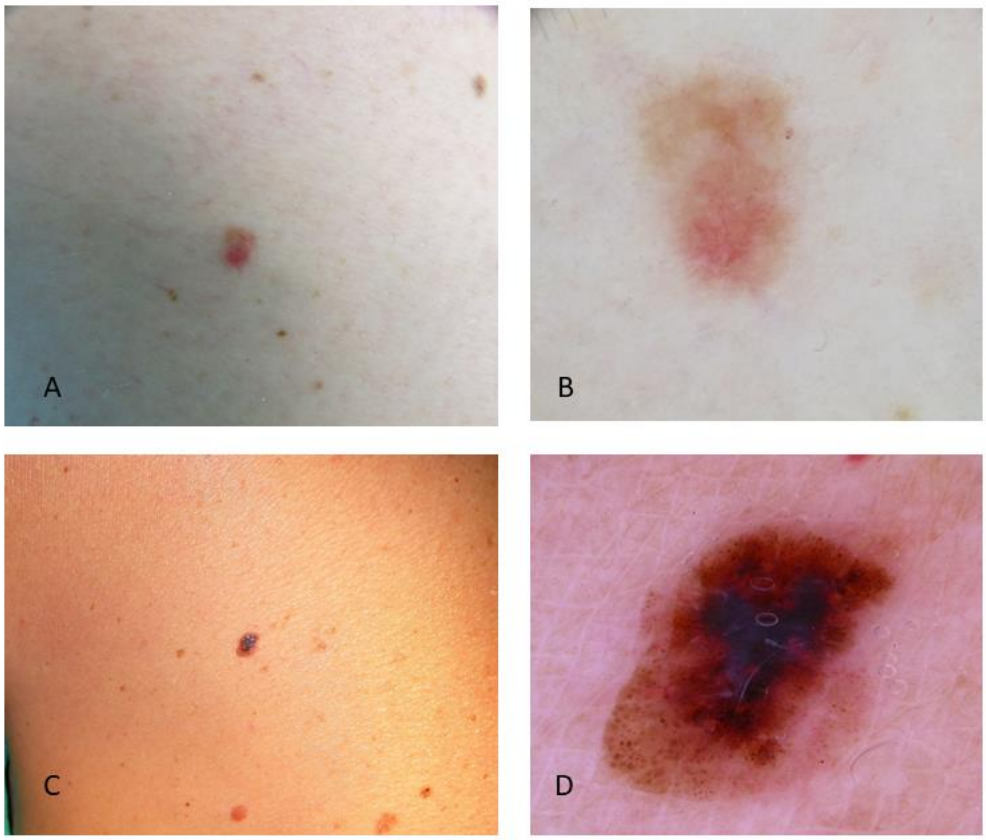


Figure 7. A) Clinical and B) dermoscopic images of a superficial spreading melanoma of the right thigh with an unspecific dermoscopic pattern in a MITF+ patient; this patient also carries one RHC variant (R142H) of MC1R that could be responsible for the hypomelanotic aspect of this lesion; C) Clinical and D) dermoscopic images of a superficial spreading melanoma of the left shoulder with a multicomponent pattern in a MITF- patient.

Taking into account that a considerable number of melanocitic lesions belonged to the same patient, we performed again the analysis excluding this outlier patient, to reduce the risk that such a relevant number of non-independent samples could bias our results. Even without the outlier patient,

melanocitic lesions in patients MITF+ and MITF- showed a different distribution of dermoscopic patterns (p=0.001). Namely, the unspecific was the most frequent dermoscopic pattern found in DN/melanomas of MITF+ patients (40%, as opposed to 9% in MITF- lesions). Conversely, the multicomponent and the reticular-globular patterns (47% and 28% respectively in MITF- lesions) were absent in MITF+ lesions.

5. Discussion

In our study cohort, the prevalence of the p.E318K germline variant in CDKN2A/CDK4-negative patients was 2.2%, slightly more than previously reported by the Italian group of Ghiorzo et al. in a smaller series of melanoma patients (1.8%) (P. L. Ghiorzo P 2013), but in line with Spanish (1.9%) (P.-B. J. Potrony M 2016), French (2.8%) (Bertolotto C 2011), Australian (3.4-3.6%) (Sturm RA 2014) and American (2.8%) (M. J. Berwick M 2014) studies.

Considering that the p.E318K variant is not common in melanoma patients, attempts to determine its effects on MITF+ patients' phenotypical features and cancer predisposition are generally limited by sample size. To our best knowledge, the present study describes the largest cohort of MITF+ patients reported to date from a dermoscopic (DN and melanomas), in addition to a genetic, clinical, and pathological perspective.

Concerning the histotype of the first diagnosed melanoma, we validated the association between p.E318K variant and the nodular subtype previously reported by Ghiorzo et al. (P. L. Ghiorzo P 2013). Indeed, seven out of 22 p.E318K patients (32%) developed a first melanoma with nodular histotype, a significantly higher percentage than the one observed in MITF- patients (16%). Our results differ from previous studies by other groups, which did not find significant associations of p.E318K with pathological features, possibly due to the underpowered study samples (P.-B. J. Potrony M 2016) (Sturm RA 2014).

However, Potrony M. and collegues reported that during 10 years of dermatological surveillance of patients at high risk of melanoma, the only two fast-growing melanomas (growth rate greater than

0.4 mm per month) were diagnosed in MITF+ patients. Of these two lesions, one was a nodular melanoma and the other one was a superficial spreading melanoma (SSM) (P.-B. J. Potrony M 2016). However, in our MITF+ study group, all nodular melanomas were first diagnosed melanomas, identified during the dermatological screening with digital follow-up or clinical examination. Conversely, all subsequent melanomas diagnosed in our MITF+ cohort during dermatological follow-up were SSM, and Breslow thickness of melanomas in patients with multiple primary melanoma was always lower than that of the preceding ones, except for one patient, possibly reflecting the intensive dermatological follow-up after the first melanoma diagnosis. However, further investigations with larger series of patients are needed to confirm the association between the p.E318K variant and nodular-type melanoma and to study the prognostic role of this variant.

Concerning the role of the p.E318K variant in the predisposition to tumors other than melanoma, we confirm the association with renal cell carcinoma (RCC) that was previously described (Bertolotto C 2011) (P. L. Ghiorzo P 2013) (P.-B. J. Potrony M 2016) (Stoehr CG 2016) (Yokoyama S 2011). The association with pancreatic cancer that was previously observed in a smaller series of Italian patients (P. L. Ghiorzo P 2013) was not confirmed here and therefore remains to be further explored. Although none of our p.E318K patients developed RCC, 18% of them reported a positive family history, as opposed to 4% of MITF- patients. Apart from melanoma, the most frequent tumor in MITF+ patients was the basal cell carcinoma (14% of the patients), in line with previous data reported by Potrony M. et al. (P.-B. J. Potrony M 2016).

The finding that MITF+ p.E318K was associated with a higher number of histologically confirmed DN in our cohort was never reported to date, differently from CDKN2A variants, whose possible role in influencing the development of dysplastic melanocytic lesions has already been described (Goldstein 2007) (Liang X 2014).

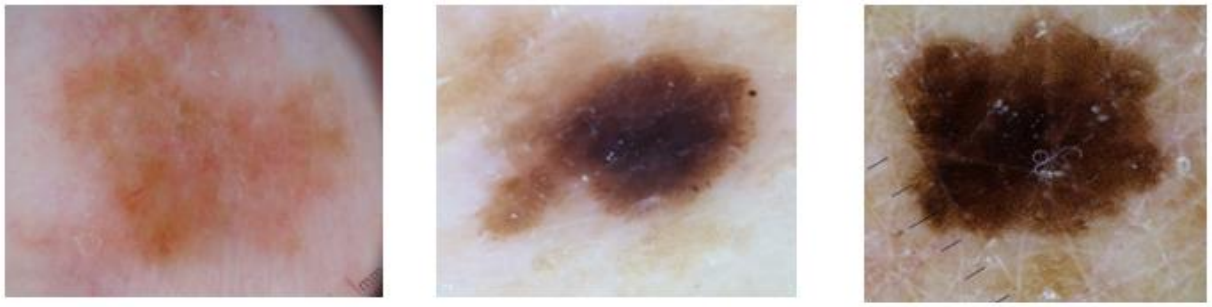
Our study confirms that MITF+ patients have an increased risk of developing multiple melanomas and show a higher total nevi count compared to controls, as previously reported (Bertolotto C 2011) (P. L. Ghiorzo P 2013) (P.-B. J. Potrony M 2016) (Yokoyama S 2011). Indeed, 28% of our MITF+

patients had more than 50 nevi with >2 mm diameter compared to 19% of MITF- patients. Previous studies also reported high nevi counts in MITF+ patients corroborating the hypothesis that MITF is involved in nevogenesis (Bassoli S 2018) (P.-B. J. Potrony M 2016) (Sturm RA 2014).

Of course, as MITF E318K is considered a medium-penetrance allele, the possibility that other additional gene's effects may have affected our results cannot be completely ruled out. However, patients with CDKN2A pathogenic variants were excluded from this study, in order to avoid a confounding effect by this gene. Moreover, MC1R variants, which influence phototype and are associated with melanoma risk (B. L. Ghiorzo P 2012) (Tagliabue E 2018), had a similar distribution in the two study groups, therefore not affecting our analyses. MC1R RHC variants have also been associated with the likelihood of developing amelanotic/hypomelanotic melanomas (Curchin C 2012). In our cohort, both MITF+ patients with amelanotic/hypomelanotic melanomas carried one RHC variants. However, due to the retrospective nature of this study, standardized information on pigmentation was not available, and therefore we could not assess the impact of RHC variants on melanoma pigmentation according to MITF germline status.

Although dermoscopic patterns of melanocytic nevi in MITF+ and MITF- patients have already been reported (Bassoli S 2018) (P.-B. J. Potrony M 2016) (Sturm RA 2014), our study is the first to assess dermoscopic characterization of DN and melanomas in MITF+ patients compared to MITF- patients. Previous studies (Bassoli S 2018) (P.-B. J. Potrony M 2016) (Sturm RA 2014) found that the predominant dermoscopic pattern of nevi the reticular one, both in MITF+ and in MITF- patients. Moreover, Sturm et al. reported that the frequency of globular nevi was greater in MITF+ patients, albeit not significant (Sturm RA 2014).

In DN and melanomas of our series of MITF+ patients, we found 3 prevalent dermoscopic patterns: unspecific, globular-homogeneous and reticular-homogeneous (Figure 8).



Melanoma with unspecific pattern | Melanoma with globular-homogeneous pattern | Melanoma with reticular-homogeneous pattern

Figure 8. Examples of the most common dermoscopic patterns of cutaneous melanomas in MITF+ patients.

The unspecific pattern was defined as devoid of structures or with too few structures to identify a pattern, except for the presence of blood vessels. This latter pattern is most frequently found in amelanotic/hypomelanotic melanocytic lesions including amelanotic/hypomelanotic nodular melanomas where it can be associated with polymorphous atypical vessels (Pizzichetta MA 2017).

While the reticular pattern is suggestive of photoinduced nevogenesis, the globular-homogeneous one with globules at the periphery of the lesion, expression of lesion growth, suggests that p.E318K variant may also act to force the continuous growth of the nevi/melanomas (Zalaudek et al., 2011).

Considering only melanomas, the prevalent pattern among the MITF+ patients was the unspecific one, a finding that was never associated with the MITF+ variant to date.

Conversely, the multicomponent pattern was prevalent among the MITF- patients, as already reported in the literature (Ciudad-Blanco et al., 2014; Pizzichetta et al., 2014).

Noteworthy, as a rule, all lesions with unspecific patterns should be biopsied, also in the context of lesions clinically appearing benign, to avoid missing melanoma (Z. I. Argenziano G 2007).

Therefore, the detection of this pattern in MITF+ patients should alert dermatologist raising the level of suspicion of malignancy.

Since among the 22 MITF+ patients, one patient developed 10 melanomas (of which 7 dermoscopic images were available) and the statistical analysis about the clinic-pathological-dermoscopic features

of the MITF+ group could have been influenced by a single patient, we repeated the analysis excluding this patient. Even though the observed pattern was actually influenced by this patient, the unspecific pattern remained prevalent in MITF+ patients and the association remained significant.

Dermoscopically, the most common patterns of DN and melanomas (multicomponent, reticular-globular) were almost absent in MITF+ patients, while the multicomponent was the most frequent pattern among MITF- patients.

6. Conclusions

Besides confirming previous results on the association of the p.E318K variant with high number of nevi (>50 units) and higher risk for melanoma and kidney cancers compared to MITF- patients, our study adds the finding that MITF+ patients have a higher risk of developing DN than MITF- patients. This result underlines the necessity for MITF+ patients to follow melanoma prevention programs, including dermatologic surveillance with digital follow-up.

In MITF+ patients, any melanocytic lesion with a dermoscopic pattern that digresses from the most commonly dermoscopic patterns reported among the MITF- patients, such as multicomponent and reticular-globular patterns, should be examined with caution to avoid missing melanomas that are devoid of structures.

Further studies through an international collaborative effort are crucial to increase the sample size and validate these findings.

7. References

Aitken JF, Youlden DR, Baade PD, Soyer HP, Green AC, Smithers. «Generational shift in melanoma incidence and mortality in Queensland, Australia, 1995–2014.» *Int J Cancer.*, 2018: 1528-1535.

Amos CI, Wang LE, Lee JE et al. «Genome-wide association study identifies novel loci predisposing to cutaneous melanoma.» *Hum Mol Genet*, 2011: 5012–5023.

Aoude LG, Wadt K, Bojesen A, et al. «A BAP1 mutation in a Danish family predisposes to uveal melanoma and other cancers.» *PLoS One*, 2013: :e72144.

Argenziano G, Soyer HP, Chimenti S, et al. «Dermoscopy of pigmented skin lesions: results of a consensus meeting via the Internet.» *J Am Acad Dermatol.* , 2003: 679-93.

Argenziano G, Soyer HP. «Dermoscopy of pigmented skin lesions--a valuable tool for early diagnosis of melanoma.» *Lancet Oncol*, 2001: 443-9.

Argenziano G, Zalaudek I, Ferrara G, et al. «Dermoscopy features of melanoma incognito: indications for biopsy.» *J Am Acad Dermatol.*, 2007: 508-13.

Barrett JH, Iles MM, Harland M et al. «Genome-wide association study identifies three new melanoma susceptibility loci.» *Nat Genet*, 2011: 1108–1113.

Bassoli S, Pellegrini C, Longo C, et al. «Clinical, dermoscopic, and confocal features of nevi and melanomas in a multiple primary melanoma patient with the MITF p.E318K homozygous mutation.» *Melanoma Res*, 2018: 166-169.

Bataille V, de Vries E. «Melanoma--Part 1: epidemiology, risk factors, and prevention.» *BMJ*, 2008: 1287–1291.

Bertolotto C, Lesueur F, Giuliano S et al. «A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma.» *Nature*, 2011: 94–98.

Berwick M, Armstrong BK, Ben-Porat L et al. «Sun exposure and mortality from melanoma.» *J Natl Cancer Inst*, 2005: 195–199.

Berwick M, Buller DB, Cust A, Gallagher R, Lee TK, Meyskens F, Pandey S, Thomas NE, Veierød MB, Ward S. «Melanoma Epidemiology and Prevention.» *Cancer Treat Res.*, 2016: 17-49.

Berwick M, Macarthur J, Orlow I et al. «MITF E318K's effect on melanoma risk independent of, but modified by, other risk factors.» *Pigment Cell Melanoma Res.*, 2014: 485-8.

Berwick M, Reiner AS, Paine S, Armstrong BK, Kricker A, Goumas C, et al. «Sun exposure and melanoma survival: a GEM study.» *Cancer Epidemiol Biomarkers Prev.*, 2014: 2145-52.

Bishop DT, Demenais F, Goldstein AM, et al. «Geographical variation in the penetrance of CDKN2A mutations for melanoma.» *J Natl Cancer Inst*, 2002: 894-903.

Bishop DT, Demenais F, Iles MM et al. «Genome-wide association study identifies three loci associated with melanoma risk.» *Nat Genet*, 2009: 920–925.

Boniol M, Autier P, Boyle P et al. «Correction. Cutaneous melanoma attributable to sunbed use: systematic review and meta-analysis.» *BMJ*, 2012: e8503.

Boniol M, Autier P, Boyle P et al. «Cutaneous melanoma attributable to sunbed use: systematic review and meta-analysis.» *BMJ*, 2012: e4757.

Bressac-de-Paillerets B, Avril M-F, Chompret A et al. «Genetic and environmental factors in cutaneous malignant melanoma.» *Biochimie*, 2002: 67–74.

Bruno W, Pastorino L, Ghiorzo P. «Multiple primary melanomas (MPMs) and criteria for genetic assessment: MultiMEL, a multicenter study of the Italian Melanoma Intergroup.» *J Am Acad Dermatol.* , 2016: 325-32.

Caini S, Gandini S, Sera F et al. «Meta-analysis of risk factors for cutaneous melanoma according to anatomical site and clino-pathological variant.» *Eur J Cancer*, 2009: 3054–3063.

Carbone M, Ferris LK, Baumann F, et al. «BAP1 cancer syndrome: malignant mesothelioma, uveal and cutaneous melanoma, and MBAITs.» *J Transl Med*, 2012: 179.

Ciotti P, Struewing JP, Mantelli M, et al. «A single genetic origin for the G101W CDKN2A mutation in 20 melanoma-prone families.» *Am J Hum Genet.*, 2000: 311-9.

Craig A. Sinclair, M, Jennifer Kay Makin, Anita Tang, Irena Brozek, BA, Vanessa Rock,. «The Role of Public Health Advocacy in Achieving an Outright Ban on Commercial Tanning Beds in Australia.» *Am J Public Health.*, 2014: e7-e9.

Crowley NJ, Seigler HF. «Late recurrence of malignant melanoma. Analysis of 168 patients.» *Ann Surg.*, 1990: 173-177.

Curchin C, Wurm E, Jagirdar K, Sturm R, Soyer P. «Dermoscopy, reflectance confocal microscopy and histopathology of an amelanotic melanoma from an individual heterozygous for MC1R and tyrosinase variant alleles.» *Australas J Dermatol.*, 2012: 291-4.

Cust AE, Goumas C, Holland EA et al. «MC1R genotypes and risk of melanoma before age 40 years: a population-based case-control-family study.» *Int J Cancer*, 2012: E269–E281.

Cust AE, Harland M, Makalic E, Schmidt D, Dowty JG, Aitken JF, et al. «Melanoma risk for CDKN2A mutation carriers who are relatives of population-based case carriers in Australia and the UK.» *Journal of medical genetics*, 2011: 266-272.

Cust AE, Mishra K, Berwick M. «Melanoma - role of the environment and genetics.» *Photochem Photobiol Sci*, 2018: 1853-1860.

Dalmasso B, Pastorino L, Ciccarese G et al. «CDKN2A germline mutations are not associated with poor survival in an Italian cohort of melanoma patients.» *J Am Acad Dermatol.*, 2018: doi: 10.1016/j.jaad.2018.07.060.

Dimitriou F, Krattinger R, Ramelyte E, Barysch MJ, Micaletto S, Dummer R, Goldinger SM. «The World of Melanoma: Epidemiologic, Genetic, and Anatomic.» *Current Oncology Reports*, 2018: 87.

D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. «UV radiation and the skin.» *Int J Mol Sci.*, 2013: 12222–48.

Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M,. «Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012.» *Int J Cancer*, 2015: 359-386.

Forsea AM, Del Marmol V, de Vries E, Bailey EE, Geller AC. «Melanoma incidence and mortality in Europe: new estimates, persistent disparities.» *Br J Dermatol*, 2012: 1124-1130.

Gandini S, Sera F, Cattaruzza MS et al. «Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure.» *Eur J Cancer*, 2005: 45-60.

Garbe C, Buttner P, Weiss J et al. «Associated factors in the prevalence of more than 50 common melanocytic nevi, atypical melanocytic nevi and actinic lentigines: multicenter case-control study of the Central Malignant Melanoma Registry of the German Dermatological Society.» *J Invest Dermatol*, 1994: 700–705.

Gershenwald JE, Scolyer RA, Hess KR et al. «Melanoma staging: Evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual.» *CA Cancer J Clin*, 2017: 472-492.

Ghiasvand R, Rueegg CS, Weiderpass E, Green AC, Lund E, Veierod MB. «Indoor tanning and melanoma risk: long-term evidence from a prospective population-based cohort study.» *Am J Epidemiol*, 2017: 147-156.

Ghiorzo P, Bonelli L, Pastorino L et al. «MC1R variation and melanoma risk in relation to host/clinical and environmental factors in CDKN2A positive and negative melanoma patients.» *Exp Dermatol.* , 2012: 718-20.

Ghiorzo P, Pastorino L, Queirolo P, et al. «Prevalence of the E318K MITF germline mutation in Italian melanoma patients: associations with histological subtypes and family cancer history.» *Pigment Cell Melanoma Res*, 2013: 259-62.

Ghiorzo P1, Ciotti P, Mantelli M, et al. «Characterization of ligurian melanoma families and risk of occurrence of other neoplasia.» *Int J Cancer.*, 1999: 441-8.

Goldstein AM, Chan M, Harland M, et al. «. High-risk melanoma susceptibility genes and pancreatic cancer, neural system.» *Cancer Res*, 2006: 9818–28.

Goldstein, A. M., Chan, M., Harland, M., Hayward, N. K.,Demenais, F., Bishop, D. T., et al. «Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma prone families from three continents.» *Journal of Medical Genetics,* 2007: 99–106.

Harland M, Cust AE, Badenas C, Chang YM, Holland EA, Aguilera P, et al. «Prevalence and predictors of germline CDKN2A mutations for melanoma cases from Australia, Spain and the United Kingdom.» *Hereditary cancer in clinical practice.*, 2014: 20.

Harrison S, McLennan R, Spear R et al. «Sun exposure and melanocytic naevi in young Australian children.» *Lancet*, 1994: 1529–1532.

Helgadottir H, Hoiom V, Tuominen R, et al. «Germline CDKN2A mutation status and survival in familial melanoma cases.» *J Natl Cancer Inst*, 2016: 108.

Helgadottir H, Tuominen R, Olsson H, Hansson J, Hoiom V. «Cancer risks and survival in patients with multiple primary melanomas: association with family history of melanoma and germline CDKN2A mutation status. .» *J Am Acad Dermatol*, 2017: 893-901.

Horn S, Figl A, Rachakonda PS, et al. «TERT promoter mutations in familial and sporadic melanoma.» *Science*, 2013: 959-61.

Hornung RL, Magee KH, Lee WJ et al. «Tanning facility use: are we exceeding food and drug administration limits?» *J Am Acad Dermatol*, 2003: 655–661.

Jameson JL, Kopp P. «Principi di genetica umana.» In *Harrison. Principi di medicina interna*, di Braunwald, Fauci, Hauser, Longo, Jameson Kasper, 427. Mc Graw Hill, 2005.

Kamb A, Shattuck-Eidens D, Eeles R, Liu Q, Gruis NA, Ding W, et al. «Analysis of the p16 gene (CDKN2) as a candidate for the chromosome 9p melanoma susceptibility locus.» *Nature genetics.*, 1994: 23-6.

Koludrovic D, Davidson I. «MITF, the Janus transcription factor of melanoma.» *Future Oncol.* , 2013: 235-44.

Køster B, Meyer MK, Andersson TM, Engholm G, Dalum P. «Sunbed use 2007-2015 and skin cancer projections of campaign results 2007-2040 in the Danish population: repeated cross-sectional surveys.» *BMJ Open*, 2018: e022094.

Lachiewicz AM, Berwick M, Wiggins CL et al. «Epidemiologic support for melanoma heterogeneity using the surveillance, epidemiology, and end results program.» *J Invest Dermatol*, 2008: 1340–1342.

Langley R, Barnhill R, Mihm M, Fitzpatrick T, Sober A. «Neoplasms: cutaneous melanoma.» In *Fitzpatrick's Dermatology in general medicine*, di Eisen A, Wolff K, Austen K, Goldsmith L, Katz S. Freedberg I, 917. McGraw Hill, 2003.

Leachman SA, Carucci J, Kohlmann W, et al. «Selection criteria for genetic assessment of patients with familial melanoma.» *J Am Acad Dermatol*, 2009: 677.e1-677.e14.

Leachman SA, Lucero OM, Sampson JE, Cassidy P, Bruno W, Queirolo P, Ghiorzo P. «Identification, genetic testing, and management of hereditary melanoma.» *Cancer Metastasis Rev*, 2017: 67-90.

Levine H, Afek A, Shamiss A, Derazne E, Tzur D, Astman N, et al. «Country of origin, age at migration and risk of cutaneous melanoma: a migrant cohort study of 1,100,000 Israeli men.» *Int J Cancer*, 2013: 486-94.

Liang X, Pfeiffer RM2, Li WQ, et al. «Association of genetic variants in CDK6 and XRCC1 with the risk of dysplastic nevi in melanoma-prone families.» *J Invest Dermatol.*, 2014: 481-487.

Linet M, Malker HS, Chow W et al. «Occupational risks for cutaneous melanoma among men in Sweden.» *J Occup Environ Med*, 1995: 1127–1135.

Lyth J, Falk M, Maroti M, Eriksson H, Ingvar C. «Prognostic risk factors of first recurrence in patients with primary stages I-II cutaneous malignant melanoma - from the population-based Swedish melanoma register.» *J Eur Acad Dermatol Venereol.*, 2017: 1468-1474.

M, Ohh. «Tumor strengths and frailties: Cancer SUMmOns Otto's metabolism.» *Nat Med.* , 2012: 30-1.

Manolio TA, Collins FS, Cox NJ, Goldstein DB et al. «Finding the missing heritability of complex diseases.» *Nature.*, 2009: 747-53.

Mantelli M, Barile M, Ciotti P, et al. «High prevalence of the G101W germline mutation in the CDKN2A (p16INK4a) gene in 62 Italian malignant melanoma families.» *Am J Med Genet*, 2002: 214-221.

Markovic SN, Erickson LA, Rao RD, et al. «Malignant melanoma in the 21st century, part 1: epidemiology, risk factors, screening, prevention, and diagnosis.» *Mayo Clin Proc.*, 2007: 364-380.

Mayer JE, Swetter SM, Fu T, Geller AC. «Screening, early detection, education, and trends for melanoma: current status (2007-2013) and future directions: Part II. Screening, education, and future directions.» *J Am Acad Dermatol.* , 2014: 611.e1-611.e10.

Nelemans PJ, Scholte R, Groenendal H et al. «Melanoma and occupation-results of a case-control study in Netherlands.» *Br J Ind Med*, 1993: 642–646.

Newton-Bishop JA, Beswick S, Randerson-Moor J et al. «Serum 25-hydroxyvitamin D3 levels are associated with Breslow thickness at presentation and survival from melanoma.» *J Clin Oncol*, 2009: 5339–5344.

NK, Hayward. «Genetics of melanoma predisposition.» *Oncogene*, 2003: 3053-62.

Olsen CM, Carroll HJ, Whiteman DC. «Familial melanoma: a meta-analysis and estimates of attributable fraction.» *Cancer Epidemiol Biomarkers Prev.*, 2010: 65-73.

Olsen CM, Zens MS, Green AC et al. «Biologic markers of sun exposure and melanoma risk in women: pooled case-control analysis.» *Int J Cancer*, 2011: 713-723.

Pampena R, Kyrgidis A, Lallas A et al. «A meta-analysis of nevus-associated melanoma: Prevalence and practical implications.» *J Am Acad Dermatol.*, 2017: 938-945.

Pho L, Grossman D, Leachman SA. «Melanoma genetics: a review of genetic factors and clinical phenotypes in familial melanoma.» *Curr Opin Oncol.*, 2006: 173-179.

Pizzichetta MA, Kittler H, Stanganelli I, et al. «Dermoscopic diagnosis of amelanotic/hypomelanotic melanoma.» *Br J Dermatol.*, 2017: 538-540.

Potrony M, Badenas C, Aguilera P et al. «Update in genetic susceptibility in melanoma.» *Ann Transl Med.*, 2015: 210.

Potrony M, Puig-Butille JA, Aguilera P, et al. «Prevalence of MITF p.E318K in Patients With Melanoma Independent of the Presence of CDKN2A Causative Mutations.» *JAMA Dermatol*, 2016: 405-12.

Pukkala E, Aspholm R, Auvinen A et al. «Incidence of cancer among Nordic airline pilots over five decades: occupational cohort study.» *Br Med J*, 2002: 567–569.

Pukkala E, Auvinen A, Wahlberg G et al. «Incidence of cancer among Finnish airline cabin attendants 1967–1992.» *Br Med J*, 1995: 649–652.

Puntervoll HE, Yang XR, Vetti HH, et al. «Melanoma prone families with CDK4 germline mutation: phenotypic profile and associations with MC1R variants.» *J Med Genet*, 2013: 264-270.

Puntervoll HE, Yang XR, Vetti HH, et al. «Melanoma prone families with CDK4 germline mutation: phenotypic profile and associations with MC1R variants.» *J Med Genet*, 2013: 264-70.

Raimondi S, Sera F, Gandini S et al. «MC1R variants, melanoma and red hair color phenotype: a meta-analysis.» *Int J Cancer*, 2008: 2753–2760.

Read J, Wadt KA, Hayward NK. «Melanoma genetics.» *J Med Genet*, 2016: 1-14.

Rongioletti F, Guadagno A, Campisi C, et al. «Atypical Spitz Tumor Arising on a Congenital Linear Plaque-Type Blue Nevus: A Case Report With a Review of the Literature on Plaque-Type Blue Nevus.» *Am J Dermatopathol.*, 2015: 915-9.

Rongioletti F, Smoller BR. «Unusual histological variants of cutaneous malignant melanoma with some clinical and possible prognostic correlations.» *J Cutan Pathol.*, 2005: 589-603.

Rosendahl CO, Grant-Kels JM, Que SK. «Dysplastic nevus: Fact and fiction.» *J Am Acad Dermatol.* , 2015: 507-12.

Shi J, Yang XR, Ballew B, et al. «Rare missense variants in POT1 predispose to familial cutaneous malignant melanoma.» *Nat Genet.* , 2014: 482-6.

Shipman AR, Clark AB, Levell NJ. «Summier European countries have lower melanoma mortality.» *Clin Exp Dermatol*, 2011: 544–547.

Sigurdson AJ, Doody MM, Rao RS et al. «Cancer incidence in the US radiology technologists health study 1983–1988.» *Cancer*, 2003: 3080–3089.

Soura E, Eliades PJ, Shannon K, Stratigos AJ, Tsao H. «Hereditary melanoma: Update on syndromes and management: Emerging melanoma cancer complexes and genetic counseling.» *J Am Acad Dermatol.*, 2016: 411-20.

Soyer HP, Argenziano G, Wellenhof R, Zalaudek I. «Introduction: the 3 point checklist.» In *Dermoscopy: the essential*, di Argenziano G, Wellenhof R, Zalaudek I. Soyer HP, 1-2. Elsevier, 2012.

Stoehr CG, Walter B, Denzinger S, et al. «The Microphthalmia-Associated Transcription Factor p.E318K Mutation Does Not Play a Major Role in Sporadic Renal Cell Tumors from Caucasian Patients.» *Pathobiology*, 2016: 165-9.

Stratigos AJ, Fargnoli MC, De Nicolo A et al. «MelaNostrum: a consensus questionnaire of standardized epidemiologic and clinical variables for melanoma risk assessment by the melanostrum consortium.» *J Eur Acad Dermatol Venereol.*, 2018: 2134-2141.

Sturm RA, Fox C, McClenahan P, et al. «Phenotypic characterization of nevus and tumor patterns in MITF E318K mutation carrier melanoma patients.» *J Invest Dermatol.*, 2014: 141-149.

Tagliabue E, Gandini S, Bellocco R et al. «MC1R variants as melanoma risk factors independent of at-risk phenotypic characteristics: a pooled analysis from the M-SKIP project.» *Cancer Manag Res*, 2018: 1143-1154.

Thompson JF, Scolyer RA, Kefford RF. «Cutaneous melanoma.» *Lancet*, 2005: 687-701.

Urso C, Rongioletti F, Innocenzi D, et al. «Interobserver reproducibility of histological features in cutaneous malignant melanoma.» *J Clin Pathol.* , 2005: 1194-8.

Vuong K, McGeechan K, Armstrong BK et al (. «Occupational sun exposure and risk of melanoma according to anatomical site.» *Int J Cancer.*, 2013: doi:10.1002/ijc.28603.

Walpole S, Pritchard AL, Cebulla CM,. «Comprehensive Study of the Clinical Phenotype of Germline BAP1 Variant-Carrying Families Worldwide.» *J Natl Cancer Inst.* , 2018: 1328-1341.

Whiteman D, Watt P, Purdie D et al. «Melanocytic nevi, solar keratosis, and divergent pathways to cutaneous melanoma.» *J Natl Cancer Inst*, 2003: 806–812.

Whiteman DC, Green AC, Olsen CM. « The growing burden of invasive melanoma: projections of incidence rates and numbers of new cases in six susceptible populations through 2031.» *J Invest Dermatol*, 2016: 1161-1171.

WHO. *Ultraviolet radiation (UV).* s.d. www.who.int/uv/faq/sunbeds/en/index6.html (consultato il giorno gennaio 13, 2019).

Williams PF, Olsen CM, Hayward NK et al. «Melanocortin-1-receptor and risk of cutaneous melanoma: a meta-analysis and estimates of population burden.» *Int J Cancer*, 2011: 1730–1740.

Yokoyama S, Woods SL, Boyle GM et al. «A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma.» *Nature*, 2011: 99–103.

Zuo L, Weger J, Yang Q, et al. «Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma.» *Nat Genet*, 2015: 97-9.

List of published manuscripts

- Walpole S, Pritchard AL, Cebulla CM, Pilarski R, Stautberg M, Davidorf FH, de la Fouchardière A, Cabaret O, Golmard L, Stoppa-Lyonnet D, Garfield E, Njauw CN, Cheung M, Turunen JA, Repo P, Järvinen RS, van Doorn R, Jager MJ, Luyten GPM, Marinkovic M, Chau C, Potrony M, Höiom V, Helgadottir H, Pastorino L, Bruno W, Andreotti V, Dalmasso B, Ciccarese G, Queirolo P, Mastracci L, Wadt K, Kiilgaard JF, Speicher MR, van Poppelen N, Kilic E, Al-Jamal RT, Dianzani I, Betti M, Bergmann C, Santagata S, Dahiya S, Taibjee S, Burke J, Poplawski N, O'Shea SJ, Newton-Bishop J, Adlard J, Adams DJ, Lane AM, Kim I, Klebe S, Racher H, Harbour JW, Nickerson ML, Murali R, Palmer JM, Howlie M, Symmons J, Hamilton H, Warrier S, Glasson W, Johansson P, Robles-Espinoza CD, Ossio R, de Klein A, Puig S, Ghiorzo P, Nielsen M, Kivelä TT, Tsao H, Testa JR, Gerami P, Stern MH, Paillerets BB, Abdel-Rahman MH, Hayward NK. Comprehensive Study of the Clinical Phenotype of Germline BAP1 Variant-Carrying Families Worldwide. J Natl Cancer Inst. 2018 Dec 1;110(12):1328-1341. doi: 10.1093/jnci/djy171.
- Dalmasso B, Pastorino L, Ciccarese G, Andreotti V, Grillo F, Mastracci L, Spagnolo F, Ballestrero A, Queirolo P, Bruno W, Ghiorzo P. CDKN2A germline mutations are not associated with poor survival in an Italian cohort of melanoma patients. J Am Acad Dermatol. 2018 Sep 28. pii: S0190-9622(18)32367-3. doi: 10.1016/j.jaad.2018.07.060.
- Drago F, Ciccarese G, Parodi A. Pityriasis rosea and pityriasis rosea-like eruptions: How to distinguish them? JAAD Case Rep. 2018 Sep 14;4(8):800-801. doi: 10.1016/j.jdcr.2018.04.002.
- Drago F, Herzum A, Ciccarese G, Broccolo F, Rebora A, Parodi A. Acute pain and postherpetic neuralgia related to Varicella zoster virus reactivation: Comparison between typical herpes zoster and zoster sine herpete. J Med Virol. 2018 Sep 4. doi: 10.1002/jmv.25304.

- Ciccarese G, Trave I, Herzum A, Gariazzo L, Cozzani E, Rebora A, Parodi A, Drago F. Dermatological infections in organ transplant recipients: a retrospective study on 222 patients. J Eur Acad Dermatol Venereol. 2018 Jun 28. doi: 10.1111/jdv.15153S
- Drago F, Ciccarese G, Herzum A, Rebora A, Parodi A. Pityriasis Rosea during Pregnancy: Major and Minor Alarming Signs. Dermatology. 2018;234(1-2):31-36. doi: 10.1159/000489879.
- Drago F, Herzum A, Ciccarese G, Parodi A. May syphilis protect against human papillomavirus infection? An example of heterologous immunity. G Ital Dermatol Venereol. 2018 Mar 29. doi: 10.23736/S0392-0488.18.05985-0.
- Ciccarese G, Parodi A, Drago F, Herzum A, Drago F. Pityriasis rosea in a patient with retrovirus infection: a reply. Postepy Dermatol Alergol. 2018 Feb;35(1):116-117. doi: 10.5114/ada.2018.73172.
- Drago F, Ciccarese G, Cordara V, Paudice M, Herzum A, Parodi A. Oral psoriasis and SIBO: is there a link? J Eur Acad Dermatol Venereol. 2018 Sep;32(9):e368-e369. doi: 10.1111/jdv.14953.
- Cozzani E, Ciccarese G, Drago F, Gerbaldo D, Parodi A. First report of genital ulcer due to Morganella morganii infection. G Ital Dermatol Venereol. 2018 Apr;153(2):291-293.
- Drago F, Ciccarese G, Herzum A, Drago F, Rebora A, Parodi A. The association between cigarettes smoke, small intestine bacterial overgrowth and rosacea. G Ital Dermatol Venereol. 2018 Feb 26. doi: 10.23736/S0392-0488.18.05919-9.
- Ciccarese G, Drago F. Atypical presentations of pityriasis rosea: a reply. Dermatol Online J. 2017 Aug 15;23(8). pii: 13030/qt2d50q1tq.
- Giacani L, Ciccarese G, Puga-Salazar C, Dal Conte I, Colli L, Cusini M, Ramoni S, Delmonte S, D'Antuono A, Gaspari V, Drago F. Enhanced Molecular Typing of Treponema pallidum subspecies pallidum Strains From 4 Italian Hospitals Shows Geographical Differences in Strain Type Heterogeneity, Widespread Resistance to Macrolides, and Lack of Mutations

Associated With Doxycycline Resistance. Sex Transm Dis. 2018 Apr;45(4):237-242. doi: 10.1097/OLQ.0000000000000741.

- Ciccarese G, Parodi A, Drago F. Pediatric pityriasis rosea. Turk J Med Sci. 2017 Aug 23;47(4):1302-1305. doi: 10.3906/sag-1701-127.
- Drago F, Ciccarese G, Gariazzo L, Cioni M, Parodi A. Acute localized exanthem due to Coxsackievirus A4. Infez Med. 2017 Sep 1;25(3):274-276.
- Ciccarese G, Parodi A, Rebora A, Drago F. The usefulness of investigating the possible underlying conditions in rosacea. J Eur Acad Dermatol Venereol. 2017 Aug 28. doi: 10.1111/jdv.14547
- Drago F, Ciccarese G, Parodi A. Human Herpesvirus-7 Papular Rash: A Comment to Brazzelli et al. Acta Derm Venereol. 2017 Oct 2;97(9):1152-1154.
- Drago F, Ciccarese G, Parodi A. HHV-6 reactivation as a cause of fever in autologous hematopoietic stem cell transplant recipients: A reply. J Infect. 2017 Aug 4. pii: S0163-4453(17)30257-8.
- Drago F, Ciccarese G, Parodi A. Effects of the treatment for small intestine bacterial overgrowth on rosacea. J Dermatol. 2017 Aug 7. doi: 10.1111/1346-8138.13985.
- Drago F, Ciccarese G, Parodi A. Can Unilateral Pityriasis Rosea be Considered a Form of Superimposed Lateralized Exanthem? J Clin Diagn Res. 2017 Jun;11(6):WL01. doi: 10.7860/JCDR/2017/27855.10050.
- Ciccarese G, Broccolo F, Rebora A, Parodi A, Drago F. Oropharyngeal lesions in pityriasis rosea. J Am Acad Dermatol. 2017 Jul 17. pii: S0190-9622(17)31935-7.
- Drago F, Ciccarese G, Broccolo F, Rebora A, Parodi A. Atypical hand, foot, and mouth disease in adults. J Am Acad Dermatol. 2017 Aug;77(2):e51-e56. doi: 10.1016/j.jaad.2017.03.046.
- Rebora A, Ciccarese G, Drago F. Postfebrile telogen effluvium. G Ital Dermatol Venereol. 2017 Jul 11. doi: 10.23736/S0392-0488.17.05643-7.

- Drago F, Ciccarese G, Cogorno L, Calvi C, Marsano LA, Parodi A. Prevention of non-melanoma skin cancers with nicotinamide in transplant recipients: a case-control study. Eur J Dermatol. 2017 Aug 1;27(4):382-385.
- Drago F, Ciccarese G. Update on infections with human herpesviruses 6A, 6B, and 7: A reply. Med Mal Infect. 2017 Jun;47(4):301-302. doi: 10.1016/j.medmal.2017.02.005.
- Ciccarese G, Gariazzo L, Cioni M, Rivara G, Drago F. Are genital ulcers always sexually transmitted? First report of scrotal ulcer caused by Serratia marcescens infection. Int J Dermatol. 2017 Aug;56(8):e160-e162. doi: 10.1111/ijd.13494.
- Drago F, Ciccarese G. Pityriasis Rosea: An Update on Etiopathogenesis and Management of Difficult Aspects - A Reply.Indian J Dermatol. 2017 Jan-Feb;62(1):95.
- Drago F, Ciccarese G, Anselmi L, Parodi A. Scrotal ulcer due to Serratia marcescens. Eur J Dermatol. 2017 Jun 1;27(3):309-310.
- Delmonte S, Sidoti F, Ribero S, Dal Conte I, Curtoni A, Ciccarese G, Stroppiana E, Stella ML, Costa C, Cavallo R, Rebora A, Drago F. Recurrent herpes labialis and HSV-1 herpes genitalis: which is the link? G Ital Dermatol Venereol. 2017 Feb 8.
- Drago F, Gasparini G, Ciccarese G, Campisi C, Parodi A. Atypical exanthemas in the Emergency Department. G Ital Dermatol Venereol. 2017 Feb;152(1):95-96.
- Ciccarese G, Herzum A, Rebora A, Drago F. Prevalence of genital, oral, and anal HPV infection among STI patients in Italy. J Med Virol. 2017 Jun;89(6):1121-1124.
- Gallo R, Pastorino C, Gasparini G, Ciccarese G, Parodi A. Scutellaria baicalensis extract: a novel botanical allergen in cosmetic products? Contact Dermatitis. 2016 Dec;75(6):387-388.
- Drago F, Ciccarese G, Gasparini G, Cogorno L, Javor S, Toniolo A, Broccolo F. Contemporary infectious exanthems: an update. Future Microbiol. 2017 Feb;12:171-193.
- Drago F, Ciccarese G, Brigati C, Parodi A. Strongyloides Autoinfection Manifesting as Larva Currens in an Immunocompetent Patient. J Cutan Med Surg. 2016 Nov;20(6):617-618.

- Drago F, Merlo G, Ciccarese G, Agnoletti AF, Cozzani E, Rebora A, Parodi A. Changes in neurosyphilis presentation: a survey on 286 patients. J Eur Acad Dermatol Venereol. 2016 Nov;30(11):1886-1900.
- Drago F, Ciccarese G, Agnoletti AF, Sarocchi F, Parodi A. Neuro sweet syndrome: a systematic review. A rare complication of Sweet syndrome. Acta Neurol Belg. 2017 Mar;117(1):33-42.
- Drago F, Ciccarese G, Tomasini CF, Calamaro P, Boggio M, Rebora A, Parodi A. First report of tertiary syphilis presenting as lipoatrophic panniculitis in an immunocompetent patient. Int J STD AIDS. 2017 Mar;28(4):408-410.
- Drago F, Herzum A, Ciccarese G, Dezzana M, Casazza S, Pastorino A, Bandelloni R, Parodi A. Ureaplasma parvum as a possible enhancer agent of HPV-induced cervical intraepithelial neoplasia: Preliminary results. J Med Virol. 2016 Dec;88(12):2023-2024.
- Drago F, Ciccarese G, Rebora A. Recurrent Jarisch-Herxheimer reaction in late latent syphilis. J Eur Acad Dermatol Venereol. 2016 Dec;30(12):e225-e226.
- Drago F, Ciccarese G, Zangrillo F, Gasparini G, Cogorno L, Riva S, Javor S, Cozzani E, Broccolo F, Esposito S, Parodi A. A Survey of Current Knowledge on Sexually Transmitted Diseases and Sexual Behaviour in Italian Adolescents. Int J Environ Res Public Health. 2016 Apr 13;13(4):422. doi: 10.3390/ijerph13040422.
- Drago F, Javor S, Ciccarese G, Parodi A. Gianotti-Crosti syndrome as presenting sign of cytomegalovirus infection: A case report and a critical appraisal of its possible cytomegalovirus etiology. J Clin Virol. 2016 May;78:120-2. doi: 10.1016/j.jcv.2016.03.009.
- Drago F, Ciccarese G, Parodi A. Nicotinamide for Skin-Cancer Chemoprevention. N Engl J Med. 2016 Feb 25;374(8):789-90. doi: 10.1056/NEJMc1514791.
- Drago F, Agnoletti AF, Ciccarese G, Cozzani E, Parodi A. Two cases of oligosymptomatic neurosyphilis in immunocompetent patients: Atypical neurosyphilis presentation. Int J STD AIDS. 2016 Feb;27(2):155-6. doi: 10.1177/0956462415578546.

- Drago F, Ciccarese G, Merlo G, Sartoris G, Parodi A. Is the Standard Treatment for Early Syphilis Sufficient to Prevent Cardiovascular and Neurologic Syphilis? Am J Cardiol. 2016 Jan 15;117(2):310-1. doi: 10.1016/j.amjcard.2015.10.048. Epub 2015 Nov 6. No abstract available.
- Drago F, Agnoletti A, Ciccarese G, Guadagno A, Cozzani E, Parodi A. Pigmentogenes Pityriasis rosea: an atypical presentation of the exanthem. G Ital Dermatol Venereol. 2016 Dec;151(6):732-733. Epub 2015 Nov 19. No abstract available.
- Drago F, Ciccarese G, Thanasi H, Agnoletti AF, Cozzani E, Parodi A. Facial involvement in pityriasis rosea: differences among Caucasian and dark-skinned patients. G Ital Dermatol Venereol. 2016 Oct;151(5):571-2. Epub 2015 Nov 18. No abstract available.
- Drago F, Cogorno L, Broccolo F, Ciccarese G, Parodi A. A fatal case of DRESS induced by strontium ranelate associated with HHV-7 reactivation. Osteoporos Int. 2016 Mar;27(3):1261-1264. doi: 10.1007/s00198-015-3384-7.

Submitted articles

- Ciccarese G*, Dalmasso B*, Bruno W, Queirolo P, Pastorino L, Andreotti V, Spagnolo F, Tanda E, Ponti G, Massone C, Drago F, Parodi A, Ghigliotti G, Pizzichetta MA, Ghiorzo P. CLINICAL, PATHOLOGICAL AND DERMOSCOPIC PHENOTYPE OF MITF p.E318K CARRIER CUTANEOUS MELANOMA PATIENTS. Submitted to: Journal of Translational Medicine.
- Gasparini G*, Ciccarese G*, Cozzani E, Bruno W, Parodi A. A rare case of four synchronous cutaneous melanomas: genotype-phenotype correlation. Submitted to: Acta Dermato-venereologica.

*Co-first authorship.