ISSN 0001-5555

# ActaDV

# ACTA DERMATO-VENEREOLOGICA

Volume 100 2020 **Theme issue**

## Advances in Dermatology and Venereology

A Non-profit International Journal for
Interdisciplinary Skin Research, Clinical and Experimental
Dermatology and Sexually Transmitted Diseases

## Frontiers in Dermatology and Venereology

- A series of theme issues in relation to the 100-year anniversary of ActaDV

Official Journal of
- European Society for Dermatology and Psychiatry

Affiliated with
- The International Forum for the Study of Itch

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# ACTA DERMATO-VENEREOLOGICA

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*Acta Dermato-Venereologica*
*S:t Johannesgatan 22, SE-753 12 Uppsala, Sweden*

**Editorial Manager,** Mrs Agneta Andersson
Please send an E-mail

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# Skin Malignancies

*Theme Editors:*
*Veronique Bataille and Nicole Basset Seguin*

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REVIEW ARTICLE

# Melanoma Epidemiology and Sun Exposure

Sara RAIMONDI[1], Mariano SUPPA[2] and Sara GANDINI[1]
*[1]Molecular and Pharmaco-Epidemiology Unit, Department of Experimental Oncology, IEO, European Institute of Oncology IRCCS, Milan, Italy, and [2]Department of Dermatology, Université Libre de Bruxelles, Hôpital Erasme, Brussels, Belgium*

**The worldwide incidence of melanoma has increased rapidly over the last 50 years. Melanoma is the most common cancer found in the young adult population, and its incidence is very high among geriatric populations. The incidence of melanoma varies by sex, and this factor is also associated with differences in the anatomical site melanoma. Adolescent and young adult women have a higher incidence than men. This may be, in part, due to the greater use of sunbeds, as well as intentional sun exposure among girls and, in general, risky behaviours in seeking to suntan, due to socially-determined aesthetic needs. Indeed, the World Health Organization declared that there is sufficient evidence to classify exposure to ultraviolet radiation (sunbed use and sun exposure) as carcinogenic to humans. Although pigmentation characteristics, such as skin colour, hair and eye colour, freckles and number of common and atypical naevi, do influence susceptibility to melanoma, recommendations regarding prevention should be directed to the entire population and should include avoiding sunbed, covering sun-exposed skin, wearing a hat and sunglasses. Sunscreen use should not be used to prolong intentional sun exposure. Primary prevention should be focused mainly on young adult women, while secondary prevention should be focused mainly on elderly men. In fact, after the age of 40 years, incidence rates reverse, and the incidence of melanoma among men is greater than among women. This is probably due to the fact that men are less likely than women to examine their own skin or present to a dermatologist for skin examination.**

*Key words:* sunburn; sunbed; sunscreen; phenotype; melanoma; sun exposure.

Accepted Apr 27, 2020; Epub ahead of print Apr 28, 2020

Acta Derm Venereol 2020; 100: adv00136.

*Corr:* Sara Gandini, Molecular and Pharmaco-Epidemiology Unit, Department of Experimental Oncology, European Institute of Oncology, Via Ripamonti 435, Milan, Italy. E-mail: sara.gandini@ieo.it

## SIGNIFICANCE

Young women present particularly high risky behaviours in terms of melanoma risk, such as tanning, related to social determined aesthetic needs. Indeed, the highest prevalence of sunbed use is found among female adolescents. Prevention recommendations include avoiding sunbed use, covering sun-exposed skin, wearing a hat and sunglasses. Sunscreen should not be used to prolong intentional sun exposure. Primary prevention should focus on young women, and secondary prevention in older men. In fact, at older ages, the incidence of melanoma among men is greater than among women, probably because men are less likely than women to examine their own skin or present to a dermatologist for skin examination.

Melanoma arises through malignant transformation of melanocytes, pigment-containing cells. Melanoma typically occurs in the skin, but may rarely occur in the mouth, intestines, or eye. Cutaneous melanoma (CM) is the most aggressive and lethal form of all skin cancers, which occurs when unrepaired DNA damage to skin cells (most often caused by ultraviolet radiation (UVR)) triggers mutations or genetic defects that lead the skin cells to multiply rapidly and form malignant tumours. CM represents approximately 5% of all skin cancers, but it accounts for approximately three-quarters of all skin cancer deaths (1).

The worldwide incidence of melanoma has risen rapidly over the course of the last 50 years. According to GLOBOCAN 2018 (2), the expected world number of new cases of CM is 287,723 in 2018, with an age-standardized incidence rate of 3.1 per 100,000/year and a mortality rate of 0.63 per 100,000/year. In populations of European origin, incidence and mortality rates were, respectively, 11.2 and 1.7 per 100,000/year in Europe, 12.2 and 1.4 in the USA and 33.6 and 3.4 per 100,000/year in Australia and New Zealand. Worldwide, CM incidence rates vary 100-fold among different populations depending on ethnicity, with the highest rates observed in New Zealand and Australia, intermediate rates in Europe and USA, and the lowest rates in South-Central Asia. In Europe, the highest estimates of CM incidence rates were observed in Sweden and Denmark and the lowest rates in Greece. This variation is mainly attributed to exposure to UVR, and genetically determined phenotypic characteristics. Differences by ethnicity were also observed for CM subtypes and body location. Although the most common melanoma subtype among populations of European origin is superficial spreading melanoma (SSM), melanomas in the African-American population occur more often on non-sun-exposed skin, such as the palms and the soles, and acral lentiginous melanoma

doi: 10.2340/00015555-3491
Acta Derm Venereol 2020; 100: adv00136

(ALM) is the most common histopathological type (3). The age range with highest number of CM diagnoses is between 40 and 60 years. The median age at diagnosis and death are, respectively, 57 and 67 years. The incidence rates start to increase from 40 years of age; thus CM is generally considered a tumour affecting young and middle-aged people, almost a decade before most solid tumours (e.g. breast, colon, lung or prostate cancers). A study that examined incidence rates time trends of CM in 39 population-based cancer registries from 1953 to 2008 (4) found that incidence rates of melanoma increased in most European countries (primarily Southern and Eastern Europe). However, indications of a stabilization or decreasing trend were observed in Australia, New Zealand, the USA, Canada and Norway, mainly in the youngest age group (25–44 years). Possible explanations of these results include decreasing sun exposure in children following intensive preventive campaigns in these countries, and changes in the proportion of young individuals at low risk of melanoma due to immigration to these countries over recent decades.

Adjusting for age, adolescent and young adult women have higher melanoma incidence rates than men (5). This may be, in part, due to the greater use of sunbeds by girls, which is associated with increased melanoma risk (6). In general, girls have greater tanning risky behaviours and socially determined aesthetic needs (7). However, after the age of 40 years, rates reverse, and the incidence of melanoma among men is greater than that of women. Men are less likely than women to examine their own skin or seek help from dermatologists for skin examination (8). Considerable sex differences in melanoma awareness and detection practices have been reported in population-based studies (9).

Looking at mortality rates, they were found to increase in the USA and in Europe since 1980s but at much slower rates than incidence. This may be due to overdiagnosis, with diagnosis and removal of very thin, not lethal, melanomas. At all ages, mortality rates are higher in males than in females, with a cumulative mortality at 70 years of 0.37% in men and 0.17% in women in Australia. A pooled analysis of the European Organization for Research and Treatment of Cancer (EORTC) trials showed that, in both localized and advanced disease, women have a significant and independent advantage, across different clinical endpoints concerning disease progression and survival (10). This seems to depend on both biological sex trait and behavioural differences regarding primary (sun exposure, UVR protection) and secondary (skin screening) prevention (11).

We review the literature regarding UV exposure and phenotypical risk factors. A brief summary of risk estimates is presented in **Table I**.

## EPIDEMIOLOGICAL RISK FACTORS

### *Ultraviolet radiation*

According to WHO estimates, 65,161 people a year worldwide die from too much sun. Sun exposure is indeed the most significant environmental cause of skin cancer and UVR is the wavelength associated with the occurrence of this disease.

The International Agency for Research on Cancer (IARC) classified the entire spectrum of UVR as "carcinogenic to humans" (Group 1) based on substantial evidence from both basic and epidemiological research. Laboratory data and animal experiments (on DNA mutations and repair, immune function, cell integrity, cell cycle regulation, and other critical biological functions) have documented a role for both UVB and UVA radiation in skin carcinogenesis. Experiments in human volunteers have also shown that exposure to UVA and UVB can weaken the immune system through interacting and overlapping mechanisms, increasing vulnerability to cancer as well as other diseases. Furthermore, evidence

**Table I. Summary of epidemiological risk factors for melanoma development**

| Category of risk factors | Risk factors | Effect estimates | Notes |
|---|---|---|---|
| UV radiation | Sun exposure | High intermittent/intentional vs. low: approximately 60% increased risk<br>High continuous/occupational vs. low: no association | Intermittent: mainly increases risk of SSM<br>Chronic: increases risk of LMM. Decrease risk on occasionally exposed sites |
| | Sunburns | History of sunburns vs. no history: double increased risk | Increases risk of SSM and LMM, not for NM |
| | Indoor tanning | Ever exposure vs. never: approximately 20% increased risk | Evidence of dose-response effect; mainly affects young women |
| | Sunscreen use | Some evidence that high SPF may decrease risk compared with no use | Sunscreen use may increase risk if used to prolong intentional sun exposure |
| Phenotype | Eye colour | Light colours vs. dark: approximately 50% increased risk | Increased risk of NM and SSM, not for LMM |
| | Hair colour | Red vs. dark: more than triple risk<br>Blonde vs. dark: almost double risk<br>Light-brown vs. dark: approximately 60% increased risk | |
| | Freckles | High-density vs. none: more than double risk | |
| | Skin colour/type | Fair vs. dark: more than double risk<br>Phototype I vs. IV: more than double risk<br>Phototype II vs. IV: approximately 80% increased risk<br>Phototype III vs. IV: approximately 70% increased risk | Dose-response trend of risk according to level of skin type |
| | Common naevi | >100 vs. <15: almost 7-times higher risk | Total naevus count was associated mainly with intermittently sun-exposed sites (trunk and legs) |
| | Atypical naevi | ≥5 vs. 0: more than 6-times higher risk | |

LMM: lentigo maligna melanoma; NM: nodular melanoma; SPF: solar protection factor; SSM: superficial spreading melanoma.

from a large number of observational studies is generally consistent, showing a significant positive association with residing in areas with high ambient UVR through life, in early life, and even for short periods in early adult life (12). Lastly, several meta-analyses showed significant increases in melanoma risk and non-melanoma skin cancer (NMSC) with high sun exposure and indoor UV tanning (6, 13).

A study conducted in Canada estimated the current attributable and future avoidable burden of melanoma related to exposure to UVR and modifiable UVR risk behaviours. They estimated that 62.3% of melanomas in Canada were attributable to exposure to UVR and that 29.7% were attributable to the combination of sunburn (7.4%), sunbathing (17.8%), and indoor tanning (7.0%). They also concluded that a 50% reduction in modifiable UVR behaviour could avoid an estimated 11,980 melanoma cases by 2042 (14).

Recognizing the importance of establishing skin cancer prevention as a national priority, The Surgeon General's Call to Action to Prevent Skin Cancer in 2014 described prevention strategies and called on the community sectors to play a role in protecting Americans from UVR from the sun and artificial sources (15). Strategies that support goals related to lifestyle modifications to reduce the burden of melanoma included reducing the harms from indoor tanning, youth education approaches, and community-wide interventions focused on modifying healthy behaviours, including decreasing UVR exposure (16).

### *Sun exposure and sunburn*

Measurements of individual sun exposure vary between studies, but are commonly classified as "intermittent" (short, intense sun exposure through activities such as sunbathing, outdoor recreation and holidays in sunny locations), "chronic" (continuous exposure, such as occupational sun exposure) and "total" (the sum of intermittent and chronic exposures).

The first systematic review and meta-analysis, summarizing 57 studies on sun exposure and melanoma, found a 60% significant increased risk of melanoma due to recreational sun exposure (summary relative risk (SRR) of CM for intermittent sun exposure of 1.61; 95% confidence intervals (95% CI) 1.31–1.99), while no association was suggested for chronic sun exposure (SRR: 0.95; 95% CI 0.87–1.04).

Sunburn is a biological response to intermittent exposure to the sun in poorly adapted skin and in multiple analyses a stronger predictor than intermittent exposure itself (13). The SRR for sunburns, which is the main indicator of sun exposure, was 2.03 (95% CI 1.73–2.37).

Despite the clear role of sunburn in increasing CM risk, a survey conducted in USA in 2013 (Youth Risk Behavior Survey (17)) highlighted that preventive practices are not regularly followed: most respondents (57%) reported having experienced 1 or more sunburns in the prior year.

Holman et al. (18) first proposed 2 distinct biological pathways by which CM might develop. One by way of intermittent sun exposure, acting primarily as a promoter of melanoma arising on pigmented naevi and mainly of the SSM type, and the other by way of a more continuous pattern of sun exposure, leading principally to lentigo maligna melanoma (LMM). In 1992, Green (19) proposed a theory of site-dependent susceptibility of melanocytes to malignant transformation. According to this hypothesis, people with a low propensity for melanocyte proliferation (small number of common naevi) need a continuous exposure to sunlight in order to drive the clonal expansion of initiated melanocytes. The melanomas arising from this pathway are more likely to be located on chronically sun-exposed body sites, to be of LMM subtype, and to occur in older patients with a history of solar damage and NMSC. On the other hand, people with a high propensity to melanocyte proliferation are more likely to develop melanomas on intermittently sun-exposed body sites, to be of SSM or nodular (NM) histological subtypes and to occur in patients with no history of sun damage or NMSC. Thus, both pathways include early initiation by sun exposure, but later proliferation is driven, in one pathway, by accumulation of sun exposure in non-naevus-prone people and, in the other pathway, by host factors in naevus-prone people (20). In the same study by Green (19), it was found that sun exposure and phenotypic characteristics were positively associated with all the main histological subtypes of melanoma. However, NM was not found to be associated with sunburns, in contrast to LMM and SSM. LMM was not found to be associated with freckling, light eye colour and hair colour, in contrast to NM and SSM, which were significantly associated with all 3.

This 2-pathway hypothesis for melanoma was confirmed and refined by many authors who observed an inverse correlation between number of naevi and clinical signs of sun damage (20–22), and identified a few genes differentially mutated in LMM vs. SSM and NM. Briefly, melanomas characterized by mutations in *BRAF*, *NRAS* and *TERT*, and approximately 80% of melanomas carry UVR signature mutations (C-T or CC-TT), along with other genes coding for downstream components of the tyrosine kinase RAS-BRAF signal transduction pathway (e.g. *CDKN2A* and *CDK4*), were suggested to be more frequent on intermittently exposed skin (23–25). Most of these are considered "passenger" mutations and not "driver" mutations; however, this high prevalence is clearly indicative of a role for UVR in melanomogenesis as is noted also by presence of somatic mutations in normal skin. BRAF mutations, which are present in approximately 40% of CM in people of European origin, are associated with characteristics of the naevus-associated pathway: younger age at diagnosis, occurrence on the

trunk, SSM type and absence of chronic sun damage in the skin (26). *TERT* promoter mutations (associated with UVR exposure) are present in approximately 43% of CM, occur more frequently at sun-exposed sites, and tend to co-occur with *BRAF* alterations (27).

The melanocortin-1 receptor (MC1R), a pigmentation gene associated with melanoma risk (28–30), is involved in the same signalling pathway and has been found to interact positively with *BRAF* and *CDKN2A* in the aetiology of melanoma occurring on usually unexposed skin (31, 32). On the other hand, p53-positive melanomas were usually associated with features of chronical sun exposure (33), supporting the hypothesis that different molecular pathways can lead to melanoma development (34, 35).

Looking at the distribution by body site of different histological types of CM, SSM is the more frequent type on the trunk in men and legs in women, while LMM is more frequent on the face and neck (36). It is likely that melanocytes on different body sites have different characteristic in terms of differentiation: atypical naevi are more commonly found on the trunk, whilst they are very rare on the face. Similarly, intradermal naevi, which are mature melanocytic lesions, are commonly found on the face, but are much rarer on limbs. It is possible that during embryogenesis, melanocytes have different properties according to head and neck, trunk and limb locations, because of migration to different body sites, and this is likely to be influenced by key developmental genes.

The complex interplay between sun exposure, pigmentary characteristics and melanocytic naevi was investigated in a meta-analysis including 24 studies for a total of 16,180 cases of melanoma (37). Considering each measure of sun exposure (intermittent, chronic, sunburns and actinic damage) SRRs for CM risk were 1.31 (95% CI 0.94–1.81) and 1.77 (95% CI 1.30–2.41) respectively for occasionally vs. usually sun-exposed body sites. Chronic sun exposure was weakly, but significantly, negatively associated with CM on occasionally sun-exposed sites. Overall, these results suggest that sun exposure is associated with CM on all body sites (except for mucosal), but in particular with CM on head and neck in older individuals.

The apparently protective effect of chronic sun exposure on CM on occasionally exposed sites and, at most, weakly causal effect on usually exposed sites is puzzling. Enhanced melanin production and melanosome delivery to keratinocytes (38) and increased thickness of the top layers of the epidermis due to continuing sun exposure may be a possible explanation; however, they would not be expected to reduce incidence to a level below that present in the absence of sun exposure. Other possible explanations are the lower melanin content, sunburn, and lower DNA repair capacity of intermittently exposed skin compared with habitually exposed skin. Sunburn can lead to cell proliferation in replacing apoptotic cells, and habitually exposed skin may have somewhat thicker stratum corneum, and thus models protection from tanning, and some upregulation of DNA repair pathways exemplified by fewer thymine dimers after repeated low exposure (39–41). However, it is important to note that the reference category for calculating RRs in epidemiological studies of melanoma and sun exposure is "low sun exposure", not "no sun exposure".

Migrant studies provide convincing evidence that childhood and adolescence are critical periods for the development of melanoma in adulthood. Indeed, it was found that adults were at increased risk of melanoma if they spent their childhood in sunny locations or if they received above average intermittent sun exposure during vacations and/or recreation. In an Australian case-control study published in 1984 (42), earlier age at arrival of immigrants to Australia was a melanoma risk predictor with little residual effect of duration of residence. Specifically, children who migrate from a less sunny country before the age of 10 years had similar incidence rates of native-born Australians, while the estimated incidence in those arriving after age 15 years was approximately a quarter of the native-born rates. Similarly, in a European case-control study (43), age <10 years old at arrival in a sunny location of residence (i.e. the Mediterranean, subtropics, or tropics) conferred a 4-fold increased risk of developing melanoma.

Studies investigating the role of residence in childhood provide further evidence that sun exposure in childhood and adolescence is more closely associated with melanoma risk than adult sun exposure. A case-control study nested in the Nurses' Health Study cohort (44) showed an increased melanoma risk in women whose residence during the ages 15–20 years was more equatorial in latitude, whereas latitude of residence after 30 years of age was not significantly related to melanoma risk. Finally, in another study of 474 cases and 926 controls, those who lived near the coast before the age of 15 years had an increased risk of melanoma compared with those who never lived far away from the coast (odds ratio (OR)=1.6; 95% CI 1.0–2.6) (45).

### *Sunbeds and indoor tanning*

Sunbeds and sunlamps used for tanning purposes represent the major source of deliberate exposure to UVR. Indoor UVR tanning has been widely practiced in Northern Europe and the USA since the 1980s and this trend has gained popularity in sunnier countries, such as Australia. Modern indoor UVR tanning equipment emits mainly in the UVA range, but a fraction (<5%) of this spectrum is in the UVB range, which is needed to induce a deep, long-lasting tan. Both UVA and UVB radiation cause DNA damage and immunosuppression (6, 46–48). Moreover, powerful UVR tanning units may be 10–15 times

stronger than the midday sun in the Mediterranean Sea area, and repeated exposure to large amounts of UVA, delivered to the skin in relatively short periods (typically 10–20 min) constitutes a new experience for human beings. There are several types and denominations of tanning devices (sunbeds, tanning beds/booths/canopies, and solarium): the term "sunbeds" is commonly used to generally define them all.

In 2012, an updated meta-analysis (6) summarized 27 epidemiological studies that quantified risk of CM associated with artificial UVR tanning. The SRR estimate for "ever" vs. "never use" of indoor tanning was 1.20 (95% CI 1.08–1.34) and the risk was independent of skin sensitivity or population and a dose-response effect was evident. When the analysis was restricted to 18 studies with a population-based sampling of cases and controls, the SRR increased to 1.25 (95% CI 1.09–1.43). The analysis restricted to exposure at a young age in 13 studies showed consistent results. For those starting first exposure to sunbeds before the age of 35 years, and increased risk of 1.59 (95% CI 1.36–1.85) was estimated with no significant between-study heterogeneity and no indication of publication bias. Studies on exposure to indoor tanning and NMSC showed a significantly increased risk of basal cell carcinoma (SRR=1.29; 95% CI 1.08–1.53) and of squamous cell carcinoma (SCC) (SRR=1.67; 95% CI 1.29–2.17). Based on the results of a meta-analysis published in 2009, it could be estimated that of 63,942 new CM cases diagnosed each year in Western Europe, 3,438 (5.4%) could be caused by sunbed use. Women represented the majority of this burden, with 2,341 estimated cases (6.9% of all melanoma cases in women) induced by sunbed use; while the figure for men was 1,096 cases annually (3.7% of all cases in men). Taking a melanoma incidence to mortality ratio of 3.7 for European men and 4.7 for European women in EU15 countries, approximately 498 women and 296 men would die each year from a melanoma caused by artificial UVR tanning.

In 2009, Hirst et al. (46) estimated the numbers of potential skin cancers that could be prevented through regulation of solarium and the associated cost-savings to the Federal Government in Australia (for each 100,000 people: 18–31 melanomas, 200–251 SCCs and $AU 256,054 associated costs).

In a paper published the following year, Hery et al. (49) noted a sharp increase in melanoma incidence among young women in Iceland, which began after 1990 with a peak in 2000. At the same time, the prevalence of sunbeds in Iceland rapidly increased, from 1979 to 1988, suggesting a possible link between the 2 observed trends. However, another possible explanation could be the increase in melanoma screening, which occurred all over Europe in the 1990s. Authors also observed a decline in melanoma rates among women after 2001, following a reduction in prevalence of sunbeds. However, it should be taken into account that the lag time between exposure and melanoma onset is quite long and the decline in melanoma incidence is unlikely to be due to the reduced use of sunbeds in the early 2000s.

Some authors hypothesized that indoor tanning could act as a protective factor for melanoma risk, by preventing sunburns. Recently 2 publications expressed scepticism about the carcinogenicity of indoor tanning (50, 51). Some authors have used the lack of randomized clinical trials (which would be unethical) to imply that the relationship between sunbed use and melanoma is not causal. Suppa & Gandini (52) recently showed, however, that the large amount of data coming from observational studies in fact provides enough information to infer that sunbed use does cause melanoma: they were able to demonstrate the applicability of all epidemiological criteria for causality to the relationship between sunbed use and melanoma. They found that recent studies have reinforced previous knowledge about the detrimental effects of first sunbed exposure at young age, especially in women (53, 54). In fact, new insights on sunbed use have emerged, such as its relevance for the development of additional primary melanomas (55), its association with melanoma of the lower limbs (most common in women) (56) and its correlation with other melanoma risk factors, including high naevus count, atypical naevi and sun damage (57).

The large body of evidence prompted both the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) of the European Commission (58) and the WHO (59) to state that there is no safe limit for exposure to UV radiation from sunbeds.

Interestingly, an Italian survey on 4,703 subjects after the ban on sunbed use before 18 years of age estimated the overall prevalence of sunbed use to be as high as 20%, with higher proportion of female, young and highly-educated users (60). Moreover, participants at high risk of melanoma were those who used sunbeds more frequently: subjects with freckles and with red hair had the higher odds of using sunbeds than subjects without freckles and with dark hair (OR were, respectively, 1.89; 95% CI 1.27–2.80 and 3.92; 95% CI 1.91–8.06). Another Italian survey on 3,089 students highlighted the important role of parents on indoor tanning practices of children (61). Indeed, students who attended a targeted educational intervention were more aware that sunbed use cannot prevent sunburns ($p = 0.03$) than those who did not attend; however, sunbed use by parents influenced the desire to use a sunbed more than participation in the educational intervention ($p < 0.0001$).

## OTHER EPIDEMIOLOGICAL RISK FACTORS RELATED TO ULTRAVIOLET RADIATION

The association of sun exposure with melanoma risk is influenced by other factors such as phenotype.

*Phenotypic characteristics*

Pigmentation characteristics, such as skin colour, hair and eye colour, and freckles are well-established host risk factors for melanoma.

A previous meta-analysis found SRR for blue, green and hazel eye colour compared with dark eye colour of 1.47 (95% CI 1.28–1.69), 1.61 (95% CI 1.06–2.45) and 1.52 (95% CI 1.26–1.83), respectively (62). According to hair colour, the highest association with melanoma was found for red-haired individuals, who have a more than tripled risk of melanoma compared with dark-haired subjects (SRR; 95% CI 3.64; 2.56–5.37). Blond-haired and light brown-haired subjects are, as well as increased melanoma risk, compared with dark-haired subjects (SRR; 1.96; 95% CI 1.41–2.74 and 1.62; 95% CI 1.11–2.34, respectively). Looking at skin colour, light-pigmented subjects had a doubled risk of melanoma compared with darker pigmented subjects (SRR 2.06; 95% CI 1.68–2.52). This result was in agreement with the analysis of skin phototype (defined according to the Fitzpatrick classification as indicator of skin sensitivity to sun): indeed, all 3 lighter skin phototypes I, II and III increased melanoma risk compared with skin phototype IV, with a trend in the calculated SRR, that were, respectively, 2.09 (95% CI 1.67–2.58), 1.84 (95% CI 1.43–2.36) and 1.77 (95% CI 1.23–2.56). Finally, high density of freckles was associated with a significantly doubled risk of melanoma: SRR 2.10 (95% CI 1.80–2.45).

In a recently published population-based prospective study including 38,854 subjects, melanoma risk was assessed in association with pigmentation characteristics and other phenotypes, and additive interactions were explored. During a mean follow-up of 3.5 years, 642 (1.5%) participants developed melanoma. Inability to tan was a recognized risk factor (no tan vs. deep tan hazard ratio (HR) 3.11 (95% CI 1.50–6.43)), while propensity to sunburn was not associated with melanoma after tanning inability was adjusted for (63). The highest population attributable fractions (PAFs), helpful in estimating the burden of disease occurring within sub-groups of a population, were observed for skin phototypes I/II (0.27, 95% CI 0.21–0.31), presence of freckles (0.23, 95% CI 0.19–0.26) and blonde hair (0.23, 95% CI 0.20–0.26). For eye colour, the PAF for blue/blue-grey eye colour was higher than for green/grey/hazel eye colour (0.18 vs. 0.13), while the PAF associated with red hair colour was 0.10 (95% CI 0.09–0.11) compared with 0.23 for blonde and 0.15 for light brown hair colour.

*Common and atypical naevi*

High number of common naevi and the presence of atypical naevi are major risk factors for CM. According to a previous meta-analysis including 10,499 cases and 14,256 controls (64), the presence of more than 100 common naevi was associated with almost 7-times higher risk of melanoma compared with less than 15 common naevi: the SRR was 6.89 (95% CI 4.63–10.25). In the same meta-analysis, the SRR for the presence of at least 5 atypical naevi vs. no atypical naevi was 6.36 (95% CI 3.80–10.33). It was estimated that 42% of melanomas are attributable to having ≥25 common naevi, corresponding to 121,800 patients newly diagnosed with melanoma from an annual worldwide total of 290,000 new cases. Moreover, approximately 25% of melanoma cases are attributable to the presence of one or more atypical naevi, corresponding to an estimated number of 70,000 new cases in 2018. High total body naevus counts (≥50 common naevi) account for approximately 27% of melanoma cases, whereas individuals with few common naevi (0–10) account for only 4% of melanoma cases.

Naevi yield similar relative risks in the UK and Australia, suggesting that genetic factors are important despite different environmental exposure. Multiple naevi might also be an indicator of excessive sun exposure, and thus be associated with an increased risk of CM. A study of Australian children found that increased sun exposure in childhood was significantly associated with an increased number of naevi (65). A separate study of more than 11,000 European children found that sunburns and holidays in the south were significantly associated with high naevus counts and the occurrence of atypical naevi (66). However, it is likely that sun exposure influences smaller naevi on chronically sun-exposed sites and to a lesser extent, larger atypical lesions on intermittently exposed sites, which have more probably a genetic basis (67, 68).

Total naevus count was found to be more strongly associated with CM on intermittently sun-exposed skin (i.e. trunk and legs) than CM on chronically exposed skin (i.e. the head/neck and arms) (37). This may be related to BRAF somatic mutations, which are also more common in CM originating on trunk and legs compared with the head and neck.

A previous prospective cohort study conducted in Australia (64) found that the characteristic most strongly associated with invasive melanoma was self-reported naevus density at age 21 years [many vs. no moles HR 4.91 (95% CI 2.81–8.55)].

Looking at melanoma-related deaths in USA, a recently published prospective study using data from the Nurses' Health Study ($n$=77,288 women) and Health Professionals Follow-up Study ($n$=32,455 men) investigated cutaneous naevi and risk of melanoma death (69). During 26 years of follow-up, 2,452 melanoma cases were histologically confirmed and 196 patients died from melanoma. An increased number of naevi was associated with melanoma death: HR for ≥3 naevi compared with no naevi was 2.49 (95% CI 1.50–4.12) for women and 3.97 (95% CI 2.54–6.22) for men. Among melanoma cases, increased number of naevi was associated with melanoma death in men, but not in women. Similarly, the number of naevi was positively associated with

Breslow thickness in men only ($p$-value for trend 0.01). A possible explanation is that male patients with melanoma and high naevus counts might tend to have their melanomas diagnoses at later stages or may be related to different prevalence of melanoma body sites in men and women. Indeed, melanoma more frequently occurred in men at the head and neck or trunk (sites associated with poorer survival), while it occurred more frequently at the extremities in women (69). The observed differential associations by sex might also reflect other aetiological mechanisms: for instance, the number of naevi had been identified as a phenotypic marker of plasma sex hormone levels, with more naevi associated with higher levels of oestradiol and testosterone (70).

## SUNSCREEN USE

Studies have been inconclusive regarding sunscreen use and the development of naevi among children, with a single randomized trial showing evidence of benefit (71), while other studies have shown a positive association between sunscreen use and naevus prevalence (66, 72–74). An Italian large observational study on 1,512 children and adolescents found that sunscreen users were more likely to develop naevi compared with non-users. Moreover, unlike other paediatric analyses (75), a higher frequency of daily application of sunscreen was associated with a higher naevus count, suggesting that this association cannot be due only to residual confounding. On the other hand the use of high sun protection factor (SPF) (>30) sunscreens exclusively, compared with the use of sunscreens with SPF≤30, adequately protected skin during sun exposure and significantly reduced naevus burden. These results were confirmed by subsequent studies (76–78).

The possible explanation of these findings may be interpreted in the light of 2 considerations. First, children who apply more sunscreen are probably fair-skinned subjects with freckles who tend to be burnt by the sun easily and, consequently, lower skin-phototypes have a greater tendency to develop sunburn and naevi. Secondly, the anti-erythematous effect and a false sense of protection against sunburn conferred by frequent application of sunscreen may lead children to spend more time in the sun and to expose themselves in the middle of the day when ultraviolet rays are stronger (79).

Sunscreen use is recommended for sun protection in addition to clothing and shade (80). Sunscreen can decrease the risk of sunburn and SCC (82).

Meta-analyses of observational studies showed no effect of sunscreens on melanoma risk, but the results of the studies are difficult to interpret due to lack of adjustment for potential confounders (82).

The only randomized controlled trial showed a decreased melanoma risk of subjects who used sunscreen daily compared with discretionary sunscreen use (78). However, this trial was conducted among subjects who lived in Australia, a country with very high ambient solar radiation and high awareness of skin cancer.

Recently, the Norwegian Women and Cancer Study (83), a prospective population-based study of 143,844 women and 722 cases of melanoma, showed that sunscreen users reported significantly more sunburns and sunbathing vacations and were more likely to use indoor tanning devices. However, SPF ≥15 sunscreen use was associated with significantly decreased melanoma risk compared with SPF <15 use. The estimated decrease in melanoma (PAF) with general use of SPF ≥15 sunscreens by women age 40–75 years was 18% (95% CI 4–30%).

Primary skin cancer prevention behaviours, focusing on reducing the amount of UVR reaching the skin, include covering sun-exposed skin, wearing a hat and sunglasses, and sunscreen use. There is no high-quality experimental evidence on the efficacy of sunscreen to prevent melanoma; however it is important that patients and consumers do not stop protecting their skin until better-quality evidence emerges. The important message is that sunscreen should not be an excuse to prolong intentional sun exposure.

## REFERENCES


1. American Cancer Society. Cancer facts and figures. Atlanta: American Cancer Society; 2018.
2. GLOBOCAN 2018. Available from: http://gco.iarc.fr/databases.php. [accessed Oct 2018].
3. Mahendraraj K, Sidhu K, Lau CS, McRoy GJ, Chamberlain RS, Smith FO. Malignant melanoma in African-Americans: a population-based clinical outcomes study involving 1106 African-American patients from the Surveillance, Epidemiology, and End Result (SEER) Database (1988–2011). Medicine (Baltimore) 2017; 96: e6258.
4. Erdmann F, Lortet-Tieulent J, Schuz J, Zeeb H, Greinert R, Breitbart EW, et al. International trends in the incidence of malignant melanoma 1953–2008 – are recent generations at higher or lower risk? Int J Cancer 2013; 132: 385–400.
5. Watson M, Geller AC, Tucker MA, Guy GP Jr, Weinstock MA. Melanoma burden and recent trends among non-Hispanic whites aged 15–49 years, United States. Prev Med 2016; 91: 294–298.
6. Boniol M, Autier P, Boyle P, Gandini S. Cutaneous melanoma attributable to sunbed use: systematic review and meta-analysis. BMJ 2012; 345: e4757.
7. Gandini S, Stanganelli I, Magi S, Mazzoni L, Medri M, Agnoletti V, et al. Melanoma attributable to sunbed use and tan seeking behaviours: an Italian survey. Eur J Dermatol 2014; 24: 35–40.
8. Brady MS, Oliveria SA, Christos PJ, Berwick M, Coit DG, Katz J, et al. Patterns of detection in patients with cutaneous melanoma. Cancer 2000; 89: 342–347.
9. Miller DR, Geller AC, Wyatt SW, Halpern A, Howell JB, Cockerell C, et al. Melanoma awareness and self-examination practices: results of a United States survey. J Am Acad Dermatol 1996; 34: 962–970.
10. Joosse A, Collette S, Suciu S, Nijsten T, Lejeune F, Kleeberg UR, et al. Superior outcome of women with stage I/II cutaneous melanoma: pooled analysis of four European Organisation for Research and Treatment of Cancer phase III trials. J Clin Oncol 2012; 30: 2240–2247.
11. Courtenay WH. Constructions of masculinity and their influence on men's well-being: a theory of gender and health. Soc Sci Med 2000; 50: 1385–1401.

12. Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer. J Photochem Photobiol B 2001; 63: 8–18.
13. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Picconi O, Boyle P, et al. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer 2005; 41: 45–60.
14. O'Sullivan DE, Brenner DR, Villeneuve PJ, Walter SD, Demers PA, Friedenreich CM, et al. Estimates of the current and future burden of melanoma attributable to ultraviolet radiation in Canada. Prev Med 2019; 122: 81–90.
15. US Department of Health & Human Services. The Surgeon General's Call to Action to Prevent Skin Cancer. Washington DC: US Department of Health and Human Services; 2014.
16. LoConte NK, Gershenwald JE, Thomson CA, Crane TE, Harmon GE, Rechis R. Lifestyle modifications and policy implications for primary and secondary cancer prevention: diet, exercise, sun safety, and alcohol reduction. Am Soc Clin Oncol Educ Book 2018; 38: 88–100.
17. Centers for Disease Control and Prevention. Youth risk behavior surveillance system: 1991–2013 high school youth risk behavior survey data. [accessed July 12 2019]. Available from: https://nccd cdc gov/Youthonline/App/Default aspx.
18. Holman CD, Armstrong BK, Heenan PJ. A theory of the etiology and pathogenesis of human cutaneous malignant melanoma. J Natl Cancer Inst 1983; 71: 651–656.
19. Green A. A theory of site distribution of melanomas: Queensland, Australia. Cancer Causes Control 1992; 3: 513–516.
20. Whiteman DC, Watt P, Purdie DM, Hughes MC, Hayward NK, Green AC. Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. J Natl Cancer Inst 2003; 95: 806–812.
21. Bataille V, Sasieni P, Grulich A, Swerdlow A, McCarthy W, Hersey P, et al. Solar keratoses: a risk factor for melanoma but negative association with melanocytic naevi. Int J Cancer 1998; 78: 8–12.
22. Elwood JM, Gallagher RP. Body site distribution of cutaneous malignant melanoma in relationship to patterns of sun exposure. Int J Cancer 1998; 78: 276–280.
23. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med 2005; 353: 2135–2147.
24. Maldonado JL, Fridlyand J, Patel H, Jain AN, Busam K, Kageshita T, et al. Determinants of BRAF mutations in primary melanomas. J Natl Cancer Inst 2003; 95: 1878–1890.
25. Thomas NE, Edmiston SN, Alexander A, Millikan RC, Groben PA, Hao H, et al. Number of nevi and early-life ambient UV exposure are associated with BRAF-mutant melanoma. Cancer Epidemiol Biomarkers Prev 2007; 16: 991–997.
26. Kim SY, Kim SN, Hahn HJ, Lee YW, Choe YB, Ahn KJ. Metaanalysis of BRAF mutations and clinicopathologic characteristics in primary melanoma. J Am Acad Dermatol 2015; 72: 1036-46.e2.
27. Griewank KG, Murali R, Puig-Butille JA, Schilling B, Livingstone E, Potrony M, et al. TERT promoter mutation status as an independent prognostic factor in cutaneous melanoma. J Natl Cancer Inst 2014; 106: pii: dju246.
28. Pasquali E, Garcia-Borron JC, Fargnoli MC, Gandini S, Maisonneuve P, Bagnardi V, et al. MC1R variants increased the risk of sporadic cutaneous melanoma in darker-pigmented Caucasians: a pooled-analysis from the M-SKIP project. Int J Cancer 2015; 136: 618–631.
29. Raimondi S, Gandini S, Fargnoli MC, Bagnardi V, Maisonneuve P, Specchia C, et al. Melanocortin-1 receptor, skin cancer and phenotypic characteristics (M-SKIP) project: study design and methods for pooling results of genetic epidemiological studies. BMC Med Res Methodol 2012; 12: 116.
30. Tagliabue E, Gandini S, Bellocco R, Maisonneuve P, Newton-Bishop J, Polsky D, 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; 10: 1143–1154.
31. Box NF, Duffy DL, Chen W, Stark M, Martin NG, Sturm RA, et al. MC1R genotype modifies risk of melanoma in families segregating CDKN2A mutations. Am J Hum Genet 2001; 69: 765–773.
32. Landi MT, Bauer J, Pfeiffer RM, Elder DE, Hulley B, Minghetti P, et al. MC1R germline variants confer risk for BRAF-mutant melanoma. Science 2006; 313: 521–522.
33. Whiteman DC, Parsons PG, Green AC. P53 expression and risk factors for cutaneous melanoma: a case-control study. Int J Cancer 1998; 77: 843–848.
34. de Snoo FA, Hayward NK. Cutaneous melanoma susceptibility and progression genes. Cancer Lett 2005; 230: 153–186.
35. Rivers JK. Is there more than one road to melanoma? Lancet 2004; 363: 728–730.
36. Newell GR, Sider JG, Bergfelt L, Kripke ML. Incidence of cutaneous melanoma in the United States by histology with special reference to the face. Cancer Res 1988; 48: 5036–5041.
37. Caini S, Gandini S, Sera F, Raimondi S, Fargnoli MC, Boniol M, et al. Meta-analysis of risk factors for cutaneous melanoma according to anatomical site and clinico-pathological variant. Eur J Cancer 2009; 45: 3054–3063.
38. Lin HC, Shieh BH, Lu MH, Chen JY, Chang LT, Chao CF. A method for quantifying melanosome transfer efficacy from melanocytes to keratinocytes in vitro. Pigment Cell Melanoma Res 2008; 21: 559–564.
39. Cust AE, Mishra K, Berwick M. Melanoma – role of the environment and genetics. Photochem Photobiol Sci 2018; 17: 1853–1860.
40. de Gruijl FR. UV adaptation: pigmentation and protection against overexposure. Exp Dermatol 2017; 26: 557–562.
41. Sheehan JM, Cragg N, Chadwick CA, Potten CS, Young AR. Repeated ultraviolet exposure affords the same protection against DNA photodamage and erythema in human skin types II and IV but is associated with faster DNA repair in skin type IV. J Invest Dermatol 2002; 118: 825–829.
42. Holman CD, Armstrong BK. Cutaneous malignant melanoma and indicators of total accumulated exposure to the sun: an analysis separating histogenetic types. J Natl Cancer Inst 1984; 73: 75–82.
43. Oliveria SA, Saraiya M, Geller AC, Heneghan MK, Jorgensen C. Sun exposure and risk of melanoma. Arch Dis Child 2006; 91: 131–138.
44. Weinstock MA, Colditz GA, Willett WC, Stampfer MJ, Bronstein BR, Mihm MC,Jr, et al. Nonfamilial cutaneous melanoma incidence in women associated with sun exposure before 20 years of age. Pediatrics 1989; 84: 199–204.
45. Osterlind A. Malignant melanoma in Denmark. Occurrence and risk factors. Acta Oncol 1990; 29: 833–854.
46. Hirst N, Gordon L, Gies P, Green AC. Estimation of avoidable skin cancers and cost-savings to government associated with regulation of the solarium industry in Australia. Health Policy 2009; 89: 303–311.
47. Wehner MR, Chren MM, Nameth D, Choudhry A, Gaskins M, Nead KT, et al. International prevalence of indoor tanning: a systematic review and meta-analysis. JAMA Dermatol 2014; 150: 390–400.
48. Wehner MR, Shive ML, Chren MM, Han J, Qureshi AA, Linos E. Indoor tanning and non-melanoma skin cancer: systematic review and meta-analysis. BMJ 2012; 345: e5909.
49. Hery C, Tryggvadottir L, Sigurdsson T, Olafsdottir E, Sigurgeirsson B, Jonasson JG, et al. A melanoma epidemic in Iceland: possible influence of sunbed use. Am J Epidemiol 2010; 172: 762–767.
50. Burgard B, Schope J, Holzschuh I, Schiekofer C, Reichrath S, Stefan W, et al. Solarium use and risk for malignant melanoma: meta-analysis and evidence-based medicine systematic review. Anticancer Res 2018; 38: 1187–1199.
51. Reichrath J, Lindqvist PG, DE Gruijl FR, Pilz S, Kimball SM, Grant WB, et al. A critical appraisal of the recent reports on sunbeds from the European Commission's Scientific Committee on Health, Environmental and Emerging Risks and from the World Health Organization. Anticancer Res 2018; 38: 1111–1120.
52. Suppa M, Gandini S. Sunbeds and melanoma risk: time to close the debate. Curr Opin Oncol 2019; 31: 65–71.
53. Lazovich D, Isaksson Vogel R, Weinstock MA, Nelson HH, Ahmed RL, Berwick M. Association between indoor tanning and melanoma in younger men and women. JAMA Dermatol

2016; 152: 268–275.
54. 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; 185: 147–156.
55. Li Y, Kulkarni M, Trinkaus K, Cornelius LA. Second primary melanomas: Increased risk and decreased time to presentation in patients exposed to tanning beds. J Am Acad Dermatol 2018; 79: 1101–1108.
56. Ghiasvand R, Robsahm TE, Green AC, Rueegg CS, Weiderpass E, Lund E, et al. Association of phenotypic characteristics and UV radiation exposure with risk of melanoma on different body sites. JAMA Dermatol 2019; 155: 39–49.
57. Suppa M, Gandini S, Njimi H, Bulliard JL, Correia O, Duarte AF, et al. Association of sunbed use with skin cancer risk factors in Europe: an investigation within the Euromelanoma skin cancer prevention campaign. J Eur Acad Dermatol Venereol 2019; 33: 76–88.
58. SCHEER (Scientific Committee on Health Environmental and Emerging Risks). Opinion on Biological effects of ultraviolet radiation relevant to health with particular reference to sunbeds for cosmetic purposes, 17 November 2017. [accessed 15 December 2018]. Available from: http://ec.europa.eu/health/scientific_committees/scheer/docs/scheer_o_003.pdf.
59. World Health Organization. Artificial tanning devices: public health interventions to manage sunbeds. 2017. [accessed: 15 December 2018]. Available from https://www.who.int/uv/publications/artificial-tanning-devices/en/.
60. Stanganelli I, Gandini S, Magi S, Mazzoni L, Medri M, Agnoletti V, et al. Sunbed use among subjects at high risk of melanoma: an Italian survey after the ban. Br J Dermatol 2013; 169: 351–357.
61. Stanganelli I, Naldi L, Falcini F, Magi S, Mazzoni L, Medri M, et al. Parental use and educational campaigns on sunbed use among teenagers and adolescents. Medicine (Baltimore) 2016; 95: e3034.
62. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Zanetti R, Masini C, et al. Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. Eur J Cancer 2005; 41: 2040–2059.
63. Olsen CM, Pandeya N, Thompson BS, Dusingize JC, Green AC, Neale RE, et al. Association between phenotypic characteristics and melanoma in a large prospective cohort study. J Invest Dermatol 2019; 139: 665–672.
64. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Abeni D, Boyle P, et al. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. Eur J Cancer 2005; 41: 28–44.
65. Harrison SL, MacLennan R, Speare R, Wronski I. Sun exposure and melanocytic naevi in young Australian children. Lancet 1994; 344: 1529–1532.
66. Dulon M, Weichenthal M, Blettner M, Breitbart M, Hetzer M, Greinert R, et al. Sun exposure and number of nevi in 5- to 6-year-old European children. J Clin Epidemiol 2002; 55: 1075–1081.
67. Lopez-Ravello BM, Arias-Santiago S, Fernandez-Pugnaire MA, Ortega SS, Buendia-Eisman A. Prevalence of common and atypical melanocytic nevi in young adults and its relationship with sun protection and exposure habits. Eur J Dermatol 2015; 25: 45–51.
68. Satagopan JM, Iasonos A, Zhou Q. Prognostic and predictive values and statistical interactions in the era of targeted treatment. Genet Epidemiol 2015; 39: 509–517.
69. Li WQ, Cho E, Weinstock MA, Li S, Stampfer MJ, Qureshi AA. Cutaneous nevi and risk of melanoma death in women and men: a prospective study. J Am Acad Dermatol 2019; 80: 1284–1291.
70. Zhang M, Zhang X, Qureshi AA, Eliassen AH, Hankinson SE, Han J. Association between cutaneous nevi and breast cancer in the Nurses' Health Study: a prospective cohort study. PLoS Med 2014; 11: e1001659.
71. Gallagher RP, Rivers JK, Lee TK, Bajdik CD, McLean DI, Coldman AJ. Broad-spectrum sunscreen use and the development of new nevi in white children: a randomized controlled trial. JAMA 2000; 283: 2955–2960.
72. Azizi E, Iscovich J, Pavlotsky F, Shafir R, Luria I, Federenko L, et al. Use of sunscreen is linked with elevated naevi counts in Israeli school children and adolescents. Melanoma Res 2000; 10: 491–498.
73. Luther H, Altmeyer P, Garbe C, Ellwanger U, Jahn S, Hoffmann K, et al. Increase of melanocytic nevus counts in children during 5 years of follow-up and analysis of associated factors. Arch Dermatol 1996; 132: 1473–1478.
74. Darlington S, Siskind V, Green L, Green A. Longitudinal study of melanocytic nevi in adolescents. J Am Acad Dermatol 2002; 46: 715–722.
75. Whiteman DC, Brown RM, Purdie DM, Hughes MC. Melanocytic nevi in very young children: the role of phenotype, sun exposure, and sun protection. J Am Acad Dermatol 2005; 52: 40–47.
76. Olsen CM, Thompson BS, Green AC, Neale RE, Whiteman DC, QSkin Sun and Health Study Group. Sun protection and skin examination practices in a setting of high ambient solar radiation: a population-based cohort study. JAMA Dermatol 2015; 151: 982–990.
77. Olsen CM, Wilson LF, Green AC, Bain CJ, Fritschi L, Neale RE, et al. Cancers in Australia attributable to exposure to solar ultraviolet radiation and prevented by regular sunscreen use. Aust NZ J Public Health 2015; 39: 471–476.
78. Green AC, Williams GM, Logan V, Strutton GM. Reduced melanoma after regular sunscreen use: randomized trial follow-up. J Clin Oncol 2011; 29: 257–263.
79. Rodvall YE, Wahlgren CF, Ullen HT, Wiklund KE. Factors related to being sunburnt in 7-year-old children in Sweden. Eur J Cancer 2010; 46: 566–572.
80. Linos E, Keiser E, Fu T, Colditz G, Chen S, Tang JY. Hat, shade, long sleeves, or sunscreen? Rethinking US sun protection messages based on their relative effectiveness. Cancer Causes Control 2011; 22: 1067–1071.
81. Green A, Williams G, Neale R, Hart V, Leslie D, Parsons P, et al. Daily sunscreen application and betacarotene supplementation in prevention of basal-cell and squamous-cell carcinomas of the skin: a randomised controlled trial. Lancet 1999; 354: 723–729.
82. Dennis LK, Beane Freeman LE, VanBeek MJ. Sunscreen use and the risk for melanoma: a quantitative review. Ann Intern Med 2003; 139: 966–978.
83. Ghiasvand R, Weiderpass E, Green AC, Lund E, Veierod MB. Sunscreen use and subsequent melanoma risk: a population-based cohort study. J Clin Oncol 2016; 34: 3976–3983.

Centenary theme section: SKIN MALIGNANCIES

REVIEW ARTICLE

# It's Not All Sunshine: Non-sun-related Melanoma Risk-factors

Veronique BATAILLE[1,2]
[1]Twin Research and Genetic Epidemiology Department, Kings College, London, and [2]Dermatology Department, West Herts NHS Trust, Herts, UK. E-mail: bataille@doctors.org.uk

**There is increasing evidence that the behaviour of naevi and melanoma is under significant genetic and/or epigenetic control. Melanoma tumours behaves similarly all over the world. Many genes have now been implicated in melanoma risk and naevi number. Embryogenesis has also been important in the discovery of links between several neurological diseases and melanoma susceptibility. Telomere biology, which regulates cell senescence, is increasingly relevant in melanoma. Melanoma is often found in the context of family cancer syndromes and the identification of these families is important as screening for cancer will save lives. Melanoma is also one of the most immunogenic cancer as the behaviour of naevi and melanoma differ in patients with vitiligo or eczema. The search for non-sun-related melanoma risk factors should continue as it is likely to lead to important discoveries which will, in turn, have an impact on therapeutic targets for this tumour.**

*Key words:* telomere; naevi; vitamin D; family cancer syndromes; body mass index.

Accepted Apr 27, 2020; Epub ahead of print Apr 28, 2020

Acta Derm Venereol 2020; 100: adv00137.

*Corr:* Veronique Bataille, Twin Research and Genetic Epidemiology Department, Kings College, London, UK. E-mail: bataille@doctors.org.uk

## SIGNIFICANCE

Many risk factors for melanoma are non-UV-related and progress in the last 20 years have been instrumental in discovering melanoma genes which are involved in telomere biology, naevi number, pigmentation, body composition, energy expenditure, neural and melanocyte differentiation. Melanoma behaves in a very similar way all over the world in all Caucasian populations and many host factors are under tight genetic control. Research in these areas is important as it sheds new light on genetic and epigenetic factors which are often set early on in life and less likely to be influenced by sun exposure in adulthood. It is also unravelling pathways which could be exploited for future therapies as public health campaigns have, so far, not been very effective. Perhaps, the role of sun exposure in melanoma has been over-estimated in the past as, like all cancers, melanoma is a very complex tumour so addressing environmental exposure cannot be the only focus of our efforts.

In this review, the importance of non-sun-related melanoma risk factors are presented looking at telomere biology, genetics, gender differences, body mass index, body sites, naevi biology, immune-related factors and links to neurological disorders.

## TELOMERE AND MELANOMA

Telomeres are strand of non-coding DNA capping the end of chromosomes protecting them from decay. They are having important and complex roles in cell replication and senescence. Protecting against cancer formation is achieved by silencing telomerase which leads to telomere erosion with age. The speed of telomere attrition is under the influence of both genetic and environmental factors. Chronic illnesses and obesity have been associated with shorter telomeres. On the other hand, cancer is usually linked to longer telomeres (1). In 2007, the first report of a link between melanoma susceptibility and telomere biology was suspected with a positive association observed between high number of naevi, the strongest risk factor for melanoma, and circulating white cell telomere length (2). In 2009, case-control studies supported this finding in melanoma case control studies (3). A few years later, a very large melanoma pedigree with no previously known germline mutation, was found to have a germline mutation in the promoter of the *TERT* gene, a telomere gene (4). Mutations in the promoter of the TERT gene were then investigated at the somatic level and were found to be common in melanoma tumours (5). The same year, 11 SNPs in genes predicting white cell telomere length were published (6). Using the same 11 SNPs, genetic scores were created to assess their effects in a large melanoma case control study in the UK. These combined SNPs scores predicting telomere length were confirmed to be predictive of melanoma risk (7).

The associations between TERT promoter mutations, telomerase activity and telomere length are, however, quite complex. It has been shown recently that different SNPs within the TERT promoter have different effects on TERT expression and telomere length despite all being associated with an increased risk of melanoma (8, 9). This implies that the risk of melanoma is not solely explained by elongation of telomeres in some of these families. In rare melanoma families, POT1, another telomere gene, has been identified over the last few years (10). Recent genome wide scan analyses (GWAS) on melanoma and/or naevi number have also identified

further telomere genes (11, 12). There are more telomere genes linked to melanoma susceptibility compared to naevus count highlighting the fact telomere genes do not always drive melanoma risk via an excess of naevi (11). Mutations in telomere genes also raise the risk of many types of cancers so the documentation of all cancers in first- and second-degree relatives of melanoma patients is important. Glioma, neuroblastoma, lung cancer and melanoma are more commonly reported in rare families with telomere mutations but many cancer types can be found (13).

The fact that long telomeres are associated with a susceptibility to melanoma may be behind the observation that individuals within melanoma families with high number of naevi, have reduced cutaneous photoageing. The delayed senescence in melanocytes reflected by the presence of the atypical mole syndrome phenotype is likely to be seen in other cell types such as fibroblasts and keratinocytes (**Fig. 1**). The background for squamous cell carcinoma is, on the contrary, a very photoaged skin. SCC is more likely to be associated with shorter telomeres contrary to melanoma (14). So, by looking at skin phenotypes, short or long telomeres may have opposite effects on signs of cutaneous ageing and, in turn, on specific skin cancer risk (15). This is supported by the negative association between solar keratoses and naevi number both risk factors for melanoma despite adjusting for age (16). This dichotomy has been reported a long time ago via phenotypic studies and is known as the dual pathway to melanoma (17).

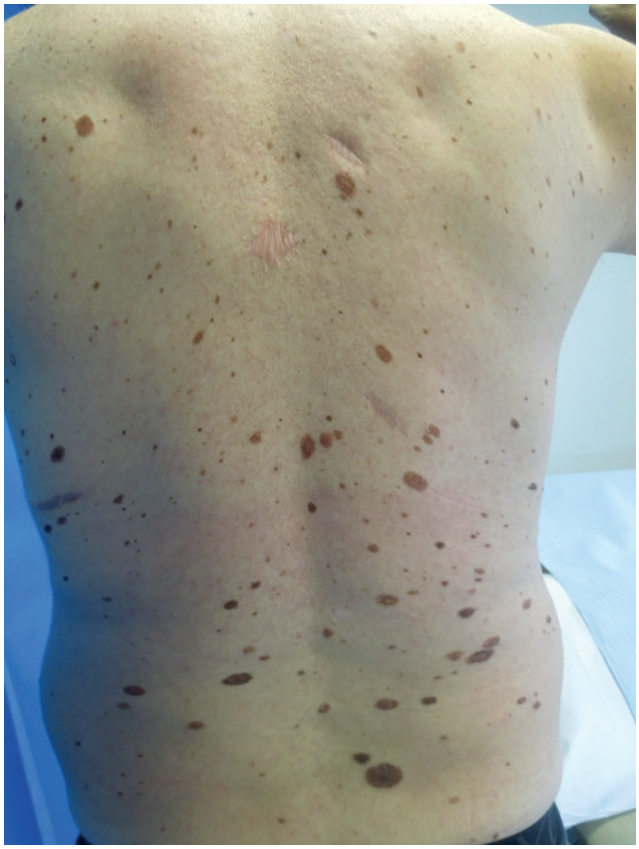

**Fig. 1. Male with the atypical mole syndrome phenotype with previous melanoma primaries.** Presence of larger atypical naevi towards the lower back.

Melanoma survival is also affected by TERT promoter mutations with worst survival for those carrying different types of mutations. This contrasts with a study published by Ribero et al. (18) showing that large number of naevi (hence predicted longer telomeres) confers a survival advantage in melanoma even in patients with positive sentinel node. However, as mention above, not all telomere gene mutations have the same effect on telomere length so this may explain opposite effects. Telomere biology is also important for potential therapeutic targets: RAS mutated melanomas represent 25% of melanoma tumours and have not had, as yet, effective gene targeted treatments. These RAS mutated melanomas appear to have a dependency on TERT which could be exploited for slowing melanoma growth (19, 20).

The balance between long telomeres leading to an increased risk of cancer versus short telomeres leading to premature ageing with frailty needs to be fine-tuned as the extreme spectrums of long and short telomere syndromes show that belonging to either of these extreme group is not advantageous (1). Most melanoma patients survive their disease and the beneficial impact of longer telomeres is likely to be apparent in old age with reduced senescence in many cell types. It could therefore be speculated that genes associated with melanoma susceptibility may have a survival advantage and have therefore remained common in Caucasian populations.

## MELANOMA AND FAMILY CANCER SYNDROMES

Melanoma is more common in cancer prone families as discussed above. However, many other non-telomere genes can be implicated in cancer susceptibility within these families. P16 or CDKN2A was one of the first melanoma gene discovered more than 20 years ago and mutations in this gene lead to an increased risk of melanoma, pancreatic cancer, lung cancer and many other tumours (21). The recruitment of melanoma families for genetic studies over the last 20 years mainly included families with multiple melanomas so family cancer syndromes were excluded. It is, however, well known that some melanoma families may present with many different cancer primaries. These family cancer syndromes are now being studied as well with collaborations from many countries via the GENOMEL consortium (www.genomel.org) with many new genes shared with other cancers being discovered. This is why melanoma germline genetic panel have become more comprehensive. The risk of melanoma in these families is higher in Australia compared to the UK so the penetrance of rare high penetrance genes such as

p16/CDKN2A is affected, in part, by sun exposure. Screening bias is also at play in Australia with many borderline melanomas excised in Australia compared to Europe in view of the active skin surveillance there. Many individuals with p16/CDKN2A mutations have the atypical mole syndrome phenotype usually evident by late teens. However, this phenotype is not always found in mutant individuals so using the naevus phenotype to select family members at risk is not reliable (22). Individuals within these families have reduced senescence in many cell types and not only melanocytes and therefore patients with high number of naevi have reduced photoageing, higher bone mineral density and better cognitive functions with age (23, 24).

Families with BPA1 mutations may present with clinically and histologically recognisable lesions typical of this syndrome called BAPOMAs. These families also have an increased risk of skin and eye melanoma, kidney cancer, mesothelioma and breast cancer (25). BRCA1 and BRCA2 families, apart from the high risk of breast and ovarian cancer, also have an increased but smaller risk of both skin and eye melanoma. However, eye melanoma is a rare tumour and there is no need to offer screening for this as retinal photography is now being offered by many opticians. Melanoma can also occur in rare retinoblastoma families because of the link between the Rb gene and the CDKN2A/CDK4 pathway. Neurofibromatosis families are at risk of melanoma because of the role of NF1 in melanocyte differentiation and growth. This syndrome is part of a group of diseases called Rasopathies where melanoma is more commonly seen such as Noonan syndrome and Leopard syndrome (26). MITF mutations predispose to melanoma and kidney cancer (27, 28). The *MITF* gene is a crucial gene in melanocyte differentiation.

Many cancer genetic clinics now include p16/CDKN2A and CDK4 in their panels as well *MITF*, *BAP1* and several telomere genes. However, it is likely that very soon all melanoma families will undergo much more comprehensive gene panel for mutation screening as they are becoming cheaper. These panels should not be limited to melanoma genes only. This will be beneficial for these families as the identification genes linked to other cancers such as colon, kidney, breast and ovary, for example, can be addressed with specific screening recommendations for the family and will save lives. Genes associated with melanoma in the context of family cancer syndromes are summarised in **Table I**.

**Table I. Genes and family cancer syndromes linked to melanoma**

| Genes | Cancers often clustering in the family with melanoma |
|---|---|
| *CDKN2A/p16 and CDK4* | Pancreas, brain and many other tumours |
| *BRAC2* | Breast, prostate, pancreas, eye melanoma |
| *POT1* | Brain, colon, cardiac angiosarcoma, and other cancers |
| *MITF* | Kidney |
| *RB1* | Retinoblastoma, osteosarcoma, soft tissue sarcoma |
| *P53* | Breast, osteosarcoma, leukaemia and other cancers (Li Fraumeni syndrome) |
| *BAP1* | Kidney, mesothelioma, eye melanoma, brain, breast |
| *PTEN* | Breast, colon, uterus, hamartomas (Cowden syndrome) |
| *CHEK2* | Breast, colon, prostate |

## BODY MASS INDEX AND MELANOMA

Melanoma risk, like all cancers, is related to body mass index (BMI) (29). Whilst it was thought to be mainly driven by increased weight, the relationship is mainly driven by height. Naevus count is also related to height rather than weight and it is speculated that growth factors are important in melanoma susceptibility. One possible explanation for this observation is telomere biology. Telomere length and cancer risk are also positively associated with height (24, 29). High BMI or obesity is, on the contrary, inversely, correlated with telomere length (30). Another observation is that patient with the atypical mole syndrome phenotype are usually taller than average but not significantly overweight or underweight with strong muscular mass. There is therefore some interesting links between melanoma susceptibility and body composition and growth. Bone mineral density is also correlated with number of naevi and this remains significant despite adjusting for telomere length, so bone senescence is also delayed in these patients as discussed above (24).

Another paradox in melanoma is the lack of cachexia in advanced melanoma. Compared to other cancers, melanoma patients in stage 4 of the disease present with weight loss very late in the evolution of their metastatic disease. There is also some evidence that melanoma patients treated with immunotherapy have different treatment responses according to fat distribution with better responses in patients with higher subcutaneous fat and strong muscle mass but not with high fat mass and low muscle mass (31).

Insulin metabolism and energy expenditure may also have a role in melanoma. However, a recent study showed that levels of IGF1 were not linked to an increased risk (32). The melanocyte-stimulating hormone (MSH) pathway is also relevant as, apart from the *MC1R* gene controlling pigmentation and other immune-related factors, other genes in the MSH pathway such as MC4R gene are also important in energy expenditure. In animal models, weight is related to colour coat pigmentation (33). The *FTO* gene, also linked to obesity, is reported in melanoma GWAS (33). However, there is some evidence that the *FTO* gene may not act via its effects on obesity as SNPs involved in melanoma differ from those reported in high BMI (34, 35). The effects may, in fact, be mediated by pathways shared between the *FTO* gene and telomere genes (36).

## VITAMIN D AND MELANOMA

Vitamin D has been found to have a significant role in melanoma survival as low levels of serum vitamin D are a negative prognostic indicator in melanoma (37, 38). On the contrary, patients with high vitamin D levels have thinner melanoma tumours but also have higher number of naevi. The relationship between high number of naevi and higher vitamin D levels is complex but despite adjusting for age and skin type, the association between high number of naevi and high vitamin D remains (39). Further adjustment for telomere length (as telomere length affects vitamin D levels as well), decreases the magnitude of the association but it remains significant. This shows that whilst telomere biology is important in the relationship between melanoma and vitamin D metabolism, other factors are at play. This has implications for public health as patients are advised to avoid sun exposure after a melanoma diagnosis and this may affect their survival. This is supported by a study showing that sun exposure after diagnosis of melanoma was protective in terms of relapse in Italy (40).

## MELANOMA AND GENDER

Melanoma behaves differently in women and men both in terms of body sites and survival. It is well established that melanoma in females are more common on the legs compared to males and the reverse is true for males where melanoma is commonest on the trunk. This difference in body sites is observed all over the world and sun exposure levels do not affect it. Furthermore, the distribution of naevi in girls versus boys is already different earlier on in life and mirrors the distribution of melanoma in adults: boys have more naevi on the torso and girls have more naevi on the limbs, especially the legs. There is therefore some sex specific melanocyte migration which does not appear to be related to sun exposure. A recent study showed that genes/loci already known to predict naevi numbers such as IRF4, DOCK8, MTAP, 9q31.2, KITLG and PLA2G6 have different effects on naevi numbers on the torso versus limbs versus head (41). It is likely that epigenetic effects with X inactivation in females explain, in part, some of these sex differences for naevi and melanoma. Females with Turner syndrome with a XO genotype have large number of naevi on the limbs and are also more prone to melanoma and brain tumours (42).

## TYPES OF NAEVI AND BODY SITES

It is evident for dermatologists that some type of naevi have a predilection for specific body sites. Intradermal naevi are more common on the face and rarely seen on distal limbs. Atypical naevi are more common on the central body and rarer on distal limbs and extremely rare on the face. This again most probably relates to specific genetic signals for melanocyte migration and growth at different body sites. Unfortunately, not many studies counting naevi have, so far, differentiated between different types of naevi (intradermal versus compound versus junctional). One twin study in Australia, has collected clinical subtypes of naevi. They have shown that SNPs in the *IRF4* gene, which was the strongest signal for their Australian naevi GWAS based on more than 1,800 adolescent twins, was having opposite effects on flat versus raised naevi. Gene may also have divergent effects according to age when comparing adolescent twins to adult twins (mean age 40–50 years) (11). The different gene effect size according to age groups shows that having very large sample size for GWAS with wide age ranges can identify differential gene expression with age. IRF4 is also a gene linked to freckling, fair skin and tanning ability which shows that skin pigmentation is tightly linked to types and number of naevi (43, 44). It is well known that the atypical mole syndrome with many junctional and intradermal naevi is rare in dark skin phototypes so pigmentation genes not only govern naevi colour, but they also have an effect on size, numbers and clinical subtypes of naevi. Visconti et al. (41) have confirmed in a recent study that body site specific genetic effects exist in females for quite a few known naevi genes/loci such as IRF4, DOCK8, MTAP, 9q31.2, KITLG and PLA2G6. In this large collaborative study, based on many cohorts, the analyses of 3,000 UK twins showed that the heritability of naevus number in females (assessed by comparing MZ to DZ female twin pairs) was the highest on the legs (69%) compared to torso (26%). Leg is also the body site where females have more naevi and a predilection for melanoma, so it is interesting to find that this site is under the strongest genetic control for naevi number.

In high risk melanoma families with the atypical mole syndrome phenotype, it is not uncommon to see large atypical naevi in the parietal area of the scalp and rarely at any other sites on the scalp (**Fig. 2**). In embryogenesis, the head development goes through successive phases which may explain the specific behaviour of naevi on specific part of the head and neck. These scalp naevi often are the first ones to appear in children in high-risk families. It is also observed that in patients with the atypical mole syndrome phenotype, atypical naevi increase in size from the upper back to the lower back especially in males which, again, is likely to be governed by genes differentially expressed at different body sites. However, what is puzzling is that many genes involved in melanocyte migration and differentiation in embryogenesis are not found in melanoma/naevi GWAS. It is likely that these early melanocyte genes interact with other gene pathways. One example for this, is the MITF gene, a very important gene early on in embryogenesis for melanoblast/melanocyte migration. Many melanoma

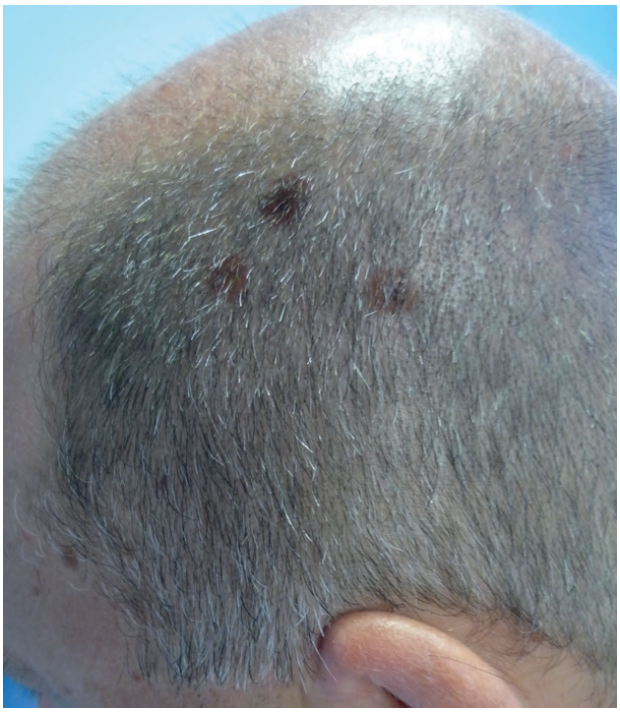

**Fig. 2. Large atypical naevi in the parietal scalp.** These are usually very stable as scalp melanoma is very rare. They are often found in patients with the atypical mole syndrome and just need monitoring and not prophylactic excision.

genes have MITF binding sites so the discovery of new melanoma genes will need to look at all these gene-gene interactions (44).

## NAEVI AND IMMUNOLOGICAL FACTORS

Naevi distribution have an inverse distribution to vitiligo. Vitiligo develops often in folds such as axillae, groins but also on the face around the eyes and mouth as well as on the hands and feet (45). Melanoma and naevi are very rarely found in vitiligo predilection sites. Vitiligo patients also have a reduced risk of melanoma. Melanoma is one of the most immunogenic cancer. It is therefore possible that immunological signals which are inhibitory for melanocyte growth explain this inverse body distribution between vitiligo and naevi/melanoma. Quite of few of the vitiligo genes are shared with melanoma and most of these are related to skin pigmentation. The same SNPs have been reported but have opposite effects in vitiligo versus melanoma which is interesting as it supports the protective effect of vitiligo on melanoma risk. However, how do the same SNPs do offer protection from melanoma in vitiligo patients is unclear (46). Immune-related genes amongst others are likely to affect these divergent associations as CTLA4, a target for the most successful melanoma therapy, is also a vitiligo gene.

Another observation is the lower number of naevi and lower incidence of melanoma in eczema cohorts and this, again, supports the fact that immunological signals in atopic patients may have an inhibitory effect on melanocytes in the skin (47).

Naevi disappear with age, especially junctional and compound naevi and the mechanisms for this process is not fully understood but senescence via genes such as p16, p21 and p53 as well as telomere genes and immune surveillance are likely to all play a role (2, 48). Patients with the atypical mole syndrome phenotype, especially within high risk families, are more likely to present with halo naevi phenomenon than controls. They also show a delayed senescence of naevi with age with large number of naevi persisting well after the age of 50 years. The presence of multiple junctional and atypical naevi after the age of 50 is a reliable sign for dermatologists that an individual is at an increased risk of melanoma.

## MELANOMA AND NEUROLOGICAL DISEASES

Naevi originates from the neural crest and it has long been observed that melanoma and Parkinson disease can cluster in some families. Many melanoma genes were later found to be Parkinson genes such as *PLA2G6, BAP1*, *DCC*, *ERBB4*, *KIT, MAPK2*, *MITF*, *PTEN*, and *TP53* (49). Pigmentation may also be important in the link between Parkinson and melanoma as fair skin is more prevalent in Parkinson cohorts and, so far, the *MC1R* gene has been implicated (50).

Charcot Marie Tooth and amyotrophic lateral sclerosis (ALS) are also neurological diseases linked to melanoma (51). The association between these neural diseases and cancer risk is puzzling as Parkinson disease and ALS have, in fact, an overall reduced risk of cancer so the link to melanoma may be because of the neural connection (51).

The NF1 gene is an important prognostic factor for melanoma at the somatic level and patients with neurofibromatosis have an increased risk of melanoma (52). NF1 positive tumours are more likely to be found in the elderly and often have a desmoplastic histology (52). Neurofibromatosis, is part of a group of diseases called Rasopathies such as Noonan syndrome, Leopard syndrome and Leguis syndromes. All these disorders are characterised by the activation of the MAP kinase pathway which is highly relevant in melanoma (26).

## SUMMARY

In summary, many risk factors for melanoma are non-UV-related and progress in the last 20 years have been instrumental in discovering melanoma genes which are involved in telomere biology, naevi number, pigmentation, body composition, energy expenditure, neural and melanocyte differentiation. Melanoma behaves in a very similar way all over the world in all Caucasian populations and many host factors are under tight genetic control. Research in these areas is important as it sheds new

light on genetic and epigenetic factors which are often set early on in life and less likely to be influenced by sun exposure in adulthood. It is also unravelling pathways which could be exploited for future therapies. Although excessive sun exposure is associated with melanoma risk, research on non-sun-related risk factors is important to redress the balance. The collection of good phenotypic and familial data as well as tumour and blood DNA is crucial for future genetic-epidemiological studies.

## REFERENCES


1. McNally EJ, Luncsford PJ, Armanios M. Long telomeres and cancer risk: the price of cellular immortality. J Clin Invest 2019; 130: pii 10.1172/JCI/20851.
2. Bataille V, Kato BS, Falchi M, Gardner J, Kimura M, Lens M, et al. Nevus size and number are associated with telomere length and represent potential markers of a decreased senescence in vivo. Cancer Epidemiol Biomarkers Prev 2007: 16: 1499–1502.
3. Caini S, Raimondi S, Johansson H, De Giorgi V, Zanna I, Palli D, et al. Telomere length and the risk of cutaneous melanoma and non-melanoma skin cancer: a review of the literature and meta-analysis. J Dermatol Sci 2015; 80: 168–174.
4. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, et al. TERT promoter mutations in familial and sporadic melanoma. Science 2013; 2339: 959–961.
5. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA, et al. Highly recurrent TERT promoter mutations in human melanoma. Science 2013; 339: 957–959.
6. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, et al. Identification of seven loci affecting mean telomere length and their association with disease. Nat Genet 2013; 45: 422–427.
7. Iles MM, Bishop DT, Taylor JC, Hayward NK, Brossard M, Cust AE. The effect on melanoma risk of genes previously associated with telomere length. J Natl Cancer Inst 2014; 106: dju267.
8. Shaugnessy M, Njaw CN, Artomov M, Tsao H. Classifying melanoma by TERT promoter mutational status. J Invest Dermatol 2020; 140: 390–394.
9. Rachaconda S, Kong H, Srinivas N, Garcia-Casado Z, Requena C, Fallah M, et al. Telomere length, telomerase reverse transcriptase promoter mutations and melanoma risk. Genes Chromosomes Cancer 2018; 57: 564–572.
10. Shi J, Yang XR, Ballew B, Rotunno M, Calista D, Fargnoli, et al. Rare missense variants in POT1 predispose to familial cutaneous malignant melanoma. Nat Genet 2014; 46: 482–486.
11. Duffy DL, Iles MM, Glass, Zhu G, Barrett JH, Holom V, et al. IRF4 variants have age-specific effects on nevus count and predispose to melanoma. Am J Hum Genet 2010; 87: 6–16.
12. Potjer TP, Bollen S, Grimbergen AJEM, Van Doorn R, Gruis NA, et al. Multigene panel sequencing of established and candidate melanoma susceptibility genes in a large cohort of Dutch non CDKN2A/CDK4 melanoma families. Int J Cancer 2019; 144: 2453–2464.
13. Telomeres Mendelian Randomization Collaboration, Haycock PC, Burgess S, Nounu A, Zheng J, Nounu A, et al. Association between telomere length and risk of cancer and non-neoplastic diseases: a Mendelian randomization study. JAMA Oncol 2017; 3: 636–651.
14. Anic GM, Sondak VK, Messina JL, Fenske NA, Zager JS, Cherpelis, et al. Telomere length and the risk of melanoma, squamous cell carcinoma and basal cell carcinoma. Cancer Epidemiol 2013; 37: 434–439.
15. Ribero S, Mangino M, Bataille V. Skin phenotypes can offer some insight about the association between telomere length and cancer susceptibility. Med Hypothesis 2016; 97: 7–10.
16. Bataille V, Sasinei P, Grulich A, Swerdlow A, McCarthy W, Hersey P, et al. Solar keratoses: a risk factor for melanoma but negative association with melanocytic nevi. Int J Cancer 1998; 78: 8–12.
17. Whiteman DC, Watt P, Purdie DM, Hughes MC, Hayward NK, Green AC. Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. J Natl Cancer Inst 2003; 95: 806–812.
18. Ribero S, Davies JR, Requena C, Carrera C, Glass D, Quaglino, et al. High number of nevi confer a favorable prognosis in melanoma. Int J Cancer 2015; 13: 1691–1698.
19. Reyes-Uribe P, Adrianzen-Ruesta MP, Deng Z, Echevarria-Vargas I, Mender I, et al. Exploiting TERT dependency as a therapeutic strategy for NRAS mutant melanoma. Oncogene 2018; 37: 4058–4072.
20. Zhang G, Wu LW, Mender I, Barzily-Rokni M, Hammond MR, Ope O, et al. Induction of telomere dysfunction prolongs disease control of therapy resistant melanoma. Clin Cancer Res 2018; 24: 4771–4784.
21. Goldstein AM, Chan M, Harland M, Hayward NK, Demenais F, Bishop DT, et al. Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. J Med Genet 2007; 44: 99–106.
22. Taylor NJ, Mitra N, Goldstein AM, Tucker MA, Avril MF, Azizi E, et al. Germline variation at CDKN2A and associations with nevus phenotypes among members of melanoma families. J Invest Dermatol 2017; 137: 2606–2612.
23. Masi S, Georgiopoulos G, Ribero S, Taddei S, Bataille V, Steves CJ. The relationship between naevus count, memory function and telomere length in the TwinsUK cohort. Pigment Cell Melanoma Res 2018; 31: 720–724.
24. Ribero S, Glass D, Aviv A, Spector TD, Bataille V. Height and bone mineral density are associated with naevus count supporting the importance of growth in melanoma susceptibility. PLoS One 2015; 10: e0116863.
25. Wiesner T, Obenauf AC, Murali R, Fried I, Griewank KG, Ulz P, et al. Germline mutations in BAP1 predispose to melanocytic tumors. Nat Genet 2011; 43: 1018–1021.
26. Halaban R, Krauthammer M. RASopathy gene mutations in melanoma. J Invest Dermatol 2016; 136: 1755–1759.
27. Bertolotto C, Lesueur F, Giuliano S, Strub T, de Lichy M, Bille K, et al. A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. Nature 2011; 480: 94–98.
28. Yokoyama S, Woods SL, Boyle GM, Aoude LG, MacGregor S, Zismann V, et al. A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma. Nature 2011; 480: 99–103.
29. Nunney L. Size matter: height, cell number and a person's risk of cancer. Proc Biol Sci 2018; 285: pii 20181743.
30. Gielen M, Hageman GJ, Antoniou EE, Nordjfall K, Mangino M, Balasubramanyam M, et al. Body mass index is negatively associated with telomere length: a collaborative cross-sectional meta-analysis of 87 observational studies. M J Clin Nutr 2018; 108: 453–475.
31. Popinat G, Cousse S, Goldfarb L, Becker S, Gardin I, Salaun M, et al. Sub-cutaneous fat measured on multislice computed tomography of pre-treatment PET/CT is a prognostic factor of stage 4 non small cell lung cancer treated with Nivolumab. Oncoimmunology 2019; 8: e1580128.
32. Bradbury KE, Appleby PN,Tipper SJ,Travis RC, Allen NE, Kvaskoff M, et al. Circulating insulin-like growth factor 1 in relation to melanoma risk in the European prospective investigation into cancer and nutrition. Int J Cancer 2019; 144: 957–966.
33. Karasik D, Zillikens MC, Hsu YH, Aghdassi A, Akesson K, Amin N, et al. Disentangling the genetic of lean mass. Am J Clin Nutr 2019; 109: 276–287.
34. Iles MM. Law MH, Stacey SN, Han J, Pfeiffer R, Harland M,et al. A variant in FTO shows association with melanoma risk not due to BMI. Nat Genet 2013; 45: 428–432.
35. Li X, Liang L, Zang M, Song F, Nan H, Wang LE, et al. Obesity related genetic variants, human pigmentation and risk of melanoma. Human Gene 2013; 132: 793–801.
36. Zhou Y, Hambly BD, McLachlan CS. FTO associations with obesity and telomere length. J Biomed Sci 2017; 24: 65.

37. Newton-Bishop JA, Beswick S, Randerson-Moor J, Chang YM, Affleck P, Elliot. Serum 25-hydroxyvitamin D3 levels are associated with breslow thickness at presentation and survival from melanoma. J Clin Oncol 2009; 27: 5439–5444.
38. O'Shea SJ, Davies JR, Newton Bishop JA. Vitamin D, Vitamin A, the primary melanoma transcriptome and survival. Br J Dermatol 2016; 175: 30–34.
39. Glass D, Lens M, Swaminathan R, Spector TD, Bataille. Pigmentation and vitamin D metabolism in Caucasians: low vitamin D serum levels in fair skin types in the UK. PLoS One 2009; 4: e6477.
40. Gandini S, De Vries E, Tosti G, Botteri E, Spadola G, Maisonneuve P, et al. Sunny holidays before and after melanoma diagnosis are respectively associated with lower Breslow thickness and lower relapse rates in Italy. PLoS One 2013; 8: e78820.
41. Visconti A, Ribero S, Sanna M, Spector TD, Bataille V, Falchi M, et al. Body site-specific genetic effects influence nevus count distribution in women. J Invest Dermatol 2019; doi: 10.1111/pcmr.12820.
42. Schoemaker MJ, Swerdlow AJ, Higgins AJ, Wright AF, Jacobs PA, et al. Mortality in women with Turner syndrome in Great Britain: a national survey. J Clin Endocrinol Metab 2008; 93: 4735–4742.
43. Visconti A, Duffy DL, Liu F, Zhu G, Wu W, Chen Y, et al. Genome wide association study in 176,678 Europeans reveals genetic loci for tanning response to sun exposure. Nat Comm 2018; 9: 1684.
44. Duffy DL, Zhu G, Li X, Sanna M, Iles MM, Jacobs LC, et al. Novel pleiotropic risk loci for melanoma and naevus density implicate mulitple biological pathways. Nat Comm 2018; 9: 4774.
45. Speeckaert R, van Geel N. Distribution patterns in generalized vitiligo. J Eur Acad Dermatol Venereol 2014; 28: 755–762.
46. Spritz R, Andersen G. Genetics of vitiligo. Dermatol Clin 2017; 35: 245–255.
47. Synnerstad I, Fredrikson M, Ternesten-Bratel A, Rosdahl I. Low risk of melanoma in patients with atopic dermatitis. J Eur Acad Dermatol Venereol 2008; 22: 1423–1428.
48. Kohli JS, Tolomio E, Frigerio S, Maurichi A, Rodolfo M, Bennett DC, et al. Common Delayed Senescence of Melanocytes from Multiple Primary Melanoma Patients. J Invest Dermatol 2017; 137: 766–768.
49. Lubbe SJ, Escott-Price V, Brice A, Gasser T, Pittman AM, Bras J, et al. Rare variants analysis of cutaneous malignant melanoma genes in Parkinson's disease. Neurobiol Aging 2016; 48: 222.e1–222.e7.
50. Tell-Marti G, Puig-Butille JA, Potrony M, Badenas C, Mila M, Malvehy J. The MC1R melanoma risk variant p.R160W is associated with Parkinson disease. Ann Neurol 2015; 77: 889–894.
51. Gibson SB, Abbott D, Farnham JM, Thai KK, McLean H, Figueroa KP, et al. Population-based risks for cancer in patients with ALS. Neurology 2016; 87: 289–294.
52. Kiuru M, Busam KJ. The NF1 gene in tumour syndromes and melanoma. Lab Invest 2017; 97: 146–157.

REVIEW ARTICLE

# Melanoma Genomics

Julia A. NEWTON-BISHOP, D. Timothy BISHOP and Mark HARLAND
*Epidemiology and Biostatistics, Institute of Medical Research at St James's, University of Leeds, Leeds, UK*

**The incidence of cutaneous melanoma continues to increase in pale skinned peoples in Europe and elsewhere. Epidemiological studies identified genetically determined phenotypes such as pale skin, freckles and red hair, and sunburn as risk factors for this cancer. The development of many melanocytic naevi is also genetically determined and a strong melanoma risk phenotype. Not surprisingly then, genome wide association studies have identified pigmentation genes as common risk genes, and to a lesser extent, genes associated with melanocytic naevi. More unexpectedly, genes associated with telomere length have also been identified as risk genes. Higher risk susceptibility genes have been identified, particularly *CDKN2A* as the most common cause, and very rarely genes such as *CDK4*, *POT1*, *TERT* and other genes in coding for proteins in the shelterin complex are found to be mutated. Familial melanoma genes are associated with an increased number of melanocytic naevi but not invariably and the atypical naevus phenotype is therefore an imperfect marker of gene carrier status. At a somatic level, the most common driver mutation is *BRAF*, second most common *NRAS*, third *NF1* and increasing numbers of additional rarer mutations are being identified such as in *TP53*. It is of note that the *BRAF* and *NRAS* mutations are not C>T accepted as characteristic of ultraviolet light induced mutations.**

*Key words:* susceptibility genes; somatic mutations; melanoma.

Accepted Apr 27, 2020; Epub ahead of print Apr 28, 2020

Acta Derm Venereol 2020; 100: adv00138.

*Corr:* Julia A. Newton-Bishop, Epidemiology and Biostatistics, Institute of Medical Research at St James's, University of Leeds, Clinical Sciences Building, St James's Hospital, Beckett Street, Leeds LS9 7TF, UK. E-mail: j.a.newton-bishop@leeds.ac.uk

## SIGNIFICANCE

Melanoma continues to increase in incidence and therefore recognizing individuals at increased risk is especially important. This review discusses the associations between inherited genes which increase risk, and how the presence of those genes is manifest in family history or skin type. Environmental exposures, namely sun exposure leading to sunburn is aetiological in the genetically predisposed.

Melanoma continues to increase in incidence in Europe; figures from the period 1995–2012 recently published showed increases in both *in situ* and invasive melanoma (1). IARC figures generated from data recorded up until 2012 were used to construct **Fig. 1**. It can be seen that the greater proportional rise in incidence in older men in the UK is mirrored in Australia albeit at a considerably higher incidence rate. Australia, however, appears to have achieved a decrease over time in incidence rates in the very young, probably related to the very active and long-standing public health activities in that country.

The common melanoma subtypes, superficial spreading melanomas (SSM), nodular melanomas (NM) and lentigo maligna melanomas (LMM) are essentially diseases of pale skinned individuals, and this observation along with the identification of reported sunburns as a significant risk factor led to the recognition that melanoma is caused by sun exposure. The comparison between rates in England and in Australia is useful as the sub-population of Australians who develop melanoma commonly claim UK ethnicity and previous genome-wide association studies confirmed inherited similarities (2): that is that this comparison in incidence therefore reflects the strong effect of sun exposure (in genetically similar people) on melanoma development.

**Fig. 2** shows a principal component analysis (PCA) from a genome-wide association study reported by the GenoMEL consortium (2). PCA analyses of inherited genetic variation effectively examines genome-wide genetic variation across the populations determining the underlying patterns. The first two components explain much of the overall pattern of variation; in this figure, each participant's genome is represented by a "dot" reflecting on a 2 dimension plot the value of that person's first two principal components – each of the principal components consists of many thousands of genetic variants across the genome. The dots in brown, orange, sky blue and dark green represent the genotype of blood samples from the UK, the Netherlands, Sydney and Brisbane respectively. The PCA did not consider the location of residence of the person or the laboratory that recruited them but when the two dimension graph is overlaid on the map of Europe, it is apparent that people recruited from the same location are together on the map and that the pattern of the geographical locations is also retained with the exception of the Australian populations which are superimposed on the map of Western Europe reflecting their ancestry. The map confirms that gene frequencies vary slowly and systematically across Europe reflecting the fact that local migration is the biggest determinant of change. For instance, one of

doi: 10.2340/00015555-3493
Acta Derm Venereol 2020; 100: adv00138

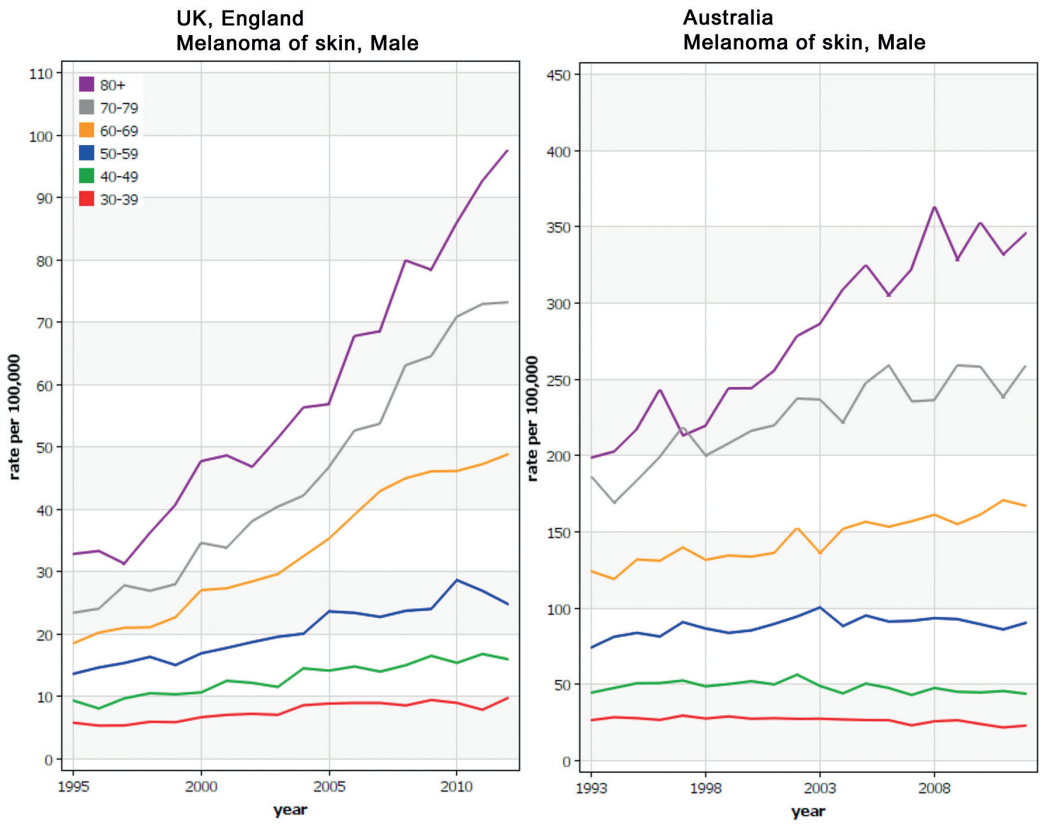


**Fig. 1. Incidence rates for melanoma in men in two genetically similar populations in England and in Australia.** The figures were generated on line using the Globocan tool (gco.iarc.fr).

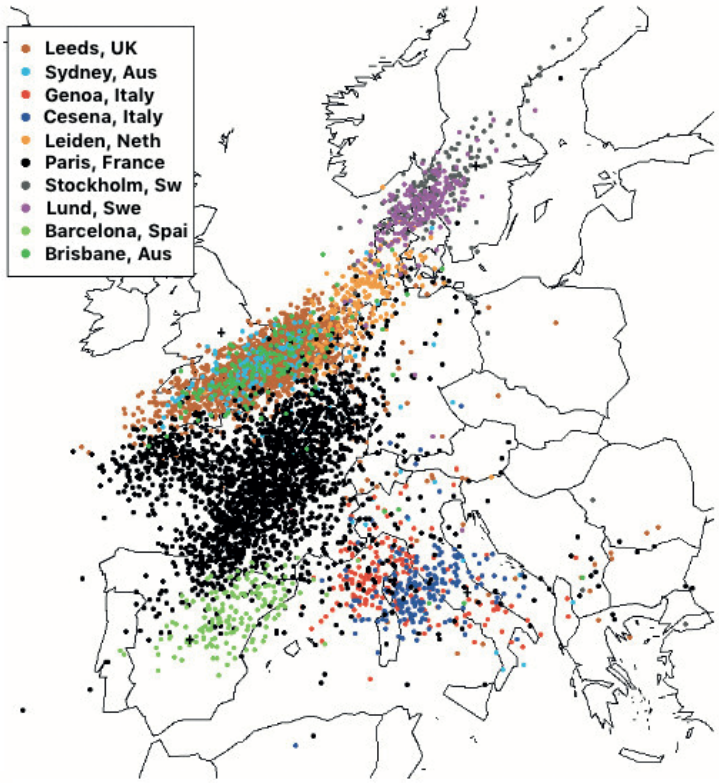


**Fig. 2. Principal components analysis (PCA) from a genome-wide association study reported by the GenoMEL consortium (2).** The coloured dots represent a measure of the genetic inheritance of participants in a genetic study of melanoma. The superimposed blue, green and terracotta dots over the UK suggests that the participants from two sites in Australia (Sydney and Brisbane) were very similar genetically to those living in the UK. This was expected as many Australian melanoma patients report ethnicity as the UK. Comparing incidence in melanoma then between the UK and Australia is to some degree comparing incidence in two populations similar genetically but with very different sun exposure histories. Figure kindly prepared by Dr Mark Iles of the University of Leeds.

**Table I. Incidence rates reported by the North American SEER registry by ethnicity. Modified from Wang et al. (3)**

| | Non-Hispanic white | Hispanic white | Asian | Black | Total |
|---|---|---|---|---|---|
| Superficial spreading melanoma | 9.05 (8.96–9.13) | 1.12 (1.05–1.19) | 0.31 (0.27–0.35) | 0.15 (0.12–0.18) | 6.18 (6.13–6.24) |
| Nodular melanoma | 1.80 (1.76–1.84) | 0.49 (0.44–0.54) | 0.14 (0.12–0.17) | 0.06 (0.04–0.08) | 1.30 (1.28–1.33) |
| Lentigo maligna melanoma | 1.87 (1.83–1.90) | 0.23 (0.19–0.27) | 0.06 (0.05–0.08) | 0.02 (0.01–0.04) | 1.37 (1.35–1.40) |
| Acral lentiginous melanoma | 0.21 (0.20–0.22) | 0.24 (0.21–0.28) | 0.17 (0.14–0.20) | 0.19 (0.16–0.23) | 0.20 (0.19–0.22) |

Age-adjusted incidence rates per 100,000 person-years.

the genes contributing to this pattern is the variation in the lactase gene reflecting the pattern of lactose intolerance across Europe. Thus the melanoma incidence curves in Fig. 1 reflect UK and Australian melanoma patients and this PCA suggests that these are more similar than populations sampled elsewhere in Europe.

## INHERITED (GERMLINE) GENOMIC VARIATION AND MELANOMA RISK

### *Skin colour genes*

Although the markedly different incidence rates for genetically similar populations in the UK and Australia reflects the effects of very different patterns of sun exposure, cutaneous melanoma is a strongly genetically determined disease. Melanoma incidence is very strongly related to skin colour being predominantly a cancer of pale skinned individuals. **Table I** indicates that the most common melanoma subtypes, SSM, NM and the less common LMM and desmoplastic melanoma, are very much more common in fair skin, whereas the acral lentiginous melanoma (ALM) variety has approximately the same incidence in most ethnic groups. Table I reports incidence for different melanoma subtypes, SSM, NM, LMM and ALM. The ethnicity terms used are those used in North America: Non-Hispanic white (NHW), Hispanic white (HW), Asian and Black. The data show that the incidence of SSM, NM and LMM is highest in those with ethnicity associated with the palest skins, indeed there is some evidence for a gradation in incidence from typically palest to darkest skins. The data also show that the incidence of ALM does not differ with ethnicity and therefore inherited pigment genes.

### *Melanocytic naevi genes*

The second risk phenotype is the presence of greater numbers of melanocytic naevi (4), both of the "common" or banal type and the presence of larger naevi described clinically as atypical naevi and histologically as dysplastic naevi. Twin studies have reported evidence for high heredity for this phenotype in the order of around 65% (5, 6). Thus the two phenotypes most predictive of melanoma risk (pale skin and the presence of many naevi) are shown to be predominantly genetically determined.

### *New low-medium penetrance loci*

Genome wide association studies have increased steadily in power to identify larger numbers of inherited genetic variation associated with increased risk of the common subtypes of melanoma (7, 8) and indeed with the risk phenotypes as a result of collaboration between multiple research groups. The role of inherited pigmentation genes in melanoma susceptibility is clear but there are also a number of genetic loci associated with increased numbers of melanocytic naevi and with telomere length. Telomeres are nucleotide repeat sequences which protect the ends of chromosomes, from excessive shortening and becoming tangled during cell division. Genes such as that coding for telomerase and additional genes coding for proteins in the so-called shelterin complex play an important role in maintaining telomeres. A number of inherited genetic variants are reported to determine telomere length and a genetic score predicting longer telomeres has been shown to strongly predict melanoma risk (9). In short, common genes associated with paler skin and in particular skin which tends to burn in the sun (predominantly the gene coding for the melanocortin receptor 1, MC1R); others which are associated with having more naevi; and genes associated with longer telomeres are melanoma risk genes, and to a large degree explain variation in melanoma incidence in different populations worldwide. Further genes associated with risk will certainly be found and other pathways may therefore be identified: a recent genome wide association study of naevi reported some evidence of pathways not previously supposed to be associated with naevus pathogenesis (8).

The low to medium penetrance (risk) genes identified in genome wide association studies each increase risk a little and melanoma occurs essentially in individuals who have inherited several risk alleles and who like the sun, in particular intermittent sun exposure. The likelihood is that risk of melanoma increases progressively with higher numbers of the risk alleles.

## RARE INHERITED MUTATIONS

Rarer inherited mutations are associated with a high risk of melanoma (high penetrance) so that clustering of cases occurs in families. The definition usually used to define a melanoma family is at least 3 cases in close relatives. The commonest high penetrance susceptibility gene is *CDKN2A* which notably codes for two quite distinct proteins: $p16^{INK4a}$ and $p14^{ARF}$. $P16^{INK4a}$ is a cyclin-dependent kinase inhibitor in the RB1 cell cycle control pathway, and $p14^{ARF}$ binds the p53-stabilizing protein MDM2 in the p53 signalling pathway. The *CDKN2A* gene is therefore involved in the regulation of two critical cell cycle regulatory

pathways. A very small number of melanoma families have causal mutations in the gene which codes for CDK4 to which p16 binds and these families appear to have a very similar phenotype to those with *CDKN2A* mutations (10).

Mutation carriers are more likely to have multiple primaries than those without such mutations (11), a little earlier age of onset and pancreatic cancer occurs in some CDKN2A families reported from mainland Europe and the USA. Studies in specific founder *CDKN2A* mutation families from Sweden (12) and the Netherlands have reported increased rates of cancers associated with smoking (13) but the risks of cancers other than melanoma and pancreatic cancer are not yet sufficiently well established to infer screening requirements, see https://www.ncbi.nlm.nih.gov/books/NBK7030/. That risks remain unclear to some extent reflects bias of ascertainment: in order to identify new high risk inherited cancer genes, researchers typically tested families who had multiple cases of the same cancer. Work is ongoing currently within GenoMEL (www.genomel.org) to address this deficiency.

Familial melanoma has been recognised for many years and between 1994 (14) and 2013, only *CDKN2A* and *CDK4* mutations were recognised as familial melanoma genes. These mutations were identified not least because the majority of affected families are at increased risk of only melanoma, sometimes also with some pancreatic cancer and families were ascertained for investigation on the basis essentially of multiple melanoma cases. There was, in essence, a deliberate bias, in that families with multiple cases of melanoma were selected for invitation to participate in research. This was the usual method for the identification of highly penetrant genes using genetic linkage studies where co-segregation of genetic variants with the cancer was required. Now that whole exomic or genomic sequencing and "panels" of cancer genes are used to identify high risk genes in families, genes are being identified with association with melanoma and an increased number of various other cancer types. As a result, rarer mutations in additional melanoma susceptibility genes have been identified. These have been seen in less than 2% of UK families with 3 or more melanoma cases. They are predominantly genes which are involved in telomere function/maintenance, first the gene named Protection of Telomeres I (*POT1*) which was described simultaneously in two groups in melanoma families (15, 16). Additional mutations were described in other genes in the shelterin telomere protection complex of which POT1 is a subunit (17), and in *TERT* (18, 19). Telomere function is therefore clearly important in melanoma pathogenesis. Finally inherited mutations in the *BAP1* gene, which were originally reported as an inherited cause of uveal melanoma but were quickly then associated additionally with a risk of lung cancer and meningiona (20) are now recognised also to increase the risk of cutaneous melanoma (21). Subsequently the mutations were recognised as also associated with renal cancer and mesotheliomas. Unusual but generally benign "spitzoid" melanocytic lesions of the skin were reported to be part of the syndrome in 2011 (21).

The role of gene testing and screening is therefore in the process of change. As the penetrance of these genes which increase the risk of melanoma and other cancers, becomes clearer then appropriate screening should be possible and gene testing/counselling likely to be increasingly performed.

Families with inherited melanoma susceptibility to melanoma often also have more melanocytic naevi than is usual in that population. This phenotype, called the atypical mole syndrome or the dysplastic naevus syndrome was originally thought to be a key component of the Familial Melanoma "Syndrome" (22). Indeed, there is certainly an association: mutations are more likely to have larger numbers of naevi (23). However, it is recognised now that some families with the same mutation may or may not have many naevi, so that family members with normal naevi may yet be found to carry the susceptibility gene. It has been postulated that the rather variable association between inherited high risk melanoma genes and naevi may be complicated by the variable co-inheritance of common lower risk melanoma susceptibility genes (23). In the dermatology or melanoma clinic, then the factors which should alert the medical team to the possibility of inherited high-risk melanoma susceptibility are, the atypical naevus syndrome, multiple primaries, relatively early onset and the co-occurrence of pancreatic cancer in some populations at least. The single most important factor, however, is family history of cancer. So, only 2% of apparently sporadic melanomas even with 2 primaries have inherited *CDKN2A* mutations (24), but in our own studies > 50% of families with 4 or more melanoma cases have such mutations. In the dermatology or melanoma clinic then, the presence of many naevi or more than one primary should alert the team to the possibility of a higher risk but family history is the strongest evidence for highly penetrant melanoma susceptibility genes. A review published by Sancy Leachman and GenoMEL (25) made recommendations for genetic counselling, but the identification of genes such as *POT1* and *TERT* which increase the risk of cancers other than melanoma means that these recommendations will be revised as more data become available.

Melanoma is an uncommon second malignancy in inherited retinoblastoma (26) and there are reports of a possible small increase of risk in carriers of *BRCA2* mutations (27) and possibly Lynch syndrome susceptibility genes although the evidence for the latter is not at this time convincing.

## SOMATIC MELANOCYTIC NAEVUS GENOMICS

Melanocytic naevi are both markers of melanoma risk and precursors of melanoma. They are benign proliferations which arise progressively starting in the first year of life,

but which stop appearing at the age of 40 years or so. The proliferation of melanocytes sufficient to produce detectable naevi results from the development of mutations in genes predominantly in the RAS/RAF/MEK/ERK pathway. The most common mutation is *BRAF*$^{V600E}$ but *NRAS*, and less commonly *KRAS* mutations occur. The prevalence of such mutations differs between naevi of different types, recently reviewed by Roh et al. (28). Roh et al. estimated that *BRAF* mutations drive 78% of common acquired naevi, 60% of dysplastic naevi, 7% of blue and 6% of Spitz naevi. Similarly, they estimated that *NRAS* mutations drive 95% of giant pigmented congenital naevi, 70% of small/medium naevi and 2% blue and Spitz naevi. *GNAQ* mutations occur in 84% of blue naevi.

Neither *BRAF* nor *NRAS* mutations have the classical genetic signature of mutagenesis as a result of ultraviolet (UV) light exposure: C>T mutations (29), but as described above, there is clear epidemiological evidence of a relationship between naevus number and sun exposure. The precise pathogenesis of such mutations remains as yet unclear but these observations suggest a complex relationship between intermittent sun exposure and naevogenesis. It has been queried whether *BRAF* mutations might actually result from DNA damage consequent upon exposure to UVA (30).

Whatever the route, the activation of the RAS/RAF/MEK/ERK pathway appears to drive the proliferation of naevi but the mutations eg in *BRAF* also induce senescence and therefore in the majority of naevus proliferation eventually ceases, resulting in growth cessation and ultimately clinical involution. Where this senescence is overcome as a result of additional mutations, then dysplastic naevi may develop and evolve into superficial spreading melanomas. As reported by Shain et al. (31), as melanoma evolves from benign naevi through to invasive tumours, then the proportion of lesions with loss of the *CDKN2A* gene, increased expression of *TERT*, increased numbers of additional mutations and copy number changes steadily increases resulting in more aggressive tumours. An on-line data source https://www.mycancergenome.org/content/disease/melanoma/ estimates the frequency of the driver mutations in melanoma as *BRAF* in 37–50%, *CTNNB1* (2–4%), *GNA11* (1%), *GNAQ* (1%), *KIT* (2–8%), *MEK1* (6–7%), *NF1* (12%) and *NRAS* (13–25%). The proportion of each in different melanoma subtypes differs, so the same data source reported that in melanomas arising on for example the trunk 50% have *BRAF*, 20% *RAS* compared with melanomas arising in skin with sun damage, whereas *BRAF* is reported to be much lower at 10%, with 10% *NRAS* and 2% *KIT*. Acral melanomas, 15% *BRAF*, 15% *NRAS* and 15% *KIT*. Individual studies have reported additional mutations. As technologies designed to detect mutations and copy number changes become more and more accessible even in formalin fixed tissues, then the knowledge of less common genomic somatic changes in melanoma increases. Hodis et al. (32) for example reported the discovery of 6 novel melanoma genes (*PPP6C*, *RAC1, SNX31, TACC1, STK19* and *ARID2*), 3 of which: *RAC1, PPP6C* and *STK19* were recurrent. Hayward et al. (33) reported in addition significant mutation of *TP 53* in cutaneous melanoma and that the significant mutations were *BRAF*, *NRAS* and *NF1* in acral melanoma and *SF 3B1* in mucosal melanoma.

### *Large mutation burden in melanomas*

Although, the classic driver mutations of naevi do not have C>T mutations, melanomas were shown by the Sanger Institute to have the greatest number of mutations of any cancer and that these mutations were predominantly C>T (29). Mutations are not surprisingly more frequent in tumours which arose on chronically sun exposed skin (31) and the probability is that these mutations are predominantly passenger mutations: that is that they don't play a key role in tumour progression. However, overall mutation rates were reported to be highest in lung cancer and melanoma (29), both of which have good responses to checkpoint blockade and the supposition is that this is at least in part attributable to mutation derived neoantigens capable of stimulation immune responses to the melanoma cells.

### *Copy number changes*

Copy number changes have been elucidated to some extent. Hodis et al. (32) described a low prevalence of amplifications in melanoma overall: 11% *CCND1*, 6% *KIT*, 3% *CDK4*, 13% *TERT*, and 4% *MITF*. The deletions were dominated by those in *CDKN2A* (38%) and *PTEN* (25%). Overall the data support the view that copy number changes are more common in acral lentiginous melanomas than in those in sun-exposed sites. In **Table II** we have summarised some of the recent reports of copy number variation in acral lentiginous melanomas, and by comparison with the proportions reported by Hodis et al. (32) it can be seen that with this albeit limited data, copy number changes are more frequent in acral lentiginous melanoma than in melanomas arising in sun-exposed sites.

In conlusion, cutaneous melanoma is a good example of gene environment interaction, in that it is largely (but

**Table II. The recently reported data looking at copy number changes in acral lentiginous melanoma**

| Copy number alteration | Reference | *n* (%) |
|---|---|---|
| Amplification *AURKA* | Yan et al. 2018 (34) | 472 (25) |
| Amplification *GAB2/PAK1* | Chernoff et al. 2009 (35)<br>Yeh et al. 2019 (36) | 122 (22) |
| Amplification *CCND1* | Sauter et al. 2002 (37)<br>Yeh et al. 2019 (36) | 122 (21) |
| Amplification *CDK4* | Curtin et al. 2005 (38)<br>Yeh et al. 2019 (36) | 122 (22) |
| Deletion *NF1* | Liang et al. 2017 (39)<br>Yeh et al. 2019 (36) | 34 (12)<br>122 (15) |
| Inactivation *NF1 cooperating factor SPRED1* | Yeh et al. 2019 (36) | 122 (7) |
| Deletion *CDKN2A* | Liang et al. 2017 (39) | 34 (35) |
| Amplification or point mutation *TERT* promoter | Liang et al. 2017 (39) | 34 (35) |

not exclusively) a cancer of genetically determined pale skinned peoples, when they experience sun burn or sun damage. The identification of genes associated with risk from low to high risk has led to the identification of biological processes involved in tumourigenesis. The genetic changes occuring in the tumours adds more to what is known about tumourigenesis but also has lead to the evolution of treatment options for advanced disease.

## REFERENCES


1. Sacchetto L, Zanetti R, Comber H, Bouchardy C, Brewster DH, Broganelli P, et al. Trends in incidence of thick, thin and in situ melanoma in Europe. Eur J Cancer 2018; 92: 108–118.
2. Bishop DT, Demenais F, Iles MM, Harland M, Taylor JC, Corda E, et al. Genome-wide association study identifies three loci associated with melanoma risk. Nat Genet 2009; 41: 920–925.
3. Wang Y, Zhao Y, Ma S. Racial differences in six major subtypes of melanoma: descriptive epidemiology. BMC Cancer 2016; 16: 691.
4. Chang YM, Newton-Bishop JA, Bishop DT, Armstrong BK, Bataille V, Bergman W, et al. A pooled analysis of melanocytic nevus phenotype and the risk of cutaneous melanoma at different latitudes. Int J Cancer 2009; 124: 420–428.
5. Easton D, Cox G, Macdonald A, Ponder B. Genetic susceptibility to naevi – a twin study. Br J Cancer 1991; 64: 1164–1167.
6. Wachsmuth RC, Gaut RM, Barrett JH, Saunders CL, Randerson-Moor JA, Eldridge A, et al. Heritability and gene-environment interactions for melanocytic nevus density examined in a U.K. adolescent twin study. J Invest Dermatol 2001; 117: 348–352.
7. Macgregor S, Montgomery GW, Liu JZ, Zhao ZZ, Henders AK, Stark M, et al. Genome-wide association study identifies a new melanoma susceptibility locus at 1q21.3. Nat Genet 2011; 943: 1114–1148.
8. Duffy DL, Zhu G, Li X, Sanna M, Iles MM, Jacobs LC, et al. Novel pleiotropic risk loci for melanoma and nevus density implicate multiple biological pathways. Nat Commun 2018; 9: 4774.
9. Iles MM, Bishop DT, Taylor JC, Hayward NK, Brossard M, Cust AE, et al. The effect on melanoma risk of genes previously associated with telomere length. J Natl Cancer Inst 2014 Sep 17106. pii: dju267.
10. Puntervoll HE, Yang XR, Vetti HH, Bachmann IM, Avril MF, Benfodda M, et al. Melanoma prone families with CDK4 germline mutation: phenotypic profile and associations with MC1R variants. J Med Genet 2013; 50: 264–270.
11. Goldstein AM, Chan M, Harland M, Hayward NK, Demenais F, Bishop DT, et al. Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. J Med Genet 2007; 44: 99–106.
12. Helgadottir H, Hoiom V, Jonsson G, Tuominen R, Ingvar C, Borg A, et al. High risk of tobacco-related cancers in CDKN2A mutation-positive melanoma families. J Med Genet 2014; 51: 545–552.
13. de Snoo FA, Bishop DT, Bergman W, van Leeuwen I, van der Drift C, van Nieuwpoort FA, et al. Increased risk of cancer other than melanoma in CDKN2A founder mutation (p16-Leiden)-positive melanoma families. Clin Cancer Res 2008; 14: 7151–7157.
14. 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. Nat Genet 1994; 8: 22–26.
15. Robles-Espinoza CD, Harland M, Ramsay AJ, Aoude LG, Quesada V, Ding Z, et al. POT1 loss-of-function variants predispose to familial melanoma. Nat Genet 2014; 46: 478–481.
16. Wong K, Robles-Espinoza CD, Rodriguez D, Rudat SS, Puig S, Potrony M, et al. Association of the POT1 germline missense variant p.I78T with familial melanoma. JAMA Dermatol 2019; 155: 604–609.
17. Aoude LG, Pritchard AL, Robles-Espinoza CD, Wadt K, Harland M, Choi J, et al. Nonsense mutations in the shelterin complex genes ACD and TERF2IP in familial melanoma. J Natl Cancer Inst 2014; 107. pii: dju408.
18. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, et al. TERT promoter mutations in familial and sporadic melanoma. Science 2013; 339: 959–961.
19. Harland M, Petljak M, Robles-Espinoza CD, Ding Z, Gruis NA, van Doorn R, et al. Germline TERT promoter mutations are rare in familial melanoma. Fam Cancer 2016; 15: 139–144.
20. Abdel-Rahman MH, Pilarski R, Cebulla CM, Massengill JB, Christopher BN, Boru G, et al. Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. J Med Genet 2011; 48: 856–859.
21. Wiesner T, Obenauf AC, Murali R, Fried I, Griewank KG, Ulz P, et al. Germline mutations in BAP1 predispose to melanocytic tumors. Nat Genet 2011; 43: 1018–1021.
22. Bale SJ, Chakravarti A, Greene MH. Cutaneous malignant melanoma and familial dysplastic nevi: evidence for autosomal dominance and pleiotropy. Am J Hum Genet 1986; 38: 188–196.
23. Taylor NJ, Mitra N, Goldstein AM, Tucker MA, Avril MF, Azizi E, et al. Germline Variation at CDKN2A and Associations with Nevus Phenotypes among Members of Melanoma Families. J Invest Dermatol 2017; 137: 2606–2612.
24. 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. Hered Cancer Clin Pract 2014; 12: 20.
25. Leachman SA, Carucci J, Kohlmann W, Banks KC, Asgari MM, Bergman W, et al. Selection criteria for genetic assessment of patients with familial melanoma. J Am Acad Dermatol 2009; 61: 677e1–14.
26. Traboulsi EI, Zimmerman LE, Manz HJ. Cutaneous malignant melanoma in survivors of heritable retinoblastoma. Arch Ophthalmology 1988; 106: 1059–1061.
27. Breast Cancer Linkage C. Cancer risks in BRCA2 mutation carriers. J Natl Cancer Inst 1999; 91: 1310–1316.
28. Roh MR, Eliades P, Gupta S, Tsao H. Genetics of melanocytic nevi. Pigment Cell Melanoma Res 2015; 28: 661–672.
29. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013; 500: 415–421.
30. Thomas NE, Berwick M, Cordeiro-Stone M. Could BRAF mutations in melanocytic lesions arise from DNA damage induced by ultraviolet radiation? J Invest Dermatol 2006; 126: 1693–1696.
31. Shain AH, Yeh I, Kovalyshyn I, Sriharan A, Talevich E, Gagnon A, et al. The genetic evolution of melanoma from precursor lesions. N Engl J Med 2015; 373: 1926–1936.
32. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, et al. A landscape of driver mutations in melanoma. Cell 2012; 150: 251–263.
33. Hayward NK, Wilmott JS, Waddell N, Johansson PA, Field MA, Nones K, et al. Whole-genome landscapes of major melanoma subtypes. Nature 2017; 545: 175–180.
34. Yan J, Yu J, Wu X, Xu T, Yu H, Dai J, et al. Increased AURKA gene copy number correlates with poor prognosis and predicts the efficacy of high-dose interferon therapy in acral melanoma. J Cancer 2018; 9: 1267–1276.
35. Chernoff KA, Bordone L, Horst B, Simon K, Twadell W, Lee K, et al. GAB2 amplifications refine molecular classification of melanoma. Clin Cancer Res 2009; 15: 4288–4291.
36. Yeh I, Jorgenson E, Shen L, Xu M, North JP, Shain AH, et al. Targeted genomic profiling of acral melanoma. J Natl Cancer Inst 2019; 111: 1068–1077.
37. Sauter ER, Yeo UC, von Stemm A, Zhu W, Litwin S, Tichansky DS, et al. Cyclin D1 is a candidate oncogene in cutaneous melanoma. Cancer Res 2002; 62: 3200–3206.
38. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med 2005; 353: 2135–2147.
39. Liang WS, Hendricks W, Kiefer J, Schmidt J, Sekar S, Carpten J, et al. Integrated genomic analyses reveal frequent TERT aberrations in acral melanoma. Genome Res 2017; 27: 524–532.

REVIEW ARTICLE

# Melanoma Risk and Melanocyte Biology

Juliette U. BERTRAND[1–3], Eirikur STEINGRIMSSON[4], Fanélie JOUENNE[5], Brigitte BRESSAC-DE PAILLERETS[6] and Lionel LARUE[1–3]

*[1]Curie Institute, PSL Research University, INSERM U1021, Normal and Pathological Development of Melanocytes, [2]CNRS UMR 3347, University Paris-Sud, University Paris-Saclay, Orsay, France, [3]Equipe Labellisée Ligue Contre le Cancer, [4]Department of Biochemistry and Molecular Biology, Biomedical Center, Faculty of Medicine, University of Iceland, Reykjavik, Iceland, [5]Laboratory of Biological Pharmacology, St Louis Hospital, Paris, France, and [6]Department of Biopathology, INSERM U1186, Gustave Roussy, University Paris-Saclay, Villejuif, France*

**Cutaneous melanoma arises from melanocytes following genetic, epigenetic and allogenetic (i.e. other than epi/genetic) modifications. An estimated 10% of cutaneous melanoma cases are due to inherited variants or *de novo* mutations in approximately 20 genes, found using linkage, next-generation sequencing and association studies. Based on these studies, 3 classes of predisposing melanoma genes have been defined based on the frequency of the variants in the general population and lifetime risk of developing a melanoma: (*i*) ultra-rare variants with a high risk, (*ii*) rare with a moderate risk, and (*iii*) frequent variants with a low risk. Most of the proteins encoded by these genes have been shown to be involved in melanoma initiation, including proliferation and senescence bypass. This paper reviews the role(s) of these genes in the transformation of melanocytes into melanoma. It also describes their function in the establishment and renewal of melanocytes and the biology of pigment cells, if known.**

*Key words:* melanocyte stem cells; embryonic development; germline mutation; inherited melanoma.

Accepted Apr 27, 2020; Epub ahead of print Apr 28, 2020

Acta Derm Venereol 2020; 100: adv00139.

*Corr:* Lionel Larue, Institut Curie, PSL Research University, INSERM U1021, Normal and Pathological Development of Melanocytes, Orsay, France. E-mail: lionel.larue@curie.fr

## SIGNIFICANCE

Inherited variants or de novo mutations in approximately 20 genes have been shown to contribute to approximately 10% of cases of cutaneous melanoma. This paper evaluates the function(s) of these proteins in the establishment of the lineage during embryogenesis, melanogenesis, renewal and, of course, during melanomagenesis.

Cutaneous melanoma results from transformation of melanocytes (Mcs). Melanoma accounts for only 10% of skin cancers, but is responsible for approximately 80% of skin cancer-related deaths; the remaining skin cancers are basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and Merkel cell carcinomas. The incidence of melanoma has increased steadily over the last 5 decades. According to Berk-Krauss et al. (1), the overall mortality from 2013-2016 in the US among caucasians was 6%. Melanoma accounts for approximately 2% of all cancers diagnosed annually worldwide (2, 3). Phototype (**Table I**) and geographical location are two key risk factors in the epidemiology of cutaneous melanoma: melanoma incidence is highest in Australia/New Zealand, followed by the USA and Northern Europe, and mostly affects Caucasian populations of phototypes I and II (4). It has been shown that for individuals living in similar environments in the USA, Caucasians have a 25 times higher risk of developing melanoma than African Americans (phototypes IV to VI). The risk of melanoma in red-haired individuals is approximately 3 times that observed in other Caucasians (5). Caucasians were found to have a 4–5 times higher risk of developing and dying from melanoma in Australia than in Europe, showing the effect of the environment. The incidence of melanoma is 56 per 100,000 in Australia and 14 in 100,000 in France, with a similar mortality (approximately 1/100,000).

The frequency of melanoma is lower in dark-skinned individuals than in light-skinned individuals. This may be explained in part by the melanoma inducers present in the environment (ultraviolet radiation). Epidemiological studies have revealed other melanoma inducers, including heavy metals, some pesticides, and alcohol consumption, but none of these were associated with phototypes (6–9). These fundamental data reveal the importance of genetics (mainly senescence, pigmentation and DNA repair genes) and the environment (mainly sun/ultraviolet (UV) exposure) in melanomagenesis.

Melanomagenesis is a multistep process that can be divided into 2 main stages: initiation and progression.

**Table I. Phototypes: Fitzpatrick classification**

| Phototype | Skin colour | Hair colour | Eye colour | Sunburn | Tanning | Freckles |
|---|---|---|---|---|---|---|
| I | Very light | Red/blonde | Light | ++++ | – | +++ |
| II | Very light | Blonde/brown | Light | +++ | + | ++* |
| III | Light | Blonde/brown | – | + | ++ | +/– |
| IV | Tan | Brown/dark/black | – | + | +++ | – |
| V | Dark | Black | Dark | +/– | +++ | – |
| VI | Dark | Black | Dark | – | +++ | – |

The 6 phototypes classification is based on skin, hair, and eye colours, on the ability to tan and sunburn.
*Freckles appear after sun exposure.

doi: 10.2340/00015555-3494
Acta Derm Venereol 2020; 100: adv00139

Melanoma initiation requires proliferation of Mcs and bypassing of senescence. After a certain number of divisions, cells enter senescence and, in order to bypass senescence and allow the further growth of the cells, the cell cycle and the length of the telomeres have to be boosted.

Mcs are melanin-producing cells that arise from neural crest cells (NCC) (sometimes called the 4th embryonic layer), a transient population of cells arising from the dorsal part of the neural tube (10). Information concerning the establishment of the Mcs was largely obtained from mouse and chicken studies. The NCC delaminates and migrates away from the neural tube. NCC derivatives exist as single cells throughout development and spread via 2 major migration pathways. Melanoblasts are NCC derivatives migrating in the space between the somites and the non-neural ectoderm (dorsolateral pathway). These cells are the precursors of Mcs and are characterized by an ability to produce the pigment melanin. They are specified in a cell-free area between the dorsal part of the neural tube, the ectoderm and the dorsal part of the somites. This area is known as the migration staging area (MSA), the site at which founder melanoblasts receive proliferation, survival and migratory signals (11, 12). Melanoblasts arising from the dorsolateral pathway travel through the developing dermis. From mid-gestation onwards, the dermal melanoblasts start to cross the basal layer and colonize the epidermis (13). The epidermal melanoblasts then concentrate around the placodes of hair follicles (HF) before entering the forming hair follicles (13, 14). They colonize the future bulge, to generate the melanocyte stem cells (McSC), and the hair bulb, to generate the differentiated Mcs of the first hair cycle ("embryonic" hair), before resting. In addition to colonizing the hair follicle in humans and pigs, melanoblasts also remain in the epidermis in the interfollicular regions. Mcs located in the interfollicular regions are responsible for the tanning response and the protection against UV. Furry animals do not require such protection, since the hair efficiently protects the skin against this type of radiation.

Hair renewal and pigmentation are concomitant processes. Mcs disappear during catagen. In early anagen, McSCs re-enter the cell cycle and divide, for self-renewal and the generation of transit amplifying cells (TAC). These cells migrate and differentiate into Mcs, which participate in the pigmentation of the first "adult" hair. Cutaneous melanoma may arise from the McSCs, TACs and/or from the Mcs (15).

Approximately 20 genes have been found to be constitutively mutated in the germline and associated with a risk of melanoma. Three classes of genes have been defined on the basis of the frequency of the variation and the risk of developing melanoma: ultra-rare variants with a high risk, rare variants with a moderate risk, and frequent variants with a low risk (**Fig. 1**). It has been estimated that ultra-rare variants conferring high risk (~10 genes) account altogether for 2% of the total risk of melanoma. These genes include $p16^{INK4A}$ and $p14^{ARF}$ (located at the same locus, *CDKN2A*), *CDK4, BAP1, RAD51B, POLE, TERT, POT1, ACD*, and *TERF2IP*. Rare variants of *MC1R* and *MITF* confer a moderate risk of melanoma and finally, frequent variants of melanocortin 1 receptor (*MC1R), OCA2, ASIP, TYR, TYRP1, MATP, SCLC45A2, KIT*, and *PARP1* are estimated to account for 12% of the risk of melanoma. It should be noted that the melanoma risk model is very complex, as genetic risk factors interacts together (rare and frequent variants modulate the risk conferred by ultra-rare variants (16)), but also with host phenotypes and environmental factors (17). Functional studies performed *in vitro* and/or *in vivo* has unravelled the role of some of these genes in melanoma. However, no systematic study has yet been performed on all 20 genes in order to evaluate their importance during the natural course of Mcs development and melanomagenesis.

This review focusses first on susceptibility genes for cutaneous melanoma and the melanoma inducers found in the environment. It then discusses the role of these genes, if known, during the various steps of Mcs and melanoma development, including in: (*i*) melanoma initiation and progression, (*ii*) the establishment of the Mcs lineage during embryonic development, (*iii*) the terminal differentiation of Mcs associated with the production and transport of melanin, and (*iv*) the transfer of melanosomes in the keratinocytes of the skin or the hair, (*v*) the renewal of Mcs from McSC, and (*vi*) the maintenance of Mcs function over time.

## CUTANEOUS MELANOMA SUSCEPTIBILITY FACTORS

The focus here is primarily on germline/constitutive mutations increasing the risk of melanoma formation and the environmental factors modulating these risks by inducing somatic mutations, epigenetic and allogenetic modifications and/or modifying the micro-environment.

### *Melanoma susceptibility genes*

Variants in melanoma susceptibility genes have been classified according to their frequency and the degree of risk. These variants are generally transmitted from germ cells, but the role of neo-mutations, micro-chimerism and somatic mutations should not be underestimated.

*Ultra-rare variants – high risk of developing melanoma*. The first genetic studies of familial melanoma mapped markers associated with melanoma risk to chromosome 9, and the 9p21 region in particular (18); subsequently, causal variants at the *CDKN2A* locus were identified (19, 20). The *CDKN2A* (cyclin-dependent kinase inhibitor 2A) gene encodes 2 proteins, p16 and p14, which inhibit cyclin-dependent kinase (CDK), thereby regulating cell

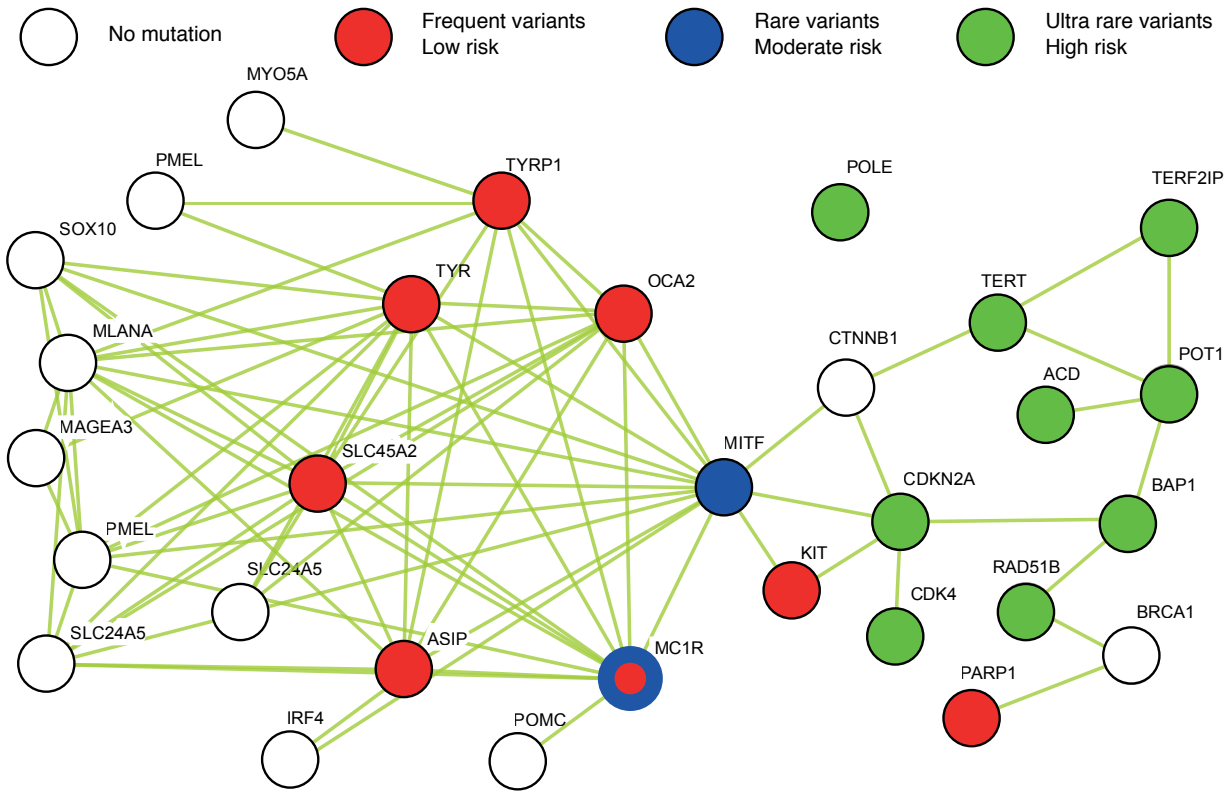


**Fig. 1. Interaction network of genes involved in melanocyte biology, and the associated melanoma risk.** The list of genes involved either in the establishment and renewal of melanocytes, in the biology of pigment cells, and/or associated with increased melanoma risk, was submitted to the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database for analysis of the gene interaction network (122). Each circle represents 1 gene, and 1 linking line represents a direct (physical) or indirect (functional) association between the proteins encoded by the 2 genes. *Green:* genes with ultra-rare variants associated with a high risk of melanoma; *blue:* genes with rare variants associated with a moderate risk of melanoma; *red:* genes with frequent variants associated with a low risk of melanoma; *white:* genes involved in melanocyte biology with no mutation currently associated with melanoma. Note, the left cluster gathers mainly genes of melanogenesis, and the right cluster gathers genes of the cell cycle, telomere length control, and DNA repair.

cycle checkpoints. *CDKN2A* variants have been found in 10–40% of familial melanoma cases, depending on geographical location, but their prevalence is very low in the general population, either never described or with a minor allele frequency (MAF) <0.001% (21, 22). *CDKN2A* variants confer a high risk of melanoma development with age- and geographical area-dependent variations: in melanoma-prone families ascertained through cancer clinics, the penetrance (a mean age-specific cumulative risk) at age 80 years was 58% in Europe, 76% in the USA and 91% in Australia (23, 24). However, at the same age penetrance was lower (28%) in population-based studies (25). Differences in melanoma risk between *CDKN2A* mutation carriers can be explained partly by the underlying pigment genes these individuals also carry and possibly also sun-exposure variants, host phenotype and sun exposure (17, 26, 27).

Mutations of *CDK4* (cyclin-dependent kinase 4), encoding another cell cycle regulator, have also been linked to melanoma formation in families with phenotypes similar to those of families with *CDKN2A* mutations (28, 29). CDK4 is a kinase that regulates G1 cell cycle progression by phosphorylating Rb proteins. *CDK4* variants are rare and are found in less than 1% of familial cases of melanoma, with a penetrance of 74% at the age of 50 years (29).

More recently, high throughput sequencing studies have added to the list of genes increasing the risk of melanoma, through the identification of rare variants in families. These genes can be divided into 2 groups: *TERT, POT1, ACD* and *TERF2IP*, which encode proteins involved in telomere length control, and *BAP1, RAD51B* and *POLE*, which encode DNA repair proteins. A variant in the *TERT* (telomerase reverse transcriptase) promoter was identified in 2 melanoma-prone families; this c.-57T>G variant is oncogenic through the creation of a new ETS transcription factor binding site leading to increased TERT expression (30, 31). Interestingly, somatic mutations of the *TERT* promoter (1 being identical) have also been detected in 33% of primary melanoma cases and 85% of metastatic cases (30). Mutations of *POT1* (protection of telomeres 1), *ACD* and *TERF2IP* were detected in exome sequencing studies of melanoma-prone families (32, 33). These 3 genes encode proteins of the shelterin complex and explain 9% of melanoma families lacking *CDKN2A* and *CDK4* mutations. Mutations of the *TERT* promoter and the shelterin complex result in longer telomeres, favouring senescence bypass.

*BAP1* (BRCA1-associated protein-1) loss of function germline mutations have been found associated to a tumour predisposition syndrome (BAP1-TPDS, OMIM 614327) including both cutaneous (0.52% of families) and uveal melanomas (CM and UM) (28.5% of families) (34–37). In addition to melanomas, the most frequent cancers of this syndrome are renal cell carcinoma, mesothelioma and multiple BCCs but the whole tumour spectrum and lifetime risk are currently unknown (38). *BAP1* is a tumour suppressor gene located at 3p21, encoding a deubiquitylase that participates in multiprotein complexes playing roles in numerous cellular processes, including DNA damage response, cell cycle regulation, cell growth, metabolism, and the regulation of inflammatory responses (38). The *BAP1* loss-of-function mutations are associated with approximately 15% risk of cutaneous melanoma. A germline *POLE* missense mutation located in the exonuclease domain of the protein (p.(Trp347Cys)) was found in a unique melanoma-prone family of 7 confirmed cases of CM and a case with UM, leading to a mutator phenotype in functional assays (39). It should be noted that POLE germline mutations are more frequent in endometrial (7–10%) and colorectal (2%) cancers (22) than in melanoma. Finally, a novel germline *RAD51B* nonsense mutation was identified in a 3-case CM family; a melanoma tissue from a carrier displayed loss of RAD51B staining in most tumoural cells, by immunohistochemistry (40). RAD51B plays an important role in DNA repair through homologous recombination, but up-to-date known germline mutations have been associated with increased risk of ovarian cancer (41).

Approximately 22% of melanoma-prone families are associated with mutations in the 9 high-risk melanoma susceptibility genes (19% for CDKN2A and 3% for the other 8 genes). However, the remaining 78% can be partly accounted for by the rare variants conferring a moderate risk and frequent variants conferring a low risk of melanoma (24).

*Rare variants – moderate risk of developing melanoma*. A unique missense variant in the microphthalmia-associated transcription factor (MITF), the MITF$^{E318K}$ variant, was linked to melanoma risk in 2 different studies. One of the studies started as a candidate gene hypothesis for melanoma and renal cancer predisposition (42), whereas the other involved whole-genome sequencing of a melanoma-prone family (43). The assumption in the first study was that MITF might be a good candidate for the following reasons: (*i*) it has been proposed to act as a melanoma oncogene (44); (*ii*) it also stimulates the transcription of hypoxia inducible factor (HIF1A), the pathway of which is targeted by kidney cancer susceptibility genes (45); and (*iii*) two members of the MITF-family of proteins, TFE3 and TFEB, have been implicated in renal cell carcinoma through somatic translocations (46). Both studies identified the same rare *MITF$^{E318K}$* variant (actual Minor Allele Frequency – MAF – in European population of 0.25%, GnomAD database) associated with a 5-fold increased risk of melanoma in genetically-enriched cases and an increased risk of 2.2 in case-controls studies (Yokoyama, 2011 #3370; Bertolotto, 2011 #2771).

The involvement of MC1R variants in melanoma is complex, since it is involved in pigmentation/phototype, naevi, UV/sun exposure (Demenais, 2010 #3357) and more recently, sex-dependence (47–49). The *MC1R* gene (16q24) is a key regulator of the synthesis of melanins (eu- and pheo-melanin) in Mcs, upon UV stimulation. Melanins are transferred to up to 40 surrounding keratinocytes to act as a natural sunscreen to absorb UV irradiation. Eumelanins are prevalent in phototypes IV to VI, while pheomelanins are responsible for red hair and freckles found in phototypes I and II (50). Beyond affecting phototypes, this 7-transmembrane receptor plays a role in DNA repair pathways and antioxidant defences for a complex photoprotective response (51). *MC1R* is highly polymorphic (> 80 variants) in Caucasian populations. Loss-of-function variants, which result in less pigmented phenotypes, are the result of human evolution associated with the classical mutation-selection events. Indeed *Homo sapiens* and *Homo neanderthalensis* migrated, and adapted from Africa to more northern and less sunny regions and subsequently lost pigmentation to allow increased vitamin D production (51). Six loss-of-function *MC1R* variants (p.ins86-87A, p.D84E, p.R142H, p.R151C, p.R160W, and p.D294H) are defined as R-type variants, as they increase the relative risk of melanoma 2–3 times; whereas another 4 variants (p.V60L, p.V92M, I155T, and p.R163Q) are classified as r-type variants as they confer melanoma risk below 2 (48). The effect is additive, as the presence of 2 or more *MC1R* "R" variants is associated with 6-fold increased risk compared to wild-type alleles. Thus, the *MC1R* gene is a moderate-penetrance melanoma susceptibility gene, but it is also a gene that modifies the risk of melanoma in patients with a *CDKN2A* mutation. Recent studies have shown that the risk conferred by *MC1R* variants seems to be independent of sun/UV exposure (52, 53) and independent of phenotype (54–56). Furthermore, *MC1R* polymorphisms seem to influence size and dermoscopic features of naevi (57). And recently, several sex-differences emerged, with *MC1R* variants associated with phototype I and II and higher melanoma risk, but better survival in females than in males (47–49).

*Frequent variants – low risk of developing melanoma*. Finally, frequent variants of genes associated with pigmentation (*OCA2, ASIP, TYR [OCA1], TYRP1 [OCA3], MATP, SCLC45A2 [OCA4], KIT,* and *PARP1*) are associated with a slight increase in risk of melanoma formation, as shown through genome wide case-control association studies (GWAS) (24, 58–60). These frequent variants have only a small individual effect on melanoma risk,

but combinations of these low-risk variants may account for up to 78% of non-familial melanomas. They are also responsible for the host phototype and are therefore important for determining the interaction between the phenotype and the environment. Further functional studies are required to decipher the associated mechanisms. These frequent pigmentation variants probably play a role as modifiers of melanoma risk in other genetic backgrounds, such as the ultra-rare and rare variants, conferring high and moderate melanoma risk, respectively.

*Environmental factors*

Sequencing studies on tumours have highlighted the genetic complexity of melanoma in terms of the somatic mutational load in the population and melanoma is the cancer with the highest mean mutation rate (61). The spectrum of driver mutations provided unequivocal genomic evidence for a direct mutagenic role of UV light in melanomagenesis (62). Somatic mutation frequencies also differ considerably between melanoma patients, showing melanoma to be a complex disease with several subtypes and multifactorial origins (63).

The melanoma risk associated with the gene variants, described above, depends heavily on the co-occurrence of other gene variants and environmental (micro- [inside the body] and macro- [outside of the body]) stresses. Protein-protein, protein-microenvironment, and gene/protein-environment interactions undoubtedly account for the complexity and diversity of melanoma. Epidemiological studies have implicated several environmental factors in the induction of cutaneous melanoma, including ultraviolet radiation (UVR), alcohol use, obesity, heavy metals and some pesticides.

UVR from the solar spectrum is the leading external cause of melanoma formation. Epidemiological studies have shown that exposure to UV is correlated with melanoma formation: intermittent rather than chronic exposure, high levels of exposure during childhood and the use of artificial UV lamps are associated with a major risk of melanoma (64, 65). Both UVA (315–400 nm) and UVB (280–315 nm) can promote melanoma formation (66). Most melanomas have many somatic mutations and most of those have a UV signature (61), i.e. ≥60% of mutations are C→T at a dipyrimidine site, with ≥5% as CC→TT changes (67).

As already mentioned, several external factors seem to be associated with melanoma, including alcohol consumption, heavy metals and pesticide exposure (6–9), but the evidence for the associations obtained for alcohol consumption, heavy metals and pesticide exposure is weaker than that obtained for UV. Epidemiological studies on farm workers, a population also exposed to UVR, have highlighted a potential risk of pesticides. The cumulative effects of UV and the pesticide carbaryl are genotoxic in human Mcs (68). Obesity is associated with melanoma initiation and progression, as it increases the risk of melanoma formation and favours melanoma growth, invasion and distant metastasis (69). Indeed, adipose tissue favours the proliferation and aggressiveness of melanoma cells through a direct dialogue, mediated by soluble factors and by exosomes, and through remodelling of the tumour microenvironment. Little is currently known about the molecular mechanisms of melanomagenesis associated with alcohol, heavy metals and pesticides. Extensive *in vitro* and *in vivo* functional studies should be performed to validate the importance of these factors in melanomagenesis.

*Melanoma susceptibility genes and environmental factors*

One epidemiological study has revealed that *CDKN2A* mutation carriers appear to have the same cumulative risk of melanoma irrespective of the ambient UV irradiation of the region in which they live (27). These results were functionally validated after exposing mice lacking $p16^{INK4A}$ to transient UV irradiations; this did not affect melanoma formation (70). Of course, this does not mean that UV irradiation has no role in melanoma initiation, as there is a strong association between UV irradiation exposure and melanoma risk for the general population. Interestingly, UVB irradiation of mice expressing $CDK4^{R24C}$, the oncogenic form of CDK4, promotes melanoma initiation, with a shorter time lag to tumour formation and faster growth (71). A germline nonsense mutation in *BAP1* (Y646X) and environmental exposure to asbestos and UV irradiation were found to contribute to the high incidence of cutaneous melanoma in a family at high risk of cancer (72). The association of UV and *MC1R* mutations has long been known to promote melanoma, and MC1R has been shown to be a potent regulator of PTEN after UV exposure; interestingly, the major Red Hair Color (RHC) *MC1R* variants R151C, R160W and D294H where shown to bind PTEN less effectively than the wild-type protein (73).

## FUNCTION OF SUSCEPTIBILITY GENES IN THE NATURAL COURSE OF MELANOMA

Carcinogenesis is a multistep process. The wild-type cell accumulates genetic, epigenetic and allogenetic (it may affect transiently, for instance, RNA, proteins and lipids; which are modulated by the micro- and macro-environment) modifications, which alter its characteristics and/or environment, leading to self-sufficiency in growth signals, a limitless potential for replication, insensitivity to anti-growth signals, the evasion of apoptosis, sustained angiogenesis and tissue invasion and metastasis (74). A model of the multistep process of melanomagenesis has been described, in which the level of complexity is reduced to provide a schematic view of the process (75).

Melanomagenesis is currently seen as a multistep process with 2 main stages: initiation and progression.

*Initiation*

Melanoma initiation is characterized by an initial "boost" of cell proliferation and the bypassing of senescence (75). It has been suggested that 25% of melanomas arise from naevi, 1–2-mm wide pigmented spots consisting of Mcs that have hyperproliferated *in situ*, but then stop proliferating and become senescent/quiescent. In the vast majority of naevi, the Mcs remain senescent throughout the life of the individual with a strict control provided by high expression of P14, P15, P16 and/or PTEN. However, in a few cases, melanomas arise due to a subsequent bypass of senescence. The remaining 75% of melanomas do not arise from naevi (76) suggesting that the initial proliferation of these cells is not affected by senescence. These 2 paths may appear different, but the molecular mechanisms are not; molecular mechanisms associated with the bypass of senescence may occur before those associated with proliferation. The RAS/MAPK signalling pathway is activated and involved in the proliferation step of most melanomas (77). Cell cycle proteins, such as CDKN2A/B and CDK4/6, and those of the PI3K/AKT and WNT/β-catenin signalling pathways are involved in senescence bypass/lack of senescence. PTEN loss and β-catenin activation can induce bypass of senescence or lack of senescence (78–81). Melanoma susceptibility genes are clearly involved in melanoma initiation.

Rare variants associated with a high risk of melanoma are involved in melanoma initiation. *CDKN2A* is certainly the best known of these genes. It encodes 2 proteins, one of which is $p16^{INK4A}$, a CDK4/6 inhibitor that represses G1/S cell cycle transition, and is known to promote senescence. Its inactivation induces cell cycle progression and the bypass of senescence. It is therefore considered to be a tumour suppressor. Senescence is controlled by the cell cycle and by telomere length. TERT, POT1, ACD and TERD2IP, which regulate telomere length, may also be tumour suppressors because their inactivation induces checkpoint bypass and promotes uncontrolled cell cycle progression.

Rare variants associated with a moderate risk of melanoma development are also involved in melanoma initiation. Indeed, a rare variant of MITF ($MITF^{E318K}$) has been shown to act through senescence bypass, leading to melanoma formation (42, 82). MITF is the main transcription factor of the Mcs lineage, where it regulates various functions, including melanogenesis/differentiation, proliferation, invasion, and senescence (83). *MC1R* variants modulate the incidence of melanoma, but it remains unclear whether this is linked to the protective effect of eumelanin against UVR or to the intrinsic role of MC1R in melanomagenesis. Studies to resolve this question are underway. One study revealed that *MC1R* mouse mutants in the $BRAF^{V600E}$ background develop more melanomas than control mice, independent of UV exposure. This highlighted the potential role of pheomelanin in inducing oxidative damage (52).

Frequent variants associated with a low risk of melanoma development are also involved in melanoma initiation. Melanoma incidence varies between populations; the higher the phototype the lower is the chance of cutaneous melanoma. Melanin, one of the key parameters for evaluating the level of the phototype, is a natural protector of the skin against external aggression. Variants in genes encoding proteins involved in melanogenesis are responsible for various forms of occulocutaneous albinism (OCA). These genes include *TYR (OCA1), OCA2 (P), TYRP1 (OCA3)*, and *SLC45A2 (OCA4)*. Recently, *OCA5*, *OCA6* and *OCA7* were identified and shown to correspond to *SLC24A5* (*OCA6*), *C10orf11* (*OCA7*), and a locus on chromosome 4q24 for *OCA5*. None of these have yet been associated with melanoma. However, further studies are required to fully evaluate the importance of these OCA genes in melanomagenesis. *OCA1* variants have a general prevalence of 1/40,000, whereas *OCA2* variants are more common in dark-skinned populations (Africa) (1/4,000 – 10,000) than in light-skinned populations (Caucasian) (1/36,000). *OCA1* and *OCA2* variants account for 80% of OCA cases and are the most strongly associated with skin cancer development. Melanomas and BCCs are more frequent in individuals with *OCA1-2* mutations than in the general population. However, SCC is the most frequent type of cancer in patients with *OCA* mutations (84). Melanoma diagnosis is particularly challenging in this population because lesions are often amelanotic. Three other genes (*ASIP, KIT*, and *PARP1*) have frequent variants associated with a low risk of developing melanoma. Mutations of the *KIT* gene affect the tyrosine kinase receptor of the corresponding protein and cause piebaldism, as do mutations of the gene encoding its ligand, Steel (KITLG). *KIT* gene mutations are present in 39% of mucosal melanomas, 36% of acral lentiginous melanomas, and 28% of skin displaying chronic solar damage. Most of the reported mutations are found in exons 9, 11, 13, and 17, and they account for between 5% and 10% of the mutations of diagnosed melanomas (85, 86).

*Progression*

Tumour progression is characterized by the dissemination of the transformed cells, followed by the formation of metastases. Dissemination involves several fundamental cellular events, including a second boost of proliferation, pseudo-epithelial-to-mesenchymal transition, migration, intravasation of the blood and lymphatic streams, resistance to anoikis, and extravasation to invade new tissues. Cells may form metastases in a suitable niche, in which the cells induce angiogenesis and proliferate.

The function of MITF in melanoma progression is complex and can be explained with a rheostat model

where the level and/or activity determine whether the Mcs or melanoma cells undergo senescence, invasion, proliferation or differentiation (83, 87, 87). MITF amplification is observed in 21% of metastatic melanomas and favours melanoma cell proliferation (44). However, MITF also represses proliferation through the regulation of p21 and p16 (88, 90). The functions of the proteins encoded by the other susceptibility genes in melanoma progression remain unknown.

## MELANOCYTE BIOLOGY AND FUNCTIONS OF SUSCEPTIBILITY GENES

We will now focus on the role of melanoma susceptibility genes in the establishment and maintenance of the Mcs lineage and pigmentation. The main function of Mcs, pigmentation, involves the intrinsic synthesis of melanin in specialized organelles called melanosomes, which are transported in Mcs and transferred to differentiating keratinocytes. Normal pigmentation is dependent on the genes involved in melanogenesis, and the transport and transfer of melanosomes and is finely regulated by extrinsic signals and cell-cell interactions.

### *Melanogenesis*

Melanogenesis is a chain of reactions occurring in melanosomes, with tyrosine as an initial substrate, and pheomelanin (yellow, orange) and eumelanin (black, brown) as final products. The first enzyme in this chain, tyrosinase (TYR), hydroxylates tyrosine to generate DOPA, and then DOPAquinone. The second enzyme, dopachrome tautomerase (DCT or TRP2), and the third enzyme, tyrosinase-related protein 1 (TYRP1), catalyse eumelanin production from DOPAquinone. Pheomelanin production from DOPAquinone is dependent on cysteine.

Melanogenesis is regulated through modulation of the level, activity and localization of these enzymes by external signals, including communication between Mcs, keratinocytes and dermal fibroblasts via secreted factors and cell-cell contact. Mcs homeostasis is controlled by a complex network of keratinocyte-derived factors that regulate Mcs proliferation and differentiation. These include melanocyte-stimulating hormone (α-MSH), endothelins (Edn), basic fibroblast growth factor (β-FGF), nerve growth factors (NGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), steel factor (SCF), leukemia inhibitory factor (LIF), hepatocyte growth factor (HGF), transforming growth factor beta (TGFβ), and Jagged1/2 (91, 92). These signals can be regulated by external factors, such as ultraviolet (UV) radiation, chemical compounds, drugs and stress.

The differences in pigmentation depend on the amount and quality of melanin (eumelanin vs. pheomelanin), which are partly controlled by the activity of *MC1R*. The MC1R receptor, which is activated by α-MSH after UV exposure, for example, induces eumelanin synthesis (93). *MC1R* variants are frequent in the Caucasian population, and lead to the expression of a receptor with normal, weak or no activity, associated with a brown, blond or red hair phenotype, respectively (94).

### *Melanosome transport*

Melanosomes are lysosome-related organelles derived from non-pigmented endosomal vesicles (known as pre-melanosome stages I and II). They mature and undergo progressive pigmentation following melanogenesis and go through stages known as stages III and IV (95).

The mature melanosomes are transported from the perinuclear area toward the periphery of the Mcs and the tips of its dendrites. Two types of movement have been observed: rapid microtubule-directed migration over long distances, and short-distance migration along actin fibres at the periphery (96). During their migration from the nucleus toward the periphery of the cell (known as centrifugal movement), melanosomes are transported by a kinesin complex on microtubules. For the reverse migration toward the nucleus (known as centripetal movement), the melanosomes are transported by a dynein complex on microtubules. The motor for peripheral migration is myosin Va (*dilute*) in a complex with melanophilin (*leaden*) and RAB27A (*ashen*), and this migration takes place along actin filaments (97).

Mutations in genes encoding these transporters are associated with abnormal pigmentation, and, in some cases, more severe syndromes, such as Griscelli syndrome type 2 (98). However, they have not, as yet, been linked to an increase in melanoma risk. Conversely, the known melanoma susceptibility genes have not been shown to participate in melanosome transport.

### *Melanosome transfer*

Melanosomes are transferred from Mcs to keratinocytes in order to deliver pigment to all epidermal keratinocytes. Several mechanisms of melanin transfer have been observed and debated: exocytosis-mediated, cytophagocytosis-mediated, tunneling nanotube-mediated and membrane vesicle-mediated transfer (99). The molecular mechanisms associated with the transfer of melanosomes to keratinocytes remain unclear. However, it has been suggested that classical pathways of exo-/endocytosis, membrane blebbing and vesicle biogenesis, filopodium formation and phagocytosis are involved. These processes involve proteins, such as Rab11b, small Rho GTPases, Cdc42 and Par-2 (100–103).

The cell body of the Mcs is located on the basement membrane, but the dendrites of the cell are in contact with 30–40 keratinocytes in the 3 dimensions of the epidermis, and these cells together form an epidermal melanin unit (104). In the basal layer, adjacent Mcs are generally separated by approximately 6 keratinocytes

(105). Melanosomes containing melanin are located in the superior part of the keratinocytes protecting the nucleus against UVR.

Albino individuals, who have Mcs but lack melanin, rarely develop melanomas. However, they develop more carcinomas (BCC and SCC) than individuals with normal pigmentation, confirming the protective effect of melanin in keratinocytes. Several issues should be raised at this point. Albinism is associated with a number of vision defects, including photophobia. These individuals therefore tend to prefer to stay out of the sun, thus leading to few melanomas. This suggests that melanin is protective for keratinocytes, but not necessarily for Mcs. Does this mean that Mcs lacking melanin are intrinsically more resistant to transformation than keratinocytes lacking melanin? If so, what are the molecular differences between these 2 cell types? Melanin, and its intermediates, such dopaquinone, and pheomelanin in particular, may damage Mcs, through oxidative stress, for example.

Melanoma susceptibility genes have not been shown to be involved in melanosome transfer. Melanosomes transfer may play no role in melanomagenesis, but this remains an open question, as the molecular mechanisms of transfer have yet to be fully elucidated.

## ESTABLISHMENT AND MAINTENANCE OF THE MELANOCYTE LINEAGE

### *Embryonic development*

In mammals, the establishment of the Mcs lineage during embryonic development involves the production of differentiated Mcs, responsible for the pigmentation of skin, hair and fur at birth, and McSC populations, responsible for maintaining pigmentation in the adult.

*Normal development*. In mice, normal Mcs development starts at approximately embryonic day 8.5 (E8.5), in midgestation, when the neural crest cells delaminate from the dorsal part of the closing neural tube and migrate into the MSA. These neural crest cells include the precursors of Mcs, melanoblasts, which proliferate and migrate between the somites (before they become the dermamyotomes, which subsequently evolve into muscle and dermis) and the ectoderm. At approximately stage E10.5, melanoblasts start to express Dct, which serves as a Mcs marker and can be easily detected by X-gal staining in Dct: LacZ mice (106). Between E11.5 and E15.5, the melanoblasts continue to proliferate and migrate through the forming dermis to cover the whole embryo. Some of these melanoblasts cross the basement membrane to colonize the epidermis, before colonizing the future hair follicles. In mice, the melanoblasts give rise to two Mcs populations at birth: the first differentiated Mcs located in the future bulb of the hair, and McSC pool located in the bulge of the hair. During the last steps of Mcs development in the embryo (from E15.5 until E19.5), the melanoblasts begin to express genes encoding enzymes required for melanin production, including Tyr and Tyrp1, which are produced in Mcs and McSC, for a few days after the birth of the McSC (107).

A second wave of melanoblast development has been described in the skin (108). NCCs give rise to several lineages, including Mcs, neurons, chromaffin cells and Schwann cells. One population of engaged precursor cells, the Schwann cell precursors (SCPs), can differentiate into either Schwann cells or Mcs. After early delamination, the SCPs migrate along the ventral pathway, between neural tube and somites, following the nerve fibres. SCPs retain a Schwann cell fate, while they remain in contact with the nerves. In the absence of signals provided by the nerve, some SCPs acquire a melanocytic fate. This second melanoblast population mostly colonizes the dorsal and lateral body walls, and seems to give rise to most of the limb Mcs.

The patterns of congenital pigmentary disorders in humans, including the congenital giant naevi that frequently display *NRAS* mutations, in particular, helped to identify a third wave of Mcs arising from the ectoderm at the time of gastrulation (109). Temporally, this is actually the first wave, because it occurs before the formation of the neural tube and the NCC formation during embryogenesis. These Mcs are responsible for the non-segmental pattern, through circular, bilateral migration centred on the midline. However, it remains unknown whether these cells contribute to mature Mcs in non-disease states.

*Pathological development*. Mcs pathology leads to pigmentation disorders of skin and/or hair, and may be associated with deafness and cognitive disorders (110, 111). Waardenburg syndrome (WS) is characterized by pigmentation and hearing disorders, sometimes associated with abnormal development of the face and limbs, and is due to the defective migration and proliferation of embryonic melanoblasts or the abnormal development of other neural crest cells. It is associated with mutations of the *MITF* and *SNAI2* genes (WSII) responsible for the pigmentary and hearing phenotypes; with mutations of the *PAX3* gene (WS I and III) affecting neural crest cell development and leading to morphological defects; and with mutations of the *EDN3, EDNRB*, and *SOX10* genes (WS IV) affecting intestinal neural cells. Piebaldism is characterized by hypopigmented patches of skin and hair and is due to the absence of Mcs in certain areas due to defective embryonic/Mcs development. Mutations of the genes coding the KIT receptor and its ligand, SCF, may cause piebaldism syndrome. Apart from MITF, no other melanoma susceptibility genes have been implicated in the embryonic development of Mcs.

### *Renewal*

*Normal renewal*. McSCs constitute a reservoir for the replacement of Mcs lost during adulthood. McSC niches

have been identified in the bulge area of hair follicles (92, 112). McSCs are characterized by a specific cell shape and localization in hair follicles. No specific molecular marker of McSCs has been identified, so a combination of markers is used to follow these cells: McSCs are considered to be Dct-positive, Ki-67-negative, BrdU-retaining cells with low or no expression of KIT and MITF. In the pigment disorder vitiligo, repigmentation often begins at the hair follicles, subsequently spreading out to generate continuous colouring of the skin. This observation is consistent with the notion that McSCs from the bulge can migrate from the hair follicles to the basal layer and differentiate into mature epidermal Mcs. Moreover, repigmentation of depigmented regions lacking hair follicles, such as the palms of the hands, is occasionally observed in patients with vitiligo, indicating that McSC niches are also present in other skin structures, such as sweat or sebaceous glands, and the dermis (113, 114).

The maintenance of hair pigmentation has been well studied in mice and humans. Renewal of the hair in the hair cycle is synchronized with a cycling renewal of the differentiated Mcs, resulting in pigmentation of the new hair. After a resting phase and destruction of the previous hair follicle, the McSCs exit quiescence, proliferate and migrate along the hair follicle as transient amplifying cells, eventually reaching the bulb, where they differentiate into pigment-producing Mcs. Bulge cell quiescence is tightly controlled by several different signals. TGFβ represses differentiation and cell cycle progression; SHH, WNT and β-catenin end the quiescence phase, activating anagen; and NOTCH controls the appropriate differentiation of Mcs (115–121).

*Failure of renewal*. The absence of Mcs renewal by McSCs can lead to unpigmented skin and hair. The McSC population is limited, despite its potential for renewal, and the number of these cells declines during ageing, resulting in physiological greying of the hair in both humans and mice.

Local depigmentation occurs in adult patients with vitiligo. Vitiligo develops as depigmentation of the skin in specific areas, characterized by a disruption of the epidermal melanin unit with the presence of very few, if any, Mcs. Interestingly, the normally pigmented skin of patients with vitiligo also displays an altered Mcs distribution in the basal layer. The number of keratinocytes between 2 adjacent basal Mcs is larger in the pigmented epidermis of individuals with vitiligo than in that of individuals without vitiligo, and the number of suprabasal Mcs in pigmented epidermis from patients with vitiligo is greater than that in a control population. Alterations to E-cadherin levels at the membrane can affect Mcs adhesion to the basal membrane (105). No melanoma susceptibility gene has yet been linked to renewal of the Mcs lineage or its pathology.

## CONCLUSION

All 20 melanoma susceptibility genes identified to date have a clear function during melanoma initiation, mainly the bypass of senescence. However, except for MITF they do not have a role in the different aspects of the life of Mcs. Susceptibility genes involved in melanogenesis (*OCA* genes, *ASIP*, and *MC1R*), in control of the cell cycle (*CDKN2A* and *CDK4*), in telomere length control (*TERT*, *POT1*, *ACD*, and *TERF2IP*), and in DNA repair (*BAP1*, *RAD51B* and *POLE*) may not be expressed nor have major function during the establishment of the Mcs lineage. As such, we understand that there is no developmental defect associated with the corresponding defective proteins, but we might expect that they may have a role during Mcs maintenance and renewal. Mutations in MITF and KIT dramatically affect the establishment of the Mcs lineage and both proteins play key roles in Mcs development and function. The importance of these genes during renewal remains unclear. Better understanding of the function of all these genes during normal Mcs renewal is crucial for advancing our understanding of their function during melanoma progression, especially during melanoma phenotype switching (86), which may use some proteins involved in McSC biology.

## ACKNOWLEDGEMENTS

This work was supported by the Ligue Contre le Cancer, INCa, ITMO Cancer, Fondation ARC (PGA), and is under the program "Investissements d'Avenir" launched by the French Government and implemented by ANR Labex CelTisPhyBio (ANR-11-LABX-0038 and ANR-10-IDEX-0001-02 PSL). This work was also supported by a grant from the Icelandic Research Fund (grant number 184861-051 to ES). BBdP is nationwide coordinator of melanoma oncogenetics for INCA.

## REFERENCES

1. Berk-Krauss J, Stein JA, Weber J, Polsky D, Geller AC. New systematic therapies and trends in cutaneous melanoma deaths among US whites, 1986–2016. Am J Public Health 2020; 110: 731–733.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359–386.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394–424.
4. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol 1988; 124: 869–871.
5. Olsen CM, Carroll HJ, Whiteman DC. Estimating the attributable fraction for melanoma: a meta-analysis of pigmentary characteristics and freckling. Int J Cancer 2010; 127: 2430–2445.
6. Mostafalou S, Abdollahi M. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicol Appl Pharmacol 2013; 268: 157–177.
7. Kubo JT, Henderson MT, Desai M, Wactawski-Wende J,

Stefanick ML, Tang JY. Alcohol consumption and risk of melanoma and non-melanoma skin cancer in the Women's Health Initiative. Cancer Causes Control 2014; 25: 1–10.
8. Rivera A, Nan H, Li T, Qureshi A, Cho E. Alcohol intake and risk of incident melanoma: a pooled analysis of three prospective studies in the United States. Cancer Epidemiol Biomarkers Prev 2016; 25: 1550–1558.
9. Smith AJ, Dieppe P, Porter M, Blom AW, National Joint Registry of E, Wales. Risk of cancer in first seven years after metal-on-metal hip replacement compared with other bearings and general population: linkage study between the National Joint Registry of England and Wales and hospital episode statistics. BMJ 2012; 344: e2383.
10. Le Douarin N, Kalcheim C. The neural crest. Second edition. Press CU, editor. Cambridge: Cambridge University Press; 1999.
11. Wehrle-Haller B, Weston JA. Soluble and cell-bound forms of steel factor activity play distinct roles in melanocyte precursor dispersal and survival on the lateral neural crest migration pathway. Development 1995; 121: 731–742.
12. Larue L, Kumasaka M, Goding CR. Beta-catenin in the melanocyte lineage. Pigment Cell Res 2003; 16: 312–317.
13. Luciani F, Champeval D, Herbette A, Denat L, Aylaj B, Martinozzi S, et al. Biological and mathematical modeling of melanocyte development. Development 2011; 138: 3943–3954.
14. Duverger O, Morasso MI. Epidermal patterning and induction of different hair types during mouse embryonic development. Birth Defects Res C Embryo Today 2009; 87: 263–272.
15. Soengas MS, Patton EE. Location, location, location: spatiotemporal cues that define the cell of origin in melanoma. Cell Stem Cell 2017; 21: 559–561.
16. Box NF, Duffy DL, Chen W, Stark M, Martin NG, Sturm RA, et al. MC1R genotype modifies risk of melanoma in families segregating CDKN2A mutations. Am J Hum Genet 2001; 69: 765–773.
17. Demenais F, Mohamdi H, Chaudru V, Goldstein AM, Newton Bishop JA, Bishop DT, et al. Association of MC1R variants and host phenotypes with melanoma risk in CDKN2A mutation carriers: a GenoMEL study. J Natl Cancer Inst 2010; 102: 1568–1583.
18. Cannon-Albright LA, Goldgar DE, Meyer LJ, Lewis CM, Anderson DE, Fountain JW, et al. Assignment of a locus for familial melanoma, MLM, to chromosome 9p13-p22. Science 1992; 258: 1148–1152.
19. Hussussian CJ, Struewing JP, Goldstein AM, Higgins PA, Ally DS, Sheahan MD, et al. Germline p16 mutations in familial melanoma. Nat Genet 1994; 8: 15–21.
20. 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. Nat Genet 1994; 8: 23–26.
21. Goldstein AM, Chaudru V, Ghiorzo P, Badenas C, Malvehy J, Pastorino L, et al. Cutaneous phenotype and MC1R variants as modifying factors for the development of melanoma in CDKN2A G101W mutation carriers from 4 countries. Int J Cancer 2007; 121: 825–831.
22. Aoude LG, Gartside M, Johansson P, Palmer JM, Symmons J, Martin NG, et al. Prevalence of germline BAP1, CDKN2A, and CDK4 mutations in an Australian population-based sample of cutaneous melanoma cases. Twin Res Hum Genet 2015; 18: 126–133.
23. Bishop DT, Demenais F, Goldstein AM, Bergman W, Bishop JN, Bressac-de Paillerets B, et al. Geographical variation in the penetrance of CDKN2A mutations for melanoma. J Natl Cancer Inst 2002; 94: 894–903.
24. Potrony M, Badenas C, Aguilera P, Puig-Butille JA, Carrera C, Malvehy J, et al. Update in genetic susceptibility in melanoma. Ann Transl Med 2015; 3: 210.
25. Begg CB, Orlow I, Hummer AJ, Armstrong BK, Kricker A, Marrett LD, et al. Lifetime risk of melanoma in CDKN2A mutation carriers in a population-based sample. J Natl Cancer Inst 2005; 97: 1507–1515.
26. Ghiorzo P, Bonelli L, Pastorino L, Bruno W, Barile M, Andreotti V, 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; 21: 718–720.
27. 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. J Med Genet 2011; 48: 266–272.
28. Zuo L, Weger J, Yang Q, Goldstein AM, Tucker MA, Walker GJ, et al. Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. Nat Genet 1996; 12: 97–99.
29. Puntervoll HE, Yang XR, Vetti HH, Bachmann IM, Avril MF, Benfodda M, et al. Melanoma prone families with CDK4 germline mutation: phenotypic profile and associations with MC1R variants. J Med Genet 2013; 50: 264–270.
30. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, et al. TERT promoter mutations in familial and sporadic melanoma. Science 2013; 339: 959–961.
31. Harland M, Petljak M, Robles-Espinoza CD, Ding Z, Gruis NA, van Doorn R, et al. Germline TERT promoter mutations are rare in familial melanoma. Fam Cancer 2016; 15: 139–144.
32. Aoude LG, Pritchard AL, Robles-Espinoza CD, Wadt K, Harland M, Choi J, et al. Nonsense mutations in the shelterin complex genes ACD and TERF2IP in familial melanoma. J Natl Cancer Inst 2014 Dec 13; 107. pii: dju408.
33. Robles-Espinoza CD, Harland M, Ramsay AJ, Aoude LG, Quesada V, Ding Z, et al. POT1 loss-of-function variants predispose to familial melanoma. Nat Genet 2014; 46: 478–481.
34. Wiesner T, Obenauf AC, Murali R, Fried I, Griewank KG, Ulz P, et al. Germline mutations in BAP1 predispose to melanocytic tumors. Nat Genet 2011; 43: 1018–1021.
35. Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E, et al. Germline BAP1 mutations predispose to malignant mesothelioma. Nat Genet 2011; 43: 1022–1025.
36. Abdel-Rahman MH, Pilarski R, Cebulla CM, Massengill JB, Christopher BN, Boru G, et al. Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. J Med Genet 2011; 48: 856–859.
37. Harbour JW, Onken MD, Roberson ED, Duan S, Cao L, Worley LA, et al. Frequent mutation of BAP1 in metastasizing uveal melanomas. Science 2010; 330: 1410–1413.
38. Walpole S, Pritchard AL, Cebulla CM, Pilarski R, Stautberg M, Davidorf FH, et al. Comprehensive study of the clinical phenotype of germline BAP1 variant-carrying families worldwide. J Natl Cancer Inst 2018; 110: 1328–1241.
39. Aoude LG, Heitzer E, Johansson P, Gartside M, Wadt K, Pritchard AL, et al. POLE mutations in families predisposed to cutaneous melanoma. Fam Cancer 2015; 14: 621–628.
40. Wadt KA, Aoude LG, Golmard L, Hansen TV, Sastre-Garau X, Hayward NK, et al. Germline RAD51B truncating mutation in a family with cutaneous melanoma. Fam Cancer 2015; 14: 337–340.
41. Song H, Dicks E, Ramus SJ, Tyrer JP, Intermaggio MP, Hayward J, et al. Contribution of Germline Mutations in the RAD51B, RAD51C, and RAD51D Genes to Ovarian Cancer in the Population. J Clin Oncol 2015; 33: 2901–2907.
42. Bertolotto C, Lesueur F, Giuliano S, Strub T, de Lichy M, Bille K, et al. A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. Nature 2011; 480: 94–98.
43. Yokoyama S, Woods SL, Boyle GM, Aoude LG, MacGregor S, Zismann V, et al. A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma. Nature 2011; 480: 99–103.
44. Garraway LA, Widlund HR, Rubin MA, Getz G, Berger AJ, Ramaswamy S, et al. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. Nature 2005; 436: 117–122.
45. Cheli Y, Ohanna M, Ballotti R, Bertolotto C. Fifteen-year quest for microphthalmia-associated transcription factor target genes. Pigment Cell Melanoma Res 2010; 23: 27–40.
46. Malouf GG, Camparo P, Molinie V, Dedet G, Oudard S, Schlei-

ermacher G, et al. Transcription factor E3 and transcription factor EB renal cell carcinomas: clinical features, biological behavior and prognostic factors. J Urol 2011; 185: 24–29.
47. Hernando B, Ibarrola-Villava M, Pena-Chilet M, Alonso S, Ribas G, Martinez-Cadenas C. Sex and MC1R variants in human pigmentation: differences in tanning ability and sensitivity to sunlight between sexes. J Dermatol Sci 2016; 84: 346–348.
48. Wendt J, Mueller C, Rauscher S, Fae I, Fischer G, Okamoto I. Contributions by MC1R variants to melanoma risk in males and females. JAMA Dermatol 2018; 154: 789–795.
49. Lira FE, Podlipnik S, Potrony M, Tell-Marti G, Calbet-Llopart N, Barreiro A, et al. Inherited MC1R variants in patients with melanoma are associated with better survival in women. Br J Dermatol 2020; 182: 138–146.
50. Nasti TH, Timares L. MC1R, eumelanin and pheomelanin: their role in determining the susceptibility to skin cancer. Photochem Photobiol 2015; 91: 188–200.
51. Herraiz C, Garcia-Borron JC, Jimenez-Cervantes C, Olivares C. MC1R signaling. Intracellular partners and pathophysiological implications. Biochim Biophys Acta Mol Basis Dis 2017; 1863: 2448–2461.
52. Mitra D, Luo X, Morgan A, Wang J, Hoang MP, Lo J, et al. An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. Nature 2012; 491: 449–453.
53. Wendt J, Rauscher S, Burgstaller-Muehlbacher S, Fae I, Fischer G, Pehamberger H, et al. Human determinants and the role of melanocortin-1 receptor variants in melanoma risk independent of UV radiation exposure. JAMA Dermatol 2016; 152: 776–782.
54. Kennedy C, ter Huurne J, Berkhout M, Gruis N, Bastiaens M, Bergman W, et al. Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skin type and hair color. J Invest Dermatol 2001; 117: 294–300.
55. Kanetsky PA, Panossian S, Elder DE, Guerry D, Ming ME, Schuchter L, et al. Does MC1R genotype convey information about melanoma risk beyond risk phenotypes? Cancer 2010; 116: 2416–2428.
56. Tagliabue E, Gandini S, Bellocco R, Maisonneuve P, Newton-Bishop J, Polsky D, 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; 10: 1143–1154.
57. Vallone MG, Tell-Marti G, Potrony M, Rebollo-Morell A, Badenas C, Puig-Butille JA, et al. Melanocortin 1 receptor (MC1R) polymorphisms' influence on size and dermoscopic features of nevi. Pigment Cell Melanoma Res 2018; 31: 39–50.
58. Gudbjartsson DF, Sulem P, Stacey SN, Goldstein AM, Rafnar T, Sigurgeirsson B, et al. ASIP and TYR pigmentation variants associate with cutaneous melanoma and basal cell carcinoma. Nat Genet 2008; 40: 886–891.
59. Bastian BC. The molecular pathology of melanoma: an integrated taxonomy of melanocytic neoplasia. Annu Review Pathol 2014; 9: 239–271.
60. Law MH, Bishop DT, Lee JE, Brossard M, Martin NG, Moses EK, et al. Genome-wide meta-analysis identifies five new susceptibility loci for cutaneous malignant melanoma. Nat Genet 2015; 47: 987–995.
61. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013; 499: 214–218.
62. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, et al. A landscape of driver mutations in melanoma. Cell 2012; 150: 251–263.
63. Rabbie R, Ferguson P, Molina-Aguilar C, Adams DJ, Robles-Espinoza CD. Melanoma subtypes: genomic profiles, prognostic molecular markers and therapeutic possibilities. J Pathol 2019; 247: 539–551.
64. Gandini S, Autier P, Boniol M. Reviews on sun exposure and artificial light and melanoma. Prog Biophys Mol Biol 2011; 107: 362–366.
65. Whiteman DC, Whiteman CA, Green AC. Childhood sun exposure as a risk factor for melanoma: a systematic review of epidemiologic studies. Cancer Causes Control 2001; 12: 69–82.
66. Noonan FP, Zaidi MR, Wolnicka-Glubisz A, Anver MR, Bahn J, Wielgus A, et al. Melanoma induction by ultraviolet A but not ultraviolet B radiation requires melanin pigment. Nat Commun 2012; 3: 884.
67. Brash DE. UV signature mutations. Photochem Photobiol 2015; 91: 15–26.
68. Ferrucio B, Tiago M, Fannin RD, Liu L, Gerrish K, Maria-Engler SS, et al. Molecular effects of 1-naphthyl-methylcarbamate and solar radiation exposures on human melanocytes. Toxicol In Vitro 2017; 38: 67–76.
69. Clement E, Lazar I, Muller C, Nieto L. Obesity and melanoma: could fat be fueling malignancy? Pigment Cell Melanoma Res 2017; 30: 294–306.
70. Hennessey RC, Holderbaum AM, Bonilla A, Delaney C, Gillahan JE, Tober KL, et al. Ultraviolet radiation accelerates NRas-mutant melanomagenesis: A cooperative effect blocked by sunscreen. Pigment Cell Melanoma Res 2017; 30: 477–487.
71. Gaffal E, Landsberg J, Bald T, Sporleder A, Kohlmeyer J, Tuting T. Neonatal UVB exposure accelerates melanoma growth and enhances distant metastases in Hgf-Cdk4(R24C) C57BL/6 mice. Int J Cancer 2011; 129: 285–294.
72. Cheung M, Kadariya Y, Talarchek J, Pei J, Ohar JA, Kayaleh OR, et al. Germline BAP1 mutation in a family with high incidence of multiple primary cancers and a potential gene-environment interaction. Cancer Lett 2015; 369: 261–265.
73. Cao J, Wan L, Hacker E, Dai X, Lenna S, Jimenez-Cervantes C, et al. MC1R is a potent regulator of PTEN after UV exposure in melanocytes. Mol Cell 2013; 51: 409–422.
74. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646–674.
75. Clark WH, Jr., Elder DE, Guerry Dt, Epstein MN, Greene MH, Van Horn M. A study of tumor progression: the precursor lesions of superficial spreading and nodular melanoma. Hum Pathol 1984; 15: 1147–1165.
76. Bevona C, Goggins W, Quinn T, Fullerton J, Tsao H. Cutaneous melanomas associated with nevi. Arch Dermatol 2003; 139: 1620–1624.
77. Cancer Genome Atlas Network. Electronic address imo, Cancer Genome Atlas N. Genomic Classification of Cutaneous Melanoma. Cell 2015; 161: 1681–1696.
78. Delmas V, Beermann F, Martinozzi S, Carreira S, Ackermann J, Kumasaka M, et al. Beta-catenin induces immortalization of melanocytes by suppressing p16INK4a expression and cooperates with N-Ras in melanoma development. Genes Dev 2007; 21: 2923–2935.
79. Dankort D, Curley DP, Cartlidge RA, Nelson B, Karnezis AN, Damsky WE, Jr., et al. Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. Nat Genet 2009; 41: 544–552.
80. Conde-Perez A, Gros G, Longvert C, Pedersen M, Petit V, Aktary Z, et al. A caveolin-dependent and PI3K/AKT-independent role of PTEN in beta-catenin transcriptional activity. Nat Commun 2015; 6: 8093.
81. McNeal AS, Liu K, Nakhate V, Natale CA, Duperret EK, Capell BC, et al. CDKN2B loss promotes progression from benign melanocytic nevus to melanoma. Cancer Discov 2015; 5: 1072–1085.
82. Bonet C, Luciani F, Ottavi JF, Leclerc J, Jouenne FM, Boncompagni M, et al. Deciphering the role of oncogenic MITF-FE318K in senescence delay and melanoma progression. J Natl Cancer Inst 2017; 109.
83. Goding CR, Arnheiter H. MITF-the first 25 years. Genes Dev 2019; 33: 983–1007.
84. Luande J, Henschke CI, Mohammed N. The Tanzanian human albino skin. Natural history. Cancer 1985; 55: 1823–1828.
85. Rivera RS, Nagatsuka H, Gunduz M, Cengiz B, Gunduz E, Siar CH, et al. C-kit protein expression correlated with activating mutations in KIT gene in oral mucosal melanoma. Virchows Arch 2008; 452: 27–32.
86. Montone KT, van Belle P, Elenitsas R, Elder DE. Proto-onco-

gene c-kit expression in malignant melanoma: protein loss with tumor progression. Mod Pathol 1997; 10: 939–944.
87. Goodall J, Carreira S, Denat L, Kobi D, Davidson I, Nuciforo P, et al. Brn-2 represses microphthalmia-associated transcription factor expression and marks a distinct subpopulation of microphthalmia-associated transcription factor-negative melanoma cells. Cancer Res 2008; 68: 7788–7794.
88. Goding CR. Commentary. A picture of Mitf in melanoma immortality. Oncogene 2011; 30: 2304–2306.
89. Carreira S, Goodall J, Aksan I, La Rocca SA, Galibert MD, Denat L, et al. Mitf cooperates with Rb1 and activates p21Cip1 expression to regulate cell cycle progression. Nature 2005; 433: 764–769.
90. Loercher AE, Tank EM, Delston RB, Harbour JW. MITF links differentiation with cell cycle arrest in melanocytes by transcriptional activation of INK4A. J Cell Biol 2005; 168: 35–40.
91. Hirobe T. How are proliferation and differentiation of melanocytes regulated? Pigment Cell Melanoma Res 2011; 24: 462–478.
92. Osawa M. Melanocyte stem cells. StemBook. Cambridge (MA) 2008.
93. Oren M, Bartek J. The sunny side of p53. Cell 2007; 128: 826–828.
94. Garcia-Borron JC, Sanchez-Laorden BL, Jimenez-Cervantes C. Melanocortin-1 receptor structure and functional regulation. Pigment Cell Res 2005; 18: 393–410.
95. Delevoye C, Giordano F, van Niel G, Raposo G. [Biogenesis of melanosomes – the chessboard of pigmentation]. Med Sci (Paris) 2011; 27: 153–162.
96. Wasmeier C, Hume AN, Bolasco G, Seabra MC. Melanosomes at a glance. J Cell Sci 2008; 121: 3995–3999.
97. Lamoreux ML, Delmas V, Larue L, Bennett D. The colors of mice: A model genetic network: Wiley-Blackwell; 2010. 312p.
98. Ohbayashi N, Mamishi S, Ishibashi K, Maruta Y, Pourakbari B, Tamizifar B, et al. Functional characterization of two RAB27A missense mutations found in Griscelli syndrome type 2. Pigment Cell Melanom Res 2010; 23: 365–374.
99. Tadokoro R, Takahashi Y. Intercellular transfer of organelles during body pigmentation. Curr Opin Genet Dev 2017; 45: 132–138.
100. Tarafder AK, Bolasco G, Correia MS, Pereira FJC, Iannone L, Hume AN, et al. Rab11b mediates melanin transfer between donor melanocytes and acceptor keratinocytes via coupled exo/endocytosis. J Invest Dermatol 2014; 134: 1056–1066.
101. Tadokoro R, Murai H, Sakai KI, Okui T, Yokota Y, Takahashi Y. Melanosome transfer to keratinocyte in the chicken embryonic skin is mediated by vesicle release associated with Rho-regulated membrane blebbing. Sci Rep 2016; 6: 38277.
102. Scott G, Leopardi S, Printup S, Madden BC. Filopodia are conduits for melanosome transfer to keratinocytes. J Cell Sci 2002; 115: 1441–1451.
103. Scott G, Leopardi S, Printup S, Malhi N, Seiberg M, Lapoint R. Proteinase-activated receptor-2 stimulates prostaglandin production in keratinocytes: analysis of prostaglandin receptors on human melanocytes and effects of PGE2 and PGF2alpha on melanocyte dendricity. J Invest Dermatol 2004; 122: 1214–1224.
104. Jimbow K, Quevedo WC, Jr., Fitzpatrick TB, Szabo G. Some aspects of melanin biology: 1950–1975. J Invest Dermatol 1976; 67: 72–89.
105. Wagner RY, Luciani F, Cario-Andre M, Rubod A, Petit V, Benzekri L, et al. Altered E-Cadherin Levels and Distribution in Melanocytes Precede Clinical Manifestations of Vitiligo. J Invest Dermatol 2015; 135: 1810–1819.
106. MacKenzie MA, Jordan SA, Budd PS, Jackson IJ. Activation of the receptor tyrosine kinase Kit is required for the proliferation of melanoblasts in the mouse embryo. Dev Biol 1997; 192: 99–107.
107. Harris ML, Levy DJ, Watkins-Chow DE, Pavan WJ. Ectopic differentiation of melanocyte stem cells is influenced by genetic background. Pigment Cell Melanoma Res 2015; 28: 223–228.
108. Adameyko I, Lallemend F, Aquino JB, Pereira JA, Topilko P, Muller T, et al. Schwann cell precursors from nerve innervation are a cellular origin of melanocytes in skin. Cell 2009; 139: 366–379.
109. Kinsler VA, Larue L. The patterns of birthmarks suggest a novel population of melanocyte precursors arising around the time of gastrulation. Pigment Cell Melanoma Res 2018; 31: 95–109.
110. Saleem MD. Biology of human melanocyte development, Piebaldism, and Waardenburg syndrome. Pediatr Dermatol 2019; 36: 72–84.
111. Pingault V, Ente D, Dastot-Le Moal F, Goossens M, Marlin S, Bondurand N. Review and update of mutations causing Waardenburg syndrome. Hum Mutat 2010; 31: 391–406.
112. Nishimura EK, Jordan SA, Oshima H, Yoshida H, Osawa M, Moriyama M, et al. Dominant role of the niche in melanocyte stem-cell fate determination. Nature 2002; 416: 854–860.
113. Okamoto N, Aoto T, Uhara H, Yamazaki S, Akutsu H, Umezawa A, et al. A melanocyte – melanoma precursor niche in sweat glands of volar skin. Pigment Cell Melanoma Res 2014; 27: 1039–1050.
114. Li L, Fukunaga-Kalabis M, Yu H, Xu X, Kong J, Lee JT, et al. Human dermal stem cells differentiate into functional epidermal melanocytes. J Cell Sci 2010; 123: 853–860.
115. Nishimura EK. Melanocyte stem cells: a melanocyte reservoir in hair follicles for hair and skin pigmentation. Pigment Cell Melanoma Res 2011; 24: 401–410.
116. Lowry WE, Blanpain C, Nowak JA, Guasch G, Lewis L, Fuchs E. Defining the impact of beta-catenin/Tcf transactivation on epithelial stem cells. Genes Dev 2005; 19: 1596–1611.
117. St-Jacques B, Dassule HR, Karavanova I, Botchkarev VA, Li J, Danielian PS, et al. Sonic hedgehog signaling is essential for hair development. Curr Biol 1998; 8: 1058–1068.
118. Aubin-Houzelstein G, Djian-Zaouche J, Bernex F, Gadin S, Delmas V, Larue L, et al. Melanoblasts' proper location and timed differentiation depend on Notch/RBP-J signaling in postnatal hair follicles. J Invest Dermatol 2008; 128: 2686–2695.
119. Schouwey K, Delmas V, Larue L, Zimber-Strobl U, Strobl LJ, Radtke F, et al. Notch1 and Notch2 receptors influence progressive hair graying in a dose-dependent manner. Dev Dyn 2007; 236: 282–289.
120. Moriyama M, Osawa M, Mak SS, Ohtsuka T, Yamamoto N, Han H, et al. Notch signaling via Hes1 transcription factor maintains survival of melanoblasts and melanocyte stem cells. J Cell Biol 2006; 173: 333–339.
121. Van Mater D, Kolligs FT, Dlugosz AA, Fearon ER. Transient activation of beta -catenin signaling in cutaneous keratinocytes is sufficient to trigger the active growth phase of the hair cycle in mice. Genes Dev 2003; 17: 1219–1224.
122. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019; 47: D607–D613.



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REVIEW ARTICLE

# Update on the Management of Basal Cell Carcinoma

Nicole BASSET-SEGUIN and Florian HERMS
*Department of Dermatology, Universite Paris 7, Hôpital Saint-Louis, 1 avenue Claude Vellefaux, FR-75010 Paris, France. E-mail: nicole.basset-seguin@aphp.fr*

**Basal cell carcinomas are the most frequent skin cancers in the fair-skinned adult population over 50 years of age. Their incidence is increasing throughout the world. Ultraviolet (UV) exposure is the major carcinogenic factor. Some genodermatosis can predispose to formation of basal cell carcinomas at an earlier age. Basal cell carcinomas are heterogeneous, from superficial or nodular lesions of good prognosis to very extensive difficult-to-treat lesions that must be discussed in multidisciplinary committees. Recent guidelines have updated the management of basal cell carcinoma. The prognosis is linked to the risk of recurrence of basal cell carcinoma or its local destructive capacity. Characteristic molecular events in these tumours are: (*i*) activation of the hedgehog pathway, which has allowed the development of hedgehog inhibitors for difficult-to-treat lesions that are not accessible to surgery or radiotherapy; (*ii*) high mutational burden, which suggests that hedgehog inhibitor refractory tumours could be offered immunotherapy; some trials are ongoing. The standard treatment for most basal cell carcinomas is surgery, as it allows excision margin control and shows a low risk of recurrence. Superficial lesions can be treated by non-surgical methods with significant efficacy.**

*Key words:* basal cell carcinoma; treatment; prognosis; surgery; radiotherapy, hedgehog inhibitors.

Accepted Apr 27, 2020; Epub ahead of print Apr 28, 2020

Acta Derm Venereol 2020; 100: adv00140.

*Corr:* Nicole Basset-Seguin, Department of Dermatology, Universite Paris 7, Hôpital Saint-Louis, 1 avenue Claude Vellefaux, FR-75010 Paris, France. E-mail: nicole.basset-seguin@aphp.fr

## SIGNIFICANCE

Basal cell carcinoma is the most frequent cancer in fair-skinned adults. The molecular background to these tumours includes activation of a cellular pathway called the "sonic hedgehog pathway". Basal cell carcinomas are induced by ultraviolet light and occur more frequently on areas of skin that are exposed to the sun. Basal cell carcinomas rarely spread to other sites in the body, although there is a risk that they will recur. There are different subtypes of these tumours with different potential to relapse. This paper gives an update of what is known about basal cell carcinomas and their treatment. The standard treatment is surgery. The prognosis for advanced basal cell carcinomas that cannot be operated on has improved with the development of systemic drugs targeting the hedgehog pathway.

Basal cell carcinoma (BCC) is a slow-growing skin tumour, which is commonly seen in dermatology. BCCs rarely metastasize, but are frequently multiple and recurrent on sun-exposed skin, with some morbidity. BCCs are a heterogeneous group of tumours, with histopathological and clinical characteristics ranging from superficial lesions to very extensive and destructive tumours. The standard treatment for BCC is surgery, but non-surgical options (medical, systemic or physical) have been developed in recent years for each end of the spectrum of these tumours: superficial lesions (sBCC) and advanced BCC (aBCC). Guidelines have been updated to help physicians with these different therapeutic strategies (1).

## EPIDEMIOLOGY OF BASAL CELL CARCINOMA

BCC is the most frequent skin cancer in fair-skinned adult patients (2). The estimated lifetime risk in this population is approximately 30% (3). The worldwide incidence of BCC is increasing continuously, but it cannot be estimated precisely as this tumour is not consistently registered. Marked geographical variations have been reported. The highest incidence is reported in Australia (up to 1,000/100,000 inhabitants per year, followed by the USA (212–407/100,000 female and male inhabitants respectively/year) and Europe (mean range from 76.21 /100,000 person-years in the UK to 157 per 100,000 person-years in 2009 in the Netherlands). This is within the range found in other European countries, such as Germany, France, Italy and Spain (4, 5). The lowest incidence is observed in Africa (<1/100,000 persons years).

BCC is most frequently seen after 50 years of age, with a female/male ratio of 2:1 (6). However, some patients develop BCC at an earlier age (<40 years). Patients with genetic predisposition syndromes, such as xeroderma pigmentosum (XP) or basal cell naevus syndrome (BCNS) can develop BCC earlier, even before 20 years of age (see the section on genetics, below). In the USA the ratio of cases of BCC to that of squamous cell carcinoma (SCC) was estimated at 4:1 and changed to 1:1 in 2012, but this is probably due to earlier SCC lesions being removed, which may have previously been treated non-surgically (7, 8).

doi: 10.2340/00015555-3495
Acta Derm Venereol 2020; 100: adv00140

The most significant risk factor for development of BCC is sun exposure, both in childhood and recreationally or occupationally in adult life (9). UVA, and mostly UVB, is implicated. This explains why most tumours are located on sun-exposed skin and are more frequent in fair-skinned people. BCC is the most highly mutated human tumour (65 mutations/megabase) (10). Another risk factor is immunosuppression, with a greater than 10-fold increase in BCC, especially in kidney transplant recipients (11).

## HISTOLOGICAL SUBTYPES

BCC develops from follicular and interfollicular keratinocyte stem cells (12, 13). Different clinical and histological types have been described with increasing invasiveness from superficial, nodular, morphoeic and basosquamous tumours **(Fig. 1)**. Nodular lesions represent 60% of all BCCs and appear as nodules or papules with telangiectasia. Superficial lesions are flat, erythematous, and scaly with well-demarcated edges; more frequently found on the trunk of younger adults; and represent 20% of all BCC. Morphoeic lesions are scar-like whitish plaques with indistinct borders. These tumours can also be ulcerated and pigmented.

In a review of 1,039 consecutive cases, Sexton et al. have found that most BCC are mixed (14). In these cases the most aggressive form defines the prognosis of the tumour. Basosquamous tumours are often found in advanced or difficult-to-treat lesions, which have been left without treatment for many years and are seen at an advanced stage. These lesions are classified as difficult-to-treat, in contrast to the former, which fall into the category of common BCC or easy-to-treat tumours unless they have specific management difficulty (1). In fact, these forms of difficult-to-treat BCC are heterogeneous and a classification system has been proposed by the European Association of Dermato-Oncology (EADO) and is under revision. These difficult-to-treat lesions often require imaging, with magnetic resonance imaging (MRI) or tomodensitometry, to determine the tumour extension.

Dermoscopy is useful to help with the diagnosis of BCC, revealing ovoid nests and globules, leaf-like areas, arborizing and superficial telangiectasias, erosions, pigmentation, but absence of pigment network. A recent study has shown that, in a comparison of naked eye examination and dermoscopy, the diagnosis sensitivity and specificity improved from 66.9% to 85% and 97.2% to 98.2%, respectively, with dermoscopy (15). Dermoscopy may also help to recognize the histopathological subtype of BCC (16).

## DIAGNOSIS OF BASAL CELL CARCINOMA

The diagnosis of BCC requires a biopsy, unless the lesion is small or clinically and dermoscopically typical, especially in non-high risk locations (trunk). A biopsy is recommended before proceeding to complex surgery or systemic treatment (1). The biopsy can confirm the diagnosis of BCC, but may not be adequate to appreciate the histological subtype in view of the heterogeneous histology.

## GENETICS OF BASAL CELL CARCINOMA

Twenty years ago, the candidate gene (germline mutation) for patients with BCNS syndrome (a genodermatose predisposing to multiple BCCs and developmental defects) was reported to be the *PTCH1* gene, leading to activation of the hedgehog pathway (Hh) (**Fig. 2**), which is a crucial event in the pathogenesis of BCC (17). *PTCH1* (located on human chromosome 9q22) encodes a transmembrane protein negatively regulating smoothened (SMO), another transmembrane protein of the pathway. When *PTCH1* binds to an extracellular ligand, such as sonic hedgehog, its negative control on SMO is relieved, allowing SMO to migrate in the cilium and activate Gli transcription factors (18, 19). Since then other germline mutations have been described in Gorlin's syndrome, targeting *PATCH2* and *SUFU* genes (20).

Activation of the Hh pathway has also been demonstrated in sporadic BCC, with 90% of the tumours bearing inactivating mutations of PATCH and 10% activation of SMO (21). These mutations are most often UV-induced: C>T transitions at dipyrimidines sites or even more specific CC>TT tandem mutations.

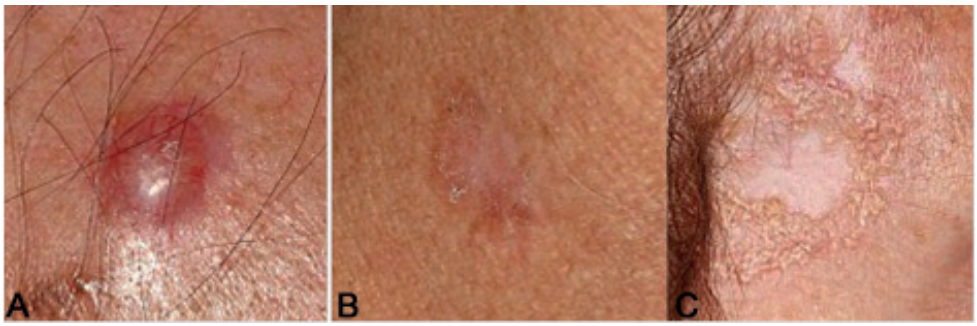


**Fig. 1. Various basal cell carcinoma (BCC) clinical subtypes.** (A) Nodular BCC. (B) Superficial BCC. (C) Morpheiform BCC.

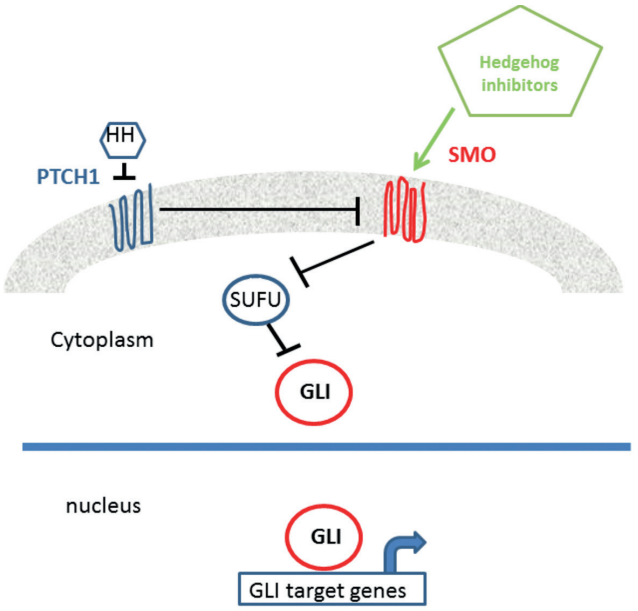


**Fig. 2. Schematic view of the hedgehog (HH) pathway.** When HH ligand binds to the transmembrane receptor PTCH1 it releases its inhibitory activity toward smoothened (SMO), which inhibits another negative regulator of the pathway *SUFU* leading to activation of *GLI* and *GLI* target genes. Hedgehog inhibitors are anti-SMO molecules. GLI is a transcription factor activated by SMO.

Inactivating the Hh pathway has been a major therapeutic goal for difficult-to-treat lesions and 2 oral-targeted therapies (hedgehog inhibitors or HhI) are currently available: vismodegib and sonidegib (1).

If the occurrence of mutations in the Hh pathway is considered to be the driver event toward formation of BCC, secondary drivers have been found in cancer genes, such as *MYCN, PPPC, SK19, LATS1, ERBB2, PIK23C, N-RAS, K-RAS, H-RAS, PTPN14, RB1*, and *FBX7* (22). Other pathways that increase the transcription factor of GLI include a recently described loss-of-function mutation in *SUFU* in sporadic BCC and a variety of non-canonical hedgehog signalling pathways (the mammalian target of rapamycin (mTOR), insulin-like growth factor (IGF)–PI3K–AKT, epidermal growth factor receptor (EGFR)–MEK–ERK, and Hippo pathways) that are independent of ligand–PTCH1 binding and SMO activation (23).

The impact of these other mutations on the histopathological characteristics and evolution of BCC or their response to systemic treatment is unknown.

Other genetic diseases can predispose patients to the formation of BC: XP due to germline mutations in DNA repair genes (24), which predispose to multiple skin tumours, including BCC, but also melanoma and squamous cell carcinoma (SCC), at an early age, as well as the Bazex-Dupre-Christol syndrome, and dominant X-linked cancer-prone genodermatosis, in which recent studies have reported mutations in the ACTRT1 gene and its enhancer, leading to activation of the Hh pathway in certain families (25).

## PROGNOSIS OF BASAL CELL CARCINOMA

BCC very rarely metastasizes; it estimated incidence of metastasis is 0.0028–0.55% (1). A recent review of published cases showed that median survival in case of distant metastases was 24 months, and 36.2% of those had systemic chemotherapies. Regional metastasis were shown to have a median survival of 87 months (26).

The major issues with BCC are local destruction and recurrence. Mortality is low. Risk of recurrence is influenced by the location of the tumour (H zone of the face), the histological subtype, perineural invasion, immunosuppression and prior recurrences. Severe forms of BCC are heterogeneous and rare. A retrospective study from the USA reported that the severe form of BCC accounted for approximately 0.8% of all cases of BCC (27), while another reported 10/100,000 persons (28). No TNM classification is available and a grading method to classify these difficult-to-treat BCC is currently being developed by the EADO group. These advanced tumours are often not measurable by Response Evaluation Criteria of Solid Tumors (RECIST criteria) and can destroy large anatomical surfaces without affecting survival (1).

## TREATMENT OF BASAL CELL CARCINOMA

### *Surgery*

Surgery is the standard treatment for the majority of BCC (**Fig. 3**). Standard excision (SE) or micrographic surgery (Mohs) can be used according to the characteristics of the tumour (size, location, previous recurrences, histology) and the skills of the surgeon. Mohs is reserved for high-risk tumours, in recurrent BCC or BCC in critical anatomical sites. A prospective randomized trial comparing SE and Mohs showed a 10-year cumulative probability of recurrence for primary BCC of 12.2% for SE and 4.4% for Mohs and for recurrent BCCs of 13.5 for SE and 3.9% for Mohs (29).

The margins used for SE depend of the BCC recurrence risk profile. Current guidelines suggest a range of peripheral margins between 2 mm and 5 mm in low-risk tumours and between 5 mm and 15 mm in high-risk lesions (1). It has been reported that the size of the BCC also correlates with the risk of subclinical extension with a 4 mm lateral margin sufficient to excise a $<2$ cm BCC, while a tumour of $>2$ cm and additional risk characteristics may need a minimal lateral margin of 13 mm for complete removal. Deep margins recommend excision to level of the fat or, in the face, to the level of fascia, perichondrium or periosteum (30).

Clinical and histological margins do not correlate, as tissue shrinkage is observed after fixation. There is no specific recommendation nor evidence-based data to re-excision in case of complete excision with narrow margins (1).

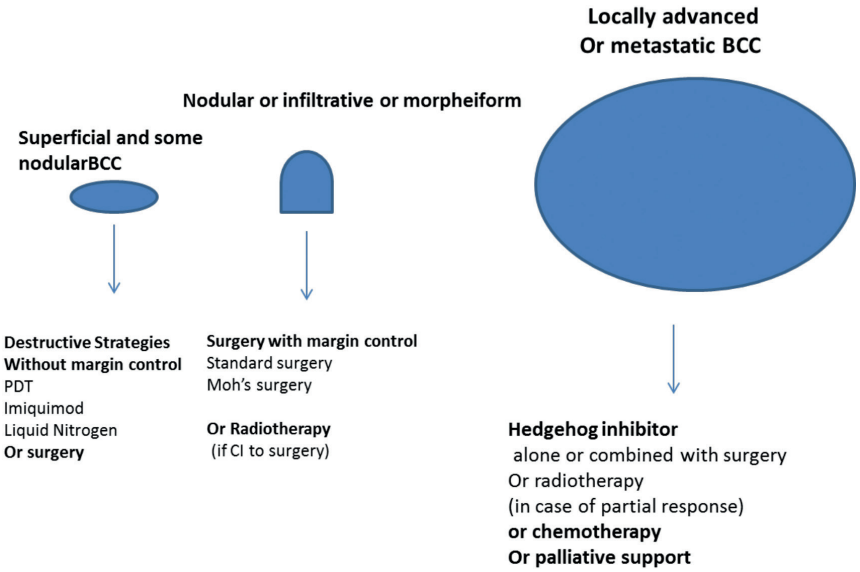


**Fig. 3. Schematic landscape of treatment options for basal cell carcinoma (BCC).**

*What to do in cases of incomplete excision?*

Incomplete excision can be reported in 4.7–24% of SE (31), and can lead to recurrence in 26–41% after 2–5 years of follow-up. If incompletely excised lesions recur, it is recommended to re-excise with wider margins, as the risk of multiple recurrence can be as high as 50% once a positive-margin BCC has recurred after surgery (32).

*Radiotherapy*

Radiotherapy is a good alternative to treat BCC, especially in elderly patients. It is recommended for patients who are not candidates for surgery (due to morbidity, patient's choice, advanced disease, etc.). Radiotherapy can use external beam radiotherapy, or brachytherapy or contact therapy, and this will depend on the size, location of the tumour, the team expertise and resources. It can also be considered, but has never been evaluated, as adjuvant therapy when re-excision of incompletely excised lesions is not possible or when there is perineural evasion.

Recent meta-analysis has reported an estimated recurrence rate of 3.5% with radiotherapy similar to that reported for surgery (1). Radiotherapy is contra-indicated in patients with BCC nevus syndrome (BCCNS), as it may cause further tumours in the field of irradiation

*Medical treatments alternative to surgery in superficial lesions*

*Imiquimod*. Imiquimod is an immune-response modifier, which is indicated for the treatment of superficial BCC and small nodular BCC in immunocompetent adults. It must be applied 5 times per week for 6 weeks. The major biological effects of imiquimod are mediated through agonistic activity towards toll-like receptors (TLR) 7 and 8 and consecutively, activation of nuclear factor kappa B (NFkB). The result of this activity is the induction of pro-inflammatory cytokines, chemokines and other mediators, leading to activation of antigen-presenting cells and other components of innate immunity and, finally, the mounting of a profound T-helper (Th1)-weighted anti-tumoural cellular immune response (33). Randomized comparative trials comparing 5% 5-fluorouracil (5-FU) with imiquimod 5% cream and MAL-PDT in patients with sBCC showed a treatment success of 72.8% for MAL-PDT, 83.4% for imiquimod, and 80.1% for 5% 5-FU at 1 year and 62.7%, 80.5% and 70%, respectively, at 5 years (31, 32).

The efficacy of imiquimod was also compared with surgery (S) for low-risk BCC and showed a successful response at 5 years, of 82.5% for imiquimod vs. 97.7% for surgery (34), confirming that imiquimod represents a good alternative to surgery for the treatment of sBCC.

Some local and general reactions can be observed with imiquimod, and patients should be informed of these.

*5-Fluorouracil*. 5-FU 5% is indicated for the treatment of sBCC (2 applications/day for 2–4 weeks), but very few studies have looked at long-term results. In the trial comparing 5-FU with imiquimod and PDT for the treatment of sBCC, 5-FU was shown to be inferior to imiquimod, but equivalent to MAL-PDT after 3 and 5 years (35).

*Physically destructive treatments*

Destructive treatments must be reserved for sBCC or small nodular BCC, as they evaluate the complete eradication of the tumour.

*Photodynamic therapy*. PDT with 5-aminolaevulinic acid (ALA) or its methyl ester (MAL) is indicated for sBCC and small nodular BCC (nBCC) less than 2 mm thickness. MAL-PDT gave clearance rates for sBCC of 92–97% and a recurrence rate at 1 year of 9%, which increased to 22% at 3 years and remained at the same rate at 5 years (36). MAL-PDT was also used and compared with surgery in nBCC and showed 91% clearance at 3 months and a sustained clearance rate of 76% at 5 years, inferior to surgery, but with cosmetic superiority. PDT with ALA nanoemulsion gel was shown to be as efficient as MAL-PDT in low-risk BCC (37).

PDT is a good option for patients with multiple superficial lesions, especially for lesions located on the back, on which application of imiquimod can be difficult.

*Cryotherapy.* Cryotherapy is indicated for low-risk BCC and has been shown to be as efficient as PDT in clinical trial (35). Its main advantage is the fact that it is an immediate procedure performed during the consultation. Its disadvantages are pain and the cosmetic results, as the treatment often leaves hypopigmented spots, which can last for years. Medical and physical treatments can be combined (i.e. PDT + imiquimod, rituximab and HhI, for example) (1).

### *Systemic treatments of difficult-to-treat or aBCC*

Treatment of aBCC must be discussed in multidisciplinary committee.

*Chemotherapy.* No clinical trial has evaluated chemotherapy for BCC. Most chemotherapies are platinum-based. The response rate is approximately 20–30%, but the duration of response does not exceed 2–3 months (26).

In addition, in elderly patients, chemotherapy can have life-threatening adverse effects. It is usually proposed as a second- or third-line treatment after failure of HhI.

*Hedgehog inhibitors.* Major progress has been achieved for the treatment of difficult-to-treat BCC with HhIs (35). Two molecules, with different pharmacokinetics, but targeting the same molecule, SMO, are available: vismodegib and sonidegib. No hed-to-head comparative studies are available. Vismodegib is indicated for laBCC (i.e. not a candidate for surgery or radiotherapy) and symptomatic metastatic BCC (mBCC) at a dose of 150 mg/day, while sonidegib is indicated for laBCC only, at a dose of 200 mg/day.

*Vismodegib*. Vismodegib was the first approved Hh inhibitor. The ERIVANCE study, an open-labelled non-randomized study, including 104 patients, showed, in the primary analysis, (using independent reviewer assessment) a 43% overall response rate (ORR) for a laBCC cohort, with 20.6% complete response (CR) and 22.2% partial response (PR). The response rate was 30.3% for the metastatic cohort (mBCC) (38). The median duration of response (DOR) was 9.5 (laBCC) and 7.6 months (mBCC). The 30-month update of ERIVANCE showed (using investigator assessment), an ORR of 60.3% for the laBCC (including 33 CR) and 48.5% for mBCC (only PR) and a DOR of 26.2 and 14.8 months, respectively (39). The median survival was 33.4 months for mBCC and was not reached for laBCC.

The STEVIE (SafeTy Events in VIsmodEgib) study, which enrolled the largest amount of patients (1,215, with 1,119 laBCC and 96 mBCC) had a main objective on safety. The secondary objective was efficacy and confirmed results obtained with the ERIVANCE study, with 68.5% of investigator-assessed objective response including 33.4 with CR for laBCC, and a median DOR of 23 months. For mBCC the ORR was observed in 36.9%, mostly PR, and a duration of response of 13.9 months (40). A subgroup analysis showed that BCCNS patients responded better to vismodegib. This was also observed in a clinical trial (41), which objective was to study the efficacy of vismodegib to shrink existing tumours and prevent formation of new BCC, both confirmed. However, long-term follow-up shows that all patients relapse after drug interruption (41).

In a recent report looking at long-term maintenance of CR after drug interruption, it was shown that 60% of patients have relapsed after 3 years of follow-up, with 40% (when BCCNS cases are excluded) having not relapsed at the time. Among relapsing patients, 48% had become eligible for surgery and 50% were vismodegib re-challenged and showed an ORR of 85% (42).

*Sonidegib*. The second HhI is sonidegib. The pivotal clinical trial Basal Cell Carcinoma Outcomes with LDE225 Treatment (BOLT) was a prospective randomized double-blinded trial comparing a once-daily dose of 200 mg with 800 mg. The 200 mg dose was approved based on the risk/benefit ratio. Evaluation used very stringent modified RECIST criteria showed a response rate of 36% (43). In the 12-month update analysis of the BOLT trial, the response rate for the 200 mg group improved to 57.6% for laBCC and 7.7% for mBCC (44). The Bolt follow-up of 30 months (45) reported a response rate of 56.1% (central review) and 71.2% (investigator review) for laBCC and 7.7% and 23.1% for mBCC. The median duration of responses was 26.1 months (laBCC) and 24.0 months (mBCC). The median survival has not been reached in the 2 groups.

Both vismodegib and sonidegib, which belong to the same class of drug, share common adverse events (most frequent: muscles cramps, dysgeusia, fatigue, hair loss and weight loss). These adverse events are observed in the majority of patients and lead to drug discontinuation in 30% of cases. No treatment-related deaths were reported. Different strategies have been proposed to prevent or manage the side-effects (46). Adverse events with sonidegib seem to be slightly less frequent and less severe, but this has not been evaluated in a comparative study. Some drug holidays have been proposed to overcome these side-effects (1)

The MIKIE trial has reported efficacy results of 2 intermittent regimens of vismodegib, and showed that it did not decrease efficacy (47). The neoadjuvant use of vismodegib has been reported in a small series, and showed that, among patients treated with vismodegib 3–6 months before surgery, only one recurred after 22 months (48). Some clinical trials are ongoing into HhI in the neo-adjuvant setting: Vismoneo (NCT02667574) and NICCI (NCT03035188).

*Topical treatment*. Earlier attempts with treatment at topical HhI failed, but a study is currently ongoing to evaluate the interest of a topical application of HhI on the face of patients with BCNS (NCT02828111).

## FOLLOW-UP

According to the type of BCC observed, the follow-up can vary. Most BCCs are discharged after confirmation of diagnosis and completeness of excision. Some high-risk patients (multiple tumours, high-risk histological subtypes, high-risk anatomical sites, immunosuppression) will need to be followed up at least each year for up to 3–5 years. Difficult-to-treat BCC, which necessitated treatment other than surgery, are followed more carefully at a rhythm decided by the multi-disciplinary board (1).

## PERSPECTIVES

BCC, being one of the most highly mutated tumours, could represent a good indication for immunotherapy.

Some isolated reports have shown response to anti-PD1 in treatment-naïve or HhI-refractory patients. In addition, a proof-of-concept study showed that pembrolizumab was efficient in patients with aBCC, but showed no increase efficacy when associated with vismodegib (49).

The efficacy of nivolumab, alone or in combination with ipilimumab, and of cemiplimab (REGN2810) is currently being investigated in patients with laBCC and mBCC in 2 independent phase 2 clinical trials (https://clinicaltrials.gov).

## CONCLUSION

BCCs are the most frequent skin cancers, and their management has been thoroughly reviewed in recently published guidelines. Most BCCs have an excellent prognosis and do not need long-term follow-up. For high-risk tumours, the follow-up schedules may need to be adapted to each clinical presentation.

The standard treatment for BCCs is surgery. The understanding of molecular events implicated in their development has allowed the development of new strategies, such as HhI and, more recently, immunotherapy, for difficult-to-treat tumours.

## REFERENCES


1. Peris K, Fargnoli MC, Garbe C, Kaufmann R, Bastholt L, Seguin NB, et al. Diagnosis and treatment of basal cell carcinoma: European consensus-based interdisciplinary guidelines. Eur J Cancer 2019; 118: 10–34.
2. Rubin AI, Chen EH, Ratner D. Basal-cell carcinoma. N Engl J Med 2005; 353: 2262–2269.
3. Dessinioti C, Antoniou C, Katsambas A, Stratigos AJ. Basal cell carcinoma: what's new under the sun. Photochem Photobiol 2010; 86: 481–491.
4. Staples MP, Elwood M, Burton RC, Williams JL, Marks R, Giles GG. Non-melanoma skin cancer in Australia: the 2002 national survey and trends since 1985. Med J Aust 2006; 184: 6–10.
5. Holm A-S, Nissen CV, Wulf HC. Basal cell carcinoma is as common as the sum of all other cancers: implications for treatment capacity. Acta Derm Venereol 2016; 96: 505–509.
6. Asgari MM, Moffet HH, Ray GT, Quesenberry CP. Trends in basal cell carcinoma incidence and identification of high-risk subgroups, 1998–2012. JAMA Dermatol 2015; 151: 976–981.
7. Rogers HW, Weinstock MA, Feldman SR, Coldiron BM. Incidence estimate of nonmelanoma skin cancer (keratinocyte carcinomas) in the U.S. Population, 2012. JAMA Dermatol 2015; 151: 1081–1086.
8. Nehal KS, Bichakjian CK. Update on keratinocyte carcinomas. N Engl J Med 2018; 379: 363–374.
9. Gallagher RP, Hill GB, Bajdik CD, Fincham S, Coldman AJ, McLean DI, et al. Sunlight exposure, pigmentary factors, and risk of nonmelanocytic skin cancer. I. Basal cell carcinoma. Arch Dermatol 1995; 131: 157–163.
10. Jayaraman SS, Rayhan DJ, Hazany S, Kolodney MS. Mutational landscape of basal cell carcinomas by whole-exome sequencing. J Invest Dermatol 2014; 134: 213–220.
11. Krynitz B, Olsson H, Lundh Rozell B, Lindelöf B, Edgren G, Smedby KE. Risk of basal cell carcinoma in Swedish organ transplant recipients: a population-based study. Br J Dermatol 2016; 174: 95–103.
12. Wang GY, So P-L, Wang L, Libove E, Wang J, Epstein EH. Establishment of murine basal cell carcinoma allografts: a potential model for preclinical drug testing and for molecular analysis. J Invest Dermatol 2011; 131: 2298–2305.
13. Youssef KK. Identification of the cell lineage at the origin of basal cell carcinoma. Nat Cell Biol 2010; 2099–2305.
14. Sexton M, Jones DB, Maloney ME. Histologic pattern analysis of basal cell carcinoma. Study of a series of 1039 consecutive neoplasms. J Am Acad Dermatol 1990; 23: 1118–1126.
15. Reiter O, Mimouni I, Gdalevich M, Marghoob AA, Levi A, Hodak E, et al. The diagnostic accuracy of dermoscopy for basal cell carcinoma: A systematic review and meta-analysis. J Am Acad Dermatol 2019; 80: 1380–1388.
16. Lallas A, Tzellos T, Kyrgidis A, Apalla Z, Zalaudek I, Karatolias A, et al. Accuracy of dermoscopic criteria for discriminating superficial from other subtypes of basal cell carcinoma. J Am Acad Dermatol 2014; 70: 303–311.
17. Epstein EH. Basal cell carcinomas: attack of the hedgehog. Nat Rev Cancer 2008; 8: 743–754.
18. Briscoe J, Thérond PP. The mechanisms of Hedgehog signalling and its roles in development and disease. Nat Rev Mol Cell Biol 2013; 14: 416–429.
19. Hanna A, Shevde LA. Hedgehog signaling: modulation of cancer properies and tumor microenvironment. Mol Cancer 2016; 15: 24.
20. Chiang A, Batra P, Sarin KY. Response to clarifying the current understanding of syndromic basal cell carcinomas. J Invest Dermatol 2019; 139: 2384–2385.
21. Reifenberger J, Wolter M, Knobbe CB, Köhler B, Schönicke A, Scharwächter C, et al. Somatic mutations in the PTCH, SMOH, SUFUH and TP53 genes in sporadic basal cell carcinomas. Br J Dermatol 2005; 152: 43–51.
22. Bonilla X, Parmentier L, King B, Bezrukov F, Kaya G, Zoete V, et al. Genomic analysis identifies new drivers and progression pathways in skin basal cell carcinoma. Nat Genet 2016; 48: 398–406.
23. Pietrobono S, Gagliardi S, Stecca B. Non-canonical hedgehog signaling pathway in cancer: activation of GLI transcription factors beyond smoothened. Front Genet 2019; 10: 556.
24. Lehmann J, Seebode C, Martens MC, Emmert S. Xeroderma pigmentosum – facts and perspectives. Anticancer Res 2018; 38: 1159–1164.
25. Bal E, Park H-S, Belaid-Choucair Z, Kayserili H, Naville M, Madrange M, et al. Mutations in ACTRT1 and its enhancer RNA elements lead to aberrant activation of Hedgehog signaling in inherited and sporadic basal cell carcinomas. Nat Med 2017; 23: 1226–1233.
26. McCusker M, Basset-Seguin N, Dummer R, Lewis K, Schadendorf D, Sekulic A, et al. Metastatic basal cell carcinoma: prognosis dependent on anatomic site and spread of disease. Eur J Cancer 2014; 50: 774–783.
27. Goldenberg G, Karagiannis T, Palmer JB, Lotya J, O'Neill C, Kisa R, et al. Incidence and prevalence of basal cell carcinoma (BCC) and locally advanced BCC (LABCC) in a large commer-

cially insured population in the United States: A retrospective cohort study. J Am Acad Dermatol 2016; 75: 957–966.e2.
28. Dacosta Byfield S, Chen D, Yim YM, Reyes C. Age distribution of patients with advanced non-melanoma skin cancer in the United States. Arch Dermatol Res 2013; 305: 845–850.
29. van Loo E, Mosterd K, Krekels GAM, Roozeboom MH, Ostertag JU, Dirksen CD, et al. Surgical excision versus Mohs' micrographic surgery for basal cell carcinoma of the face: a randomised clinical trial with 10 year follow-up. Eur J Cancer 2014; 50: 3011–3020.
30. Nahhas AF, Scarbrough CA, Trotter S. A Review of the global guidelines on surgical margins for nonmelanoma skin cancers. J Clin Aesthet Dermatol 2017; 10: 37–46.
31. Trakatelli M, Morton C, Nagore E, Ulrich C, Del Marmol V, Peris K, et al. Update of the European guidelines for basal cell carcinoma management. Eur J Dermatol 2014; 24: 312–329.
32. Masud D, Moustaki M, Staruch R, Dheansa B. Basal cell carcinomata: risk factors for incomplete excision and results of re-excision. J Plast Reconstr Aesthet Surg 2016; 69: 652–656.
33. Schön MP, Schön M. Imiquimod: mode of action. Br J Dermatol 2007; 157: 8–13.
34. Williams HC, Bath-Hextall F, Ozolins M, Armstrong SJ, Colver GB, Perkins W, et al. Surgery versus 5% imiquimod for nodular and superficial basal cell carcinoma: 5-year results of the SINS randomized controlled trial. J Invest Dermatol 2017; 137: 614–619.
35. Arits AHMM, Mosterd K, Essers BA, Spoorenberg E, Sommer A, De Rooij MJM, et al. Photodynamic therapy versus topical imiquimod versus topical fluorouracil for treatment of superficial basal-cell carcinoma: a single blind, non-inferiority, randomised controlled trial. Lancet Oncol 2013; 14: 647–654.
36. Basset-Seguin N, Ibbotson SH, Emtestam L, Tarstedt M, Morton C, Maroti M, et al. Topical methyl aminolaevulinate photodynamic therapy versus cryotherapy for superficial basal cell carcinoma: a 5 year randomized trial. Eur J Dermatol 2008; 18: 547–553.
37. Morton CA, Dominicus R, Radny P, Dirschka T, Hauschild A, Reinhold U, et al. A randomized, multinational, noninferiority, phase III trial to evaluate the safety and efficacy of BF-200 aminolaevulinic acid gel vs. methyl aminolaevulinate cream in the treatment of nonaggressive basal cell carcinoma with photodynamic therapy. Br J Dermatol 2018; 179: 309–319.
38. Sekulic A, Migden MR, Oro AE, Dirix L, Lewis KD, Hainsworth JD, et al. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. N Engl J Med 2012; 366: 2171–2179.
39. Sekulic A, Migden MR, Basset-Seguin N, Garbe C, Gesierich A, Lao CD, et al. Long-term safety and efficacy of vismodegib in patients with advanced basal cell carcinoma: final update of the pivotal ERIVANCE BCC study. BMC Cancer 2017; 17: 332.
40. Basset-Seguin N, Hauschild A, Grob J-J, Kunstfeld R, Dréno B, Mortier L, et al. Vismodegib in patients with advanced basal cell carcinoma (STEVIE): a pre-planned interim analysis of an international, open-label trial. Lancet Oncol 2015; 16: 729–736.
41. Tang JY, Ally MS, Chanana AM, Mackay-Wiggan JM, Aszterbaum M, Lindgren JA, et al. Inhibition of the hedgehog pathway in patients with basal-cell nevus syndrome: final results from the multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Oncol 2016; 17: 1720–1731.
42. Herms F, Lambert J, Grob JJ, Haudebourg L, Bagot M, Dalac S, et al. Follow-up of patients with complete remission of locally advanced basal cell carcinoma after vismodegib discontinuation: a multicenter French study of 116 patients. J Clin Oncol 2019; 37: 3275–3282.
43. Migden MR, Guminski A, Gutzmer R, Dirix L, Lewis KD, Combemale P, et al. Treatment with two different doses of sonidegib in patients with locally advanced or metastatic basal cell carcinoma (BOLT): a multicentre, randomised, double-blind phase 2 trial. Lancet Oncol 2015; 16: 716–728.
44. Dummer R, Guminski A, Gutzmer R, Dirix L, Lewis KD, Combemale P, et al. The 12-month analysis from Basal Cell Carcinoma Outcomes with LDE225 Treatment (BOLT): A phase II, randomized, double-blind study of sonidegib in patients with advanced basal cell carcinoma. J Am Acad Dermatol 2016; 75: 113–125.e5.
45. Lear JT, Migden MR, Lewis KD, Chang ALS, Guminski A, Gutzmer R, et al. Long-term efficacy and safety of sonidegib in patients with locally advanced and metastatic basal cell carcinoma: 30-month analysis of the randomized phase 2 BOLT study. J Eur Acad Dermatol Venereol 2018; 32: 372–381.
46. Lacouture ME, Dréno B, Ascierto PA, Dummer R, Basset-Seguin N, Fife K, et al. Characterization and management of hedgehog pathway inhibitor-related adverse events in patients with advanced basal cell carcinoma. Oncologist 2016; 21: 1218–1229.
47. Dréno B, Kunstfeld R, Hauschild A, Fosko S, Zloty D, Labeille B, et al. Two intermittent vismodegib dosing regimens in patients with multiple basal-cell carcinomas (MIKIE): a randomised, regimen-controlled, double-blind, phase 2 trial. Lancet Oncol 2017; 18: 404–412.
48. Ally MS, Aasi S, Wysong A, Teng C, Anderson E, Bailey-Healy I, et al. An investigator-initiated open-label clinical trial of vismodegib as a neoadjuvant to surgery for high-risk basal cell carcinoma. J Am Acad Dermatol 2014; 71: 904–911.e1.
49. Borradori L, Sutton B, Shayesteh P, Daniels GA. Rescue therapy with anti-programmed cell death protein 1 inhibitors of advanced cutaneous squamous cell carcinoma and basosquamous carcinoma: preliminary experience in five cases. Br J Dermatol 2016; 175: 1382–1386.

Centenary theme section: SKIN MALIGNANCIES

REVIEW ARTICLE

# Cutaneous Melanoma – A Review of Systemic Therapies

Karla A. LEE[1] and Paul NATHAN[2]
[1]Department of Twin Research, King's College London, London and [2]Mount Vernon Cancer Centre, Middlesex, UK

**This decade has brought significantly improved outcomes for patients with advanced melanoma with immunotherapies and targeted treatments offering utility in a variety of settings. In 2020, we can hope for durable long-term responses, and complete remission in a subset of patients with metastatic disease. In the adjuvant setting, approximately 50% improvements in recurrence-free survival are seen both with targeted and immunotherapies. Early data from neoadjuvant immunotherapy clinical trials are very promising. However, responses to treatment are heterogeneous and not always durable; further advances are required, and several emerging strategies are of particular interest. We review the systemic treatment of melanoma, discussing the treatment of unresectable stage III–IV and recurrent disease, outlining curative treatment of cutaneous melanoma in the adjuvant setting and briefly discussing neoadjuvant systemic therapies for advanced melanoma.**

*Key words:* melanoma; systemic therapy; targeted therapy; immunotherapy.

Accepted Apr 27, 2020; Epub ahead of print Apr 28, 2020

Acta Derm Venereol 2020; 100: adv00141.

*Corr:* Dr Karla Lee, St. Department of Twin Research, King's College London, SE1 9RT London, UK. E-mail: karlalee@nhs.net

## SIGNIFICANCE

Melanoma is an aggressive and rare skin cancer that can threaten the lives of patients it affects. New treatments have been introduced over the past decade which have dramatically changed the way in which patients with advanced melanoma are managed. Here we review the treatments currently available to patients with advanced melanoma, focusing firstly on patients with stage IV melanoma. We also review treatments available to reduce the risk of a melanoma returning – these treatments can be given either before ("neoadjuvantly") or after ("adjuvantly") a melanoma is surgically removed, but only the latter is currently approved.

Accounting for only 1% of all skin malignancies, melanoma represents the most aggressive and deadly form of skin cancer (1). Melanoma is predominantly a disease of Caucasian populations and affects men and women in equal measure. With a propensity to migrate to draining lymph nodes and any visceral organ, metastatic melanoma carries a poor prognosis.

Prior to 2011, outcomes were poor, with treatment for metastatic disease limited to palliative therapies that offered little or no survival benefit. In 2020, we can hope for durable long-term responses, and complete remission in a subset of patients. The use of immunotherapies and targeted therapies for melanoma in the metastatic, adjuvant and neoadjuvant settings will be reviewed here; the initial management of cutaneous melanoma is discussed separately. This review will cover the systemic treatment of melanoma, starting with a description of therapeutic agents. We will discuss the treatment of unresectable stage III–IV and recurrent disease, outline curative treatment of cutaneous melanoma in the adjuvant setting and briefly discuss neoadjuvant systemic therapies for advanced melanoma.

## CLASSES OF THERAPEUTIC AGENTS

### *Immunotherapy*

*Immune checkpoint inhibitors*. Immune checkpoint inhibitors (CPIs) are a form of immunotherapy designed to target key regulators of the immune system. Immune checkpoints provide stimulatory or inhibitory control of immunity. Tumours can use the inhibitory pathways to protect themselves from being targeted by the immune system. CPIs currently in clinical use act to block these negative pathways enabling T-cells to recognise cancer cells more efficiently. Agonists for stimulatory pathways are also in clinical development. CPIs were the first class of therapy shown to improve the overall survival (OS) for patients with advanced melanoma and provide hope of durable, long-term responses in a subset of patients. The most extensively studied immune checkpoint receptors are cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and programmed cell death protein-1 (PD-1). CTLA-4 and PD-1 induce T-cell suppression through non-overlapping mechanisms and likely impact different populations of T-cells during different phases of the immune response (CTLA-4 during priming and PD-1 during the effector phase), providing a mechanistic rationale for the combination of CTLA-4 and PD-1 blockade.

*CTLA-4*. Based on promising antitumour activity in preclinical cancer models (2), CTLA-4-blocking antibodies have been developed. Ipilimumab is a fully human monoclonal antibody of the IgG1 isotype that

inhibits CTLA-4 leading to enhanced T-cell activation. For T-cell activation to occur, two sequential signals are required (3–5). Firstly, antigens presented in context with the major histocompatibility complex (MHC) I or II on specialised antigen-presenting cells (APCs) must bind with T-cell receptors (TCRs). Following this, there is a translation of TCR stimulation into T-cell activation which requires a costimulatory signal, occurring when B7 surface molecules on the APC bind with CD28 T-cell-surface receptors. Subsequently, T-cell surface expression of CTLA-4 occurs, competitively inhibiting the binding of B7 to CD28, preventing the costimulatory signal and dampening down T-cell activation and proliferation. Treatment can be associated with mechanism-based, immune-related adverse events more frequently than anti-PD-1 treatment.

A second CTLA-4-blocking antibody, tremelimumab, has been developed. Tremelimumab is a fully human anti-CTLA-4 monoclonal antibody of the IgG2 isotype. However, tremelimumab failed to reach its primary endpoint of improved OS compared to standard-of-care chemotherapy for patients with previously untreated, unresectable stage III or IV melanoma (6). Clinical development of tremelimumab is ongoing in a number of non-melanoma cancers.

*PD-1*. Like CTLA-4, PD-1 inhibits T-cell activity and is expressed by activated T-cells. However, instead of competitively inhibiting co-stimulation by interfering with CD28/B7 ligand interaction, PD-1 negatively regulates TCR-signalling events. While CTLA-4 inhibits T-cells during the priming phase of immune responses, PD-1 is thought to inhibit activated T-cells at a later stage in peripheral tissues, playing a critical role in the maintenance of peripheral T-cell tolerance.

The first anti-PD-1 blocking antibody developed was nivolumab, a fully human monoclonal antibody of the IgG4 isotype that binds to PD-1, preventing it from interacting with its ligands. Pembrolizumab was the second anti-PD-1 blocking antibody to be used in advanced melanoma; like nivolumab, pembrolizumab is a fully human monoclonal antibody of the IgG4 isotype that binds to human PD-1 preventing ligand interaction. Nivolumab and pembrolizumab are clinically comparable in terms of efficacy and toxicity as monotherapy for inoperable melanoma (despite the absence of any head-to-head comparison), but only nivolumab is licensed for delivery as a combination with ipilimumab. The subtle preclinical and molecular differences between these two agents have been described by Fessas et al. (7). Compared with ipilimumab, anti-PD-1 blockade with pembrolizumab has been shown to have a superior clinical efficacy and improved toxicity profile with fewer SAEs and fewer patients requiring early treatment withdrawal (8).

*Oncolytic virus therapy.* Oncolytic viruses are a novel class of intratumoural immunotherapies that show promise for treating solid tumours. Talimogene laherparepvec (T-VEC) is a first-in-class, genetically modified, herpes simplex virus type 1-based oncolytic immunotherapy approved for the local treatment of unresectable cutaneous, subcutaneous and nodal lesions in patients with melanoma recurrent after initial surgery. The mechanism of action and clinical applications of T-VEC are described in detail by Raman et al. (9). The key study to note in the context of advanced melanoma is the OPTiM study which randomised 436 participants in a 2:1 ratio to receive intratumoural T-VEC or subcutaneous recombinant granulocyte macrophage colony-stimulating factor (GM-CSF). OPTiM first reported positive findings in late 2015 (10), and recently published final analyses confirmed T-VEC's association with durable complete responses that were associated with prolonged survival (11).

### *Targeted therapy*

The vast majority of cutaneous melanomas harbour mutations in genes of key signalling pathways. Yet, only a small number of these are considered to be driver mutations due to their active role(s) in cancer development and progression; the others are seen as coincidental passenger mutations that are dispensable for cancer cell viability and develop over the course of tumour evolution (12, 13). The mitogen-activated protein kinase (MAPK) pathway is a complex cascade requiring sequential phosphorylation of the different pathway components. In normal cells, when MAPK activation occurs, it leads to cell growth and differentiation. In cells harbouring $BRAF^{V600E}$ mutations, the normal process of negative feedback does not occur and this results in permanent MAPK pathway activation, leading to uncontrolled proliferation. This pathway offers various points at which the protein cascade can be blocked. Mutant BRAF is a "driver oncogene" as mutant BRAF inactivation can induce cancer cell toxicity due to an acquired dependency of cancer cells on oncogenic, mutant forms of BRAF (14). Targeted inactivation of BRAF by pharmacologic inhibitors is an archetypal example of targeted therapy in cancer (14, 15). The recognition of key molecular mutation, $BRAF^{V600E}$ mutation, provided new therapeutic opportunities and facilitated the development of promising small molecule inhibitory compounds later on. Approximately 40% of melanomas harbour a BRAF mutation (16, 17), the most common being $BRAF^{V600E}$, followed by $BRAF^{V600K}$ and rarer genotypes (18).

MEK is the next kinase down from BRAF on the MAPK cascade. BRAF inhibition is the most established form of targeted therapy in melanoma and produces rapid, but often short-lived, tumour regression in the majority of patients. When MEK inhibition is added to BRAF inhibition, increased efficacy and reduced toxicity are seen. Indeed, the combination of BRAF and MEK inhibition offer greater inhibition of MAPK signalling and result in longer durations of response, higher rates

of tumour response, and less cutaneous toxicity often observed from paradoxical MAPK pathway activation with BRAF inhibitor monotherapy (19). The development of acquired resistance to combination BRAF and MEK inhibitor therapy, along with tumour heterogeneity, are formidable obstacles in the treatment of patients with advanced melanoma.

BRAF *inhibitors*. The first BRAF inhibiting tyrosine kinase inhibitor (TKI) approved by the US Food and Drug Administration (FDA) for melanoma treatment was vemurafenib in 2011 (20). The success of vemurafenib in phase I and II settings (21, 22) and then in the BRIM-3 study (23) encouraged intensive investigation of the molecular mechanisms of pathogenesis in melanoma and development of new therapeutic strategies targeting specific molecules in the MAPK pathway. Dabrafenib followed vemurafenib and is another small molecule agent inhibiting $BRAF^{V600}$ mutation-positive melanoma cell growth, demonstrating efficacy as a monotherapy in the BREAK-3 study (24). Encorafenib is a second generation BRAF inhibitor, characterised by a substantially prolonged dissociation half-life (25), and in the phase III COLUMBUS trial demonstrated superior efficacy over vemurafenib monotherapy (26).

*MEK inhibitors*. Preclinical and early studies demonstrated that the addition of a MEK inhibitor to a BRAF inhibitor decreased tumour growth, delaying the development of resistance and reducing occurrence of skin lesions in metastatic melanoma (27). As a results, there has been considerable interest in various combinations of BRAF and MEK inhibition. Trametinib was the first MEK inhibitor approved for the treatment of BRAF-mutated metastatic melanoma naïve to BRAF-inhibition. Trametinib is approved for use in combination with dabrafenib showing efficacy both as a monotherapy when compared to investigator's choice chemotherapy (28), and when combined with dabrafenib (29, 30). Cobimetinib is another MEK inhibitor which demonstrated efficacy while used in combination with vemurafenib in the CoBRIM study (31), while bimetinib is the most recently-introduced of the MEK inhibitors and has demonstrated efficacy in the COLUMBUS study (26).

### *Chemotherapy*

Prior to recent advances, chemotherapy was the backbone of treatment for metastatic melanoma. Studies reported responses in 10–15% of patients with 5 year survival in only 2–6% of patients (32). Despite the poor survival statistics, agents such as dacarbazine or the combination of a platinum agent and a taxol were the standard of care for many years, due to a paucity of other useful therapeutic options. Currently chemotherapy is used infrequently, and primarily when immunotherapy and targeted therapy options have either failed or cannot be used.

## TREATMENT OF UNRESECTABLE STAGE III-IV AND RECURRENT MELANOMA

Systemic therapy is indicated for patients with stage III–IV melanoma in whom surgical metastasectomy is not appropriate. Patients with oligometastatic disease should be evaluated for possible metastasectomy, as complete resection of metastatic disease can achieve cure (33, 34). In such cases, adjuvant therapy would then be recommended following complete resection to reduce recurrence risk (discussed later). This section will focus on systemic therapy for inoperable melanoma.

The primary systemic therapy options for patients with metastatic melanoma are CPIs, and, where a BRAF mutation is the driver mutation, MAPK targeted therapies. The presence or absence of a BRAF mutation is currently the only reliable predictive biomarker that can influence the treatment of advanced melanoma and must promptly and accurately be determined. Many different methods for BRAF testing are currently in use internationally (35–37), but a discussion of these is beyond the scope of this review. Targeted MAPK therapy is not indicated in patients without a characteristic BRAF mutation and may indeed be harmful to this patient group.

Whether patients with $BRAF^{V600}$ mutant melanoma should receive CPIs or MAPK targeted therapy as first line therapy is not always straightforward and prospective head-to-head comparative trials of MAPK inhibitors and CPIs are lacking. A 2019 update of survival in metastatic melanoma reported exploratory analysis of survival data from selected CPI and TKI clinical trials (38). In first line therapy, mean 3-year OS proportions were 41.3% for BRAF plus MEK inhibition, 49.9% for PD-1 inhibition and 58.4% for CTLA-4 plus PD-1 inhibition. Comparison of the mean progression free survival (PFS) and OS curves of kinase inhibition and checkpoint blockade revealed a superiority of combined BRAF plus MEK inhibition within the first 12 months, later changing to a superiority of PD-1 blockers alone or in combination with CTLA-4 blockade. In second-line or higher, BRAF plus MEK inhibition was superior to anti-PD-1 monotherapy throughout the first 3 years; mean 3-year OS proportions were 42.4% for BRAF plus MEK inhibition, and 40.1% for PD-1 inhibition.

### *Checkpoint inhibitors*

**Table I** outlines key phase III CPI studies in melanoma. Nivolumab (39) and pembrolizumab (40, 41) have been established as preferred monotherapy options for inoperable melanoma given their efficacy over standard of care chemotherapies and acceptable toxicity profiles. Checkmate-067 compared nivolumab and ipilimimab as a combination with nivolumab and ipilimumab monotherapies, recently demonstrating an OS of 52% for the combination group at 5 years. This exceptional survival was associated with 59% of patients receiving the combination suffering

**Table I. Landmark checkpoint inhibitor (CPI) trials in metastatic melanoma**

| Trial | Regimen | Patients *n* | Outcome | G3/4 AEs: |
|---|---|---|---|---|
| Checkmate 066 (40)<br>Nivo 1st line | Nivo 3 mg/kg q2w vs. DTIC 1,000 mg/m$^2$ q2w | 418 | 3 years OS: 51.2% vs 21.6% mOS: 37.5 vs 11.2 months | 11.7% vs 17.6% |
| Checkmate 037 (41)<br>Nivo 2nd line | Nivo 3mg/kg q2w<br>vs. ICC | 405 | ORR: 27% vs 10%<br>mOS: 16 vs 14 mo<br>mPFS: 3.1 vs 3.7 mo | 14% vs 34% |
| Checkmate 067 (42)<br>Ipi + Nivo 1st line | Comparision of 3x 3-weekly regimens:<br>Nivo 1mg/kg + Ipi 3 mg/kg q3w vs. Nivo 3 mg/kg q2w<br>vs. Ipi 3 mg/kg x 4 doses | 945 | PFS at 60 months:<br>36%* (Ipi +Nivo) vs 29%* (Nivo) vs 8% (Ipi)<br>OS at 60 months: 52% (Ipi +Nivo) vs 44% (Nivo) vs 26% (Ipi) | 59% (Ipi+Nivo) vs 23% (Nivo) vs 28% (Ipi) |
| Keynote-006 (8)<br>Pembro 1st line | Pembro 10 mg/kg<br>q2w vs. q3w vs. Ipi 3 mg/kg q3w x 4 doses | 834 | mOS at 60 months: 32.7% vs. 15.9%<br>mPFS at 60 months: 8.4 months vs 3.4 months | 17% vs 50% |
| Keynote-002 (39)<br>Pembro 2nd line (Ipi refractory) | Pembro 2 mg/kg q3w vs. Pembro 10 mg/kg q3w vs. ICC | 180 | PFS at 28 months:<br>36% (pembro 2 mg) vs 38% (pembro 10 mg) vs 30% (ICC)<br>mOS at 28 months:13.4 (pembro 2 mg) vs 14.7 (pembro 10 mg) vs 11.0 months | 13.5% (pembro 2 mg) vs 16.8% (pembro 10 mg) vs 26.3% (ICC) |

mOS: median overall survival; HR: hazard ratio; mPFS: median progression-free survival; PD: progressive disease; G: grade; AE: adverse event; TRAE: treatment-related adverse event; Ipi: Ipilimumab; Nivo: Nivolumab; Pembro: Pembrolizumab; DTIC: Dacarbazine; ICC: investigator's choice chemotherapy.

grade 3 or 4 adverse events (42). As such, combination PD-1 and CTLA-4 blockade is usually considered only for those patients with a very good performance status, with some institutions and oncologists preferring CPI monotherapy for metastatic disease. Untreated brain metastases represent one particular clinical scenario in which combination CPI offers particular advantage and may be preferred in this instance (43).

### *MAPK pathway inhibition*

Overall response rates to vemurafenib, dabrafenib and encorafenib monotherapies are 45%, 51% and 60%, respecively (29, 44, 45). A number of studies have presented clear evidence that the combination of these agents with a MEK inhibitor provide increased efficacy with a reduction in toxicity (**Table II**). In the COLUMBUS study, encorafenib plus bimetinib showed favourable efficacy compared with encorafenib or vemurafenib monotherapy, with the combination associated with an improved tolerability profile compared with either monotherapies (26). The CoBRIM study showed improved survival of vemurafenib and cobimetinib compared with vemurafenib alone, with no significant difference in toxicity (31). Robert et al. recently analysed pooled extended survival data from COMBI-d and COMBI-v trials ($n=563$) which compared dabrafenib and trametinib with dabrafenib and vemurafenib monotherapies, respectively, reporting complete responses in 19% of patients and improved long-term outcomes, with OS rates of 71% and less toxicity seen with the combination of BRAF and MEK inhibition (29).

### *Checkpoint and MAPK inhibition combinations*

Increasing evidence suggests that BRAF and MEK inhibition has an immune-modulating effect, enhancing anti-tumour immunity (47–49). Early evidence from treatment of advanced melanoma with BRAF inhibition demonstrated increased expression of PD-1 and its

**Table II. Landmark mitogen-activated protein kinase (MAPK) targeted therapy trials in metastatic melanoma**

| Trial | Regimen | Patients *n* | Outcome | Toxicity |
|---|---|---|---|---|
| BRIM-3 (23) | Vemurafenib 960 mg BD<br>vs. DTIC 1,000 mg/m2 q3w | 675 | mOS: 13.6 vs 9.7 months<br>mPFS: 6.9 vs 1.6 months | Modification/Interruption: 38% vs 16% |
| BREAK-3 (24) | Dabrafenib vs. DTIC | 250 | mPFS: 5.1 months vs 2.7 months | G3/4 AEs:<br>12.8% vs 17.4% |
| METRIC (28) | Trametinib 2 mg/day vs. ICC | 322 | mPFS: 4.9 vs 1.5 months<br>5 year OS: 32% vs 17% | G3/4 AEs: 29% vs 12% |
| CoBRIM (31) | Vemurafenib + Cobimetinib 60 mg OD vs.<br>Vemurafenib 960 mg BD + placebo | 495 | mOS: 22.5 months vs 17.4 months<br>mPFS at 5 years: 12.6 vs 7.2 months<br>5 years OS: 30.8% vs 26.3% | G3/4 AEs:<br>60% vs 52% |
| COMBI-d (46) | Dabrafenib 150 mg BD + Trametinib 2 mg OD vs. Dabrafenib 150 mg + placebo | 423 | 3 years OS: 44% vs 32%<br>mPFS: 11.0 vs 8.8 months<br>5 years pooled resuls with COMBI-d: CR in 19%; OS rates of 71% (29) | G3/4 AEs:<br>48% vs 50% |
| COMBI-v (30) | Dabrafenib 150 mg BD + Trametinib 2 mg OD vs. Vemurafenib 960 mg BD | 704 | 3 years OS: 45% vs 32%<br>3 years PFS: 25% vs 11%<br>5 years pooled resuls with COMBI-v: CR in 19%; OS rates of 71% (29) | G3/4 AEs:<br>58% vs 66% |
| COLUMBUS (26) | Encorafenib 450 mg OD + Bimetinib 45 mg BD (Combo) vs. Encorafenib 300 mg OD<br>vs. Vemurafenib 960 mg BD | 577 | mOS: 33.6% (combo) vs. 23.5 months (enco) vs 16.9% (vem)<br>mPFS: 14.9 months (combo) vs. 9.6 months (enco) vs. 7.3 months (vem) | G3/4 events ocurred in 68% (combo), 68% (enco) and 66% (vem) |

AEs: adverse events; OD: once daily; BD: twice daily; mOS: median overall survival; HR: hazard ratio; mPFS: median progression-free survival; PD: progressive disease; G: grade; AE: adverse event; DTIC: Dacarbazine; ICC: investigator's choice chemotherapy; enco: encorafenib; vem: vemurafenib; combo: combination; CR: complete response.

ligand, PD-L1 (50), suggesting there may be a therapeutic benefit in combing BRAF inhibition with CPI. A phase 1 study showed vemurafenib and ipilimumab to have an unacceptable rate of hepatic toxicity, leading to its discontinuation (51). A preclinical study demonstrated that treatment with BRAF and MEK inhibition, in the presence of the oncogenic $BRAF^{V600}$ mutation, improved CPI anti-cancer effect without any negative impact on immune cell function (47), as had previously been thought may be the case (52). It is believed that MEK inhibition has a protective effect on $CD8^+$ T-cells due to chronic TCR stimulation (53). Such toxicity in the context of BRAF inhibition may be related to the paradoxical activation of MAPK in BRAF wild-type cells and can be ameliorated by the addition of a MEK inhibitor (54).

Preclinical data provide rationale to support testing of a triple combination of BRAF inhibition, MEK inhibition and PD-1 blockade (47, 53). A number of trials have reported relatively initial results with some 1- and 2-year data available, indicating that the combination of CPI and TKI may have a role as standard of care within the next numbers of years (**Table III**).

## ADJUVANT THERAPY FOR RESECTED MELANOMA

The role of adjuvant therapy in patients with resected stage III melanoma is a rapidly evolving field. Interferon was the first agent shown to have utility in this space, however, advances in both targeted therapies and immunotherapies have led to a number of practice-changing adjuvant trials in resected stage III and IV disease. By eliminating the micrometastatic disease that remains after surgery, adjuvant systemic therapy aims to reduce disease recurrence and ultimately improve rates of cure following surgical resection of locoregional or stage IV disease. Patients with resected stage III or IV disease have significant differences in predicted survival at 5 years ranging from approximately 80% for stage IIIa disease to less than 20% for resected stage IIId disease (58). Adjuvant treatment with either CPI or MAPK targeted therapy have dramatically changed outcomes for this patient group, with approximately 50% increased recurrence-free survival (RFS) for both treatment approaches (59–62). CPIs and MAPK targeted therapies have not been directly compared in phase III studies and there is currently no clear consensus on choice of approach for patients with a $BRAF^{V600}$ mutation in the adjuvant setting.

For patients with stage I and II primary tumours and a negative sentinel lymph node biopsy, there is presently no indication for adjuvant therapy (63). It is worth noting that patients with high risk (primary tumour >4 mm, or >2 mm with ulceration) but node negative tumours were excluded from the phase III clinical trials that evaluated nivolumab, ipilimumab and the targeted therapy doublet of dabrafenib and trametinib (62, 64, 65). As such, data on adjuvant therapy in this cohort of patients is not available and is currently under investigation.

### *Adjuvant checkpoint inhibitors*

As already discussed, CPI represents an important advance in the treatment of patients with inoperable melanoma. These results led to the evaluation of these agents in the adjuvant setting for patients at high risk of recurrence following initial surgery. Adjuvant treatment with ipilimumab at 10 mg/kg dosing was shown to have a 10% absolute improvement in OS and RFS, but toxicity and high treatment-related death rates limited its widespread use and it was never licensed for this indication in Europe (66). Only 13.4% of patients completed the full planned course of treatment, and nearly 40% of patients discontinued treatment after the first 4 doses due to treatment-related side effects. Adjuvant anti-PD-1 therapy has been tested in two large phase III studies, Checkmate 238 and Keynote 054, which have established nivolumab and pembrolizumab as the CPIs of choice for the adjuvant treatment of resected melanoma (60, 67). **Table IV** summarises the key trials in this setting.

### *Adjuvant targeted therapy*

A key study in this context is COMBI-AD, a study of 870 Stage III BRAF mutant melanoma patients in the adjuvant setting following excision and lymphadenectomy (61, 64). They were randomised to the combination arm of dabrafenib and trametinib, or to matching placebos

**Table III. Landmark check-point/tyrosine kinase inhibitor (CPI-TKI) targeted therapy trials in metastatic melanoma**

| Trial | Regimen | Patients *n* | Outcome | Toxicity |
|---|---|---|---|---|
| Keynote 022 NCT02130466 (55) | Pembrolizumab 2 mg/kg + Dabrafenib 150 mg BD + Tremetibib 2 mg OD vs. Placebo + Dabrafenib 150 mg BD + Tremetibib 2 mg OD | 120 | mPFS: 16.0 vs 10.3<br>mDOR: 18.7 months vs 12.5<br>mOS: NR vs 23.4 | G3-5 AEs: 70% vs 45% |
| IMspire150 NCT02908672 (56) | Atezolizumab 840 mg D1 and D15 + Vemurafenib 960 mg BD + Cobimetinib 60 mg/D vs. Placebo + Vemurafenib 960 mg BD + Cobimetinib 60 mg/day | 514 | PFS: 15.1 vs 10.6 months<br>2 years OS: 60.4% vs 53.1% | G3-5 AEs: 33.5% vs 28.8% |
| COMBI-i NCT02967692 (57) | Spartalizumab 400mg q4W + Dabrafenib 150 mg BD + Tremetinib 2 mg QDS | 36 | ORR: 75% (33% CR)<br>12 months PFS: 65.3%<br>12 months OS: 85.9% | 75% had G3/4 AEs |

AEs: adverse events, OD: once daily, BD: twice daily, mOS: median overall survival; HR: hazard ratio; mPFS: median progression-free survival; mDOR: median duration of response; ORR: overall response rate; NR: not reached; PD: progressive disease; G: grade; AE: adverse event; DTIC: Dacarbazine; ICC: investigator's choice chemotherapy.

**Table IV. Summary of randomised controlled trials of adjuvant therapy for patients with cutaneous melanoma**

| Trial | Agents | Patients | Primary Endpoint | 12 months RFS | Toxicity |
|---|---|---|---|---|---|
| EORTC 18071 (66) | Ipi vs. placebo | Complete resection in Stage III | Median RFS: 26-mo vs 17-months 7-year OS: 60% vs 51.3% | 64% vs. 56% | G3/4 AEs: 54% vs. 26% 1% death from Ipi AE |
| Checkmate 238 (59, 67) | Nivo vs. Ipi | Complete resection in Stage IIIB, IIIC, IV | 3-year RFS: 58% vs. 45% | 71% vs. 61% Stage III alone: 72% vs. 62% | G3/4 AEs: 14% vs. 46% 0.4% death from ipi SAE |
| COMBI-AD (61) | D&T vs. placebo | Complete resection in Stage III | RFS 4 years: 54% vs 38% 3 years OS: 86% vs. 77% | 88% vs. 56% | SAE: 36% vs. 10% 1 death D&T |
| Keynote 054 (60) | Pembro vs. placebo | Complete resection in Stage III | 12-months RFS: 75% vs. 61% | 75% vs. 61% | G 3/4 AE: 15% vs. 3% 1 death pembro |
| BRIM8 (68) | Vem vs. placebo | Complete resection: Stage IIC-IIIA/B (cohort 1) and IIIC (cohort 2) | Median DFS: Cohort 1: NR vs. 37-months Cohort 2: 23-months vs. 15-months | Cohort 1: 84% vs 66% Cohort 2: 79% vs 58% | G3/4 AE: 57% vs. 15% SAE: 16% vs. 10% |

AE: adverse event; DFS: disease-free survival; EORTC: European Organization for Research and Treatment of Cancer; Gr: grade; NR: not reached; OS: overall survival; Pembro, pembrolizumab; Ipi: Ipilimumab; Nivo: Nivolumab; Vem: Vemurafenib; plac: placebo; D&T: Dabrafenib & Trametinib; RFS: recurrence-free survival; SAE: serious adverse event.

for one year. The primary endpoint, RFS, was longer with dabrafenib and trametinib than with placebo (4-year rate: 54% vs 38%; hazard ratio [HR] 0.49, 95% CI 0.40–0.59), with treatment benefits observed irrespective of baseline factors, according to subgroup analysis (61). Vemurafenib was compared to placebo in the adjuvant BRIM8 study demonstrating efficacy but high rates of grade 3/4 toxicity (68).

## NEOADJUVANT THERAPY FOR EARLY MELANOMA

Given the success of immunotherapies and targeted therapies for the treatment of advanced melanoma, the natural extension is to identify the role of these therapies in the neoadjuvant setting, with a wealth of clinical trials currently underway. Patients with clinically detectable stage III melanoma represent a high-risk population with poor outcomes when treated with upfront surgery alone and are obvious candidates for investigation of neoadjuvant therapy. However, the clear need to carefully evaluate short-term clinical endpoints such as RFS, and long-term endpoints of neoadjuvant therapy against those of adjuvant therapy remains. Neoadjuvant therapy for melanoma is not presently standard-of-care but represents an active area of research with a large number of completed and recruiting trials with differing designs, endpoints, and methods of analysis under investigation. **Table V** illustrates those neoadjuvant (preoperative therapy) trials which have reported data.

One study of note is OPACIN-NEO study which reported in 2018 (69). OPACIN-NEO examined neoadjuvant combination CPI with 3 different regimens of ipilimumab and nivolumab. A combination of ipilimumab at 1 mg/kg combined with nivolumab at 3 mg/kg given 3-weekly for two cycles was chosen to take forward into later phase studies, as this combination had a response rate of 77%, with responders experiencing excellent outcomes to date. If more mature data confirm these early observations, this schedule will be tested in randomised phase 3 studies versus adjuvant therapies, which are the current standard-of-care systemic therapy for patients with stage III melanoma.

## FUTURE DIRECTIONS AND CONCLUSION

The investigation of new immunotherapy and/or targeted therapy combinations, such as anti-PD-1/anti-CTLA-4 CPIs with other immunotherapies (e.g. indoleamine 2,3 dioxygenase inhibitors, antilymphocyte activation 3, histone deacetylase inhibitors, Toll-like receptor 9 agonists, anti-glucocorticoid-induced tumour necrosis factor receptor, pegylated interleukin-2), combination targeted therapies (e.g. MEK and CDK4/6 co-inhibition), and the combined use of immunotherapy and continued research on targeted therapy (e.g. the triplet combination of BRAF/MEK inhibition with anti–PD-1s) are keys for the future of systemic therapy for advanced melanoma. The identification of novel therapeutic targets in the MAPK pathway provides opportunity to improve outcomes by overcoming *de novo* and acquired resistance to BRAF/MEK inhibition. Adoptive cell transfer may have a potential role in patients whose disease has progressed following CPI. Altogether, these new approaches offer potential to build upon past advances and improve long-term survival outcomes for patients with melanoma.

This decade has brought significantly improved outcomes for patients with advanced melanoma with the advent of immunotherapies and targeted treatments that have utility in a variety of settings. However, responses to treatment are heterogeneous and not always durable. Further advances are required, and several emerging strategies are of particular interest.

*Conflicts of interest:* KAL has no conflicts of interest to report. PN reports personal fees from AstraZeneca, BMS, Merck, Immunocore, Pfizer, Ipsen, 4SC, Pierre Fabre and Roche.

**Table V. Neoadjuvant trials with available data**

| Trial | Eligible patients *n* | Regimen | Median follow-up (months) | Results | TRAEs |
|---|---|---|---|---|---|
| IMMUNOTHERAPY | | | | | |
| NCT00972933 (70) 2018 | Clinical stage IIIB or IIIC and oligometastatic stage IV; *n* = 35 | Two neoadjuvant doses of ipi (10 mg/kg), surgery, followed by two adjuvant doses of ipi | 18 | RFS: 11 months<br>No pPR or pCR reported | G3 AEs: 32% |
| NCT02437279 (71) 2018 | Clinical stage III; 10 per group | Surgery plus 12-week adjuvant ip (3 mg/kg) and nivo (1 mg/kg); 6 weeks of neoadjuvant and 6 weeks of adjuvant ipi (3 mg/kg) and nivo (1 mg/kg) | 32 | 30% pCR, 40% near pCR, 0% pPR | G/43 adverse events: 90% of participants in the surgery group vs 90% of participant in the neoadjuvant therapy group |
| NCT02519322 (72) 2018 | Clinical stage III and oligometastatic stage IV 12 participants in the nivo-only group and 11 in the ipi plus nivo group | 4 doses of nivo (3 mg/kg) neoadjuvant therapy, surgery, and 24 weeks of nivo adjuvant therapy; 3 courses of ipi (3 mg/kg) plus nivo (1 mg/kg) neoadjuvant therapy, surgery, and 24 weeks of adjuvant nivo | 20 | Group A: pCR 45%<br>Group B; pCR 25%<br>RFS: 56% participants in the nivo-only group vs. 81% participants in the ipi-nivo group | Nivolumab-only:<br>8% participants had G3 AEs; ipi plus nivo:<br>73% participants had G3 AEs; No G4/5 AEs in any group |
| NCT02977052 (69) OpACIN-neo 2019 | Clinical stage III; 30 in group A; 30 in group B; and 26 in group C | Group A: two courses of ipi (3 mg/kg) plus nivo (1 mg/kg) once every 3 weeks; Group B: two courses of ipi (1 mg/kg) plus nivo (3 mg/kg) once every 3 weeks; Group C: two courses of ipi (3 mg/kg) once every 3 weeks plus two courses of nivo (3 mg/kg) once every 2 weeks | 8.3 | 43% of non-pCRs relapsed; no relapses reported in the other response groups | G3/4 AEs: 40% in group A vs 20% in group B vs 50% in group C |
| NCT01608594 (73) 2018 | Clinically detectable locally and/or regionally advanced melanoma *n* = 28 | Ipilimumab 3 or 10 mg/kg high-dose interferon | 32 | 32% pCR | At median follow-up of 32 months, 10/11 patients with either pCR or minimal residual disease remained disease free More grade 3/4 irAEs were seen with ipilimumab 10 mg/kg versus 3 mg/kg ($p = 0.042$) |
| NCT02339324 (74) 2018 | Stage 3 and 4 resected (5 x IIIB, 11 x IIIC and 4 x IV) *n* = 20 | Pembrolizumab 200 mg with high-dose interferon | 11 | 35% pCR | 90% of patients had to stop early due to G3/4 toxicities |
| TARGETED THERAPY | | | | | |
| NCT02231775 (75) 2018 | Clinical stage IIIB or IIIC and oligometastatic stage IV with BRAFV600E/V600K mutation *n* = 21 | Neoadjuvant dabrafenib (150 mg twice a day) plus trametinib (2 mg daily) for 8 weeks followed by surgery and 44 weeks of the same adjuvant treatment versus surgery | 18.6 | pPR 17% and pCR 58%<br>RFS: 19.7 mo for adjuvant systemic vs 2.9 mo for surgery group | A: G3: 47% of participants in the neoadjuvant systemic therapy group had G3 AEs |
| NCT01972347 (76) NeoCombi 2019 | Clinical stage III with BRAFV600E/V600K mutation; *n* = 35 | Dabrafenib (150 mg twice a day) plus trametinib (2 mg daily):<br>12 weeks neoadjuvant therapy and 40 weeks of adjuvant therapy | 27 | 23 mo of overall RFS (30 mo of pCR, 18 mo of non-pCR) | 57% participant had any grade 3 adverse events;<br>3% had any g G4 AEs and 26% had surgical G3 AEs; 26% had drug-related grade 3 events and 3% drug-related G4 AEs |
| Sloot et al. (77) 2016 | Stage III<br>Of 15, 6 underwent surgery | Vemurafenib 960 mg BID or Dabrafenib 150 mg QD ± Trametinib | 25.4 | pPR 33% and pCR 33% | Dose reduction or discontinuation because of toxicities occurred in 10/15 patients |
| Zippel et al. (78) 2017 | Stage III<br>*n* = 12 | Vemurafenib 960 mg BID or Dabrafenib 150 mg QD ± Trametinib 2 mg QD | 20 | pPR 62% and pCR 31% | N/a |
| Eroglu et al. (79) 2017 | Stage IIIC and IV<br>*n* = 20 | Vemurafenib<br>Dabrafenib + Trametinib<br>Encorafenib + Binimetinib | 25 | pCR 35% | Not reported |

pCR: pathological complete response; mo: months, G: grade; TRAE: treatment related adverse events; AE: adverse event; ipi: ipilimumab; nivo: nivolumab; pPR: pathological partial response; pCR: pathological complete response.

## REFERENCES


1. Domingues B, Lopes JM, Soares P, Pópulo H. Melanoma treatment in review. Immunotargets Ther 2018; 7: 35–49.
2. Keler T, Halk E, Vitale L, O'Neill T, Blanset D, Lee S, et al. Activity and safety of CTLA-4 blockade combined with vaccines in cynomolgus macaques. J Immunol 2003; 171: 6251–6259.
3. Boasberg P, Hamid O, O'Day S. Ipilimumab: unleashing the power of the immune system through CTLA-4 blockade. Semin Oncol 2010; 37: 440–449.
4. Hoos A, Ibrahim R, Korman A, Abdallah K, Berman D, Shahabi V, et al. Development of ipilimumab: contribution to a new paradigm for cancer immunotherapy. Semin Oncol 2010; 37: 533–546.
5. Weber J. Immune checkpoint proteins: a new therapeutic paradigm for cancer – preclinical background: CTLA-4 and PD-1 blockade. Semin Oncol 2010; 37: 430–439.
6. Ribas A, Kefford R, Marshall MA, Punt CJA, Haanen JB, Marmol M, et al. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. J Clin Oncol 2013; 31: 616–622.
7. Fessas P, Lee H, Ikemizu S, Janowitz T. A molecular and preclinical comparison of the PD-1-targeted T-cell checkpoint inhibitors nivolumab and pembrolizumab. Semin Oncol 2017; 44: 136–140.
8. Robert C, Ribas A, Schachter J, Arance A, Grob J-J, Mortier L, et al. Pembrolizumab versus ipilimumab in advanced melanoma (KEYNOTE-006): post-hoc 5-year results from an open-label, multicentre, randomised, controlled, phase 3 study. Lancet Oncol 2019; 20: 1239–1251.
9. Raman SS, Hecht JR, Chan E. Talimogene laherparepvec: review of its mechanism of action and clinical efficacy and safety. Immunotherapy 2019; 11: 705–723.
10. Andtbacka RH, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol 2015; 33: 2780–2788.
11. Andtbacka RHI, Collichio F, Harrington KJ, Middleton MR,

Downey G, Öhrling K, et al. Final analyses of OPTiM: a randomized phase III trial of talimogene laherparepvec versus granulocyte-macrophage colony-stimulating factor in unresectable stage III–IV melanoma. J Immunother Cancer 2019; 7: 145.
12. Clarke CN, Katsonis P, Hsu TK, Koire AM, Silva-Figueroa A, Christakis I, et al. Comprehensive genomic characterization of parathyroid cancer identifies novel candidate driver mutations and core pathways. J Endocr Soc 2019; 3: 544–559.
13. Kim HS, Minna JD, White MA. GWAS meets TCGA to illuminate mechanisms of cancer predisposition. Cell 2013; 152: 387–389.
14. Rustgi AK. BRAF: a driver of the serrated pathway in colon cancer. Cancer Cell 2013; 24: 1–2.
15. Hofer S, Berthod G, Riklin C, Rushing E, Feilchenfeldt J. BRAF V600E mutation: A treatable driver mutation in pleomorphic xanthoastrocytoma (PXA). Acta Oncol 2016; 55: 122–123.
16. Colombino M, Capone M, Lissia A, Cossu A, Rubino C, De Giorgi V, et al. BRAF/NRAS mutation frequencies among primary tumors and metastases in patients with melanoma. J Clin Oncol 2012; 30: 2522–2529.
17. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med 2005; 353: 2135–2147.
18. Menzies AM, Haydu LE, Visintin L, Carlino MS, Howle JR, Thompson JF, et al. Distinguishing clinicopathologic features of patients with V600E and V600K BRAF-mutant metastatic melanoma. Clin Cancer Res 2012; 18: 3242–3249.
19. Eroglu Z, Ribas A. Combination therapy with BRAF and MEK inhibitors for melanoma: latest evidence and place in therapy. Ther Adv Med Oncol 2016; 8: 48–56.
20. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011; 364: 2507–2516.
21. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of Mutated, Activated BRAF in Metastatic Melanoma. N Engl J Med 2010; 363: 809-819.
22. Ribas A, Kim KB, Schuchter LM, Gonzalez R, Pavlick AC, Weber JS, et al. BRIM-2: An open-label, multicenter phase II study of vemurafenib in previously treated patients with BRAF V600E mutation-positive metastatic melanoma. J Clin Oncol 2011; 29: 8509.
23. Chapman PB, Robert C, Larkin J, Haanen JB, Ribas A, Hogg D, et al. Vemurafenib in patients with BRAFV600 mutation-positive metastatic melanoma: final overall survival results of the randomized BRIM-3 study. Annals Oncol 2017; 28: 2581–2587.
24. Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet 2012; 380: 358–365.
25. Koelblinger P, Thuerigen O, Dummer R. Development of encorafenib for BRAF-mutated advanced melanoma. Curr Opin Oncol 2018; 30: 125–133.
26. Ascierto PA, Dummer R, Gogas HJ, Flaherty KT, Arance A, Mandala M, et al. Update on tolerability and overall survival in COLUMBUS: landmark analysis of a randomised phase 3 trial of encorafenib plus binimetinib vs vemurafenib or encorafenib in patients with BRAF V600-mutant melanoma. Eur J Cancer 2020; 126: 33–44.
27. Kim KB, Kefford R, Pavlick AC, Infante JR, Ribas A, Sosman JA, et al. Phase II study of the MEK1/MEK2 inhibitor Trametinib in patients with metastatic BRAF-mutant cutaneous melanoma previously treated with or without a BRAF inhibitor. J Clin Oncol 2013; 31: 482–489.
28. Robert C, Flaherty K, Nathan P, Hersey P, Garbe C, Milhem M, et al. Five-year outcomes from a phase 3 METRIC study in patients with BRAF V600 E/K-mutant advanced or metastatic melanoma. Eur J Cancer 2019; 109: 61–69.
29. Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. N Engl J Med 2014; 371: 1877–1888.
30. Robert C, Grob JJ, Stroyakovskiy D, Karaszewska B, Hauschild A, Levchenko E, et al. Five-year outcomes with dabrafenib plus trametinib in metastatic melanoma. N Engl J Med 2019; 381: 626–636.
31. McArthur GA, Dréno B, Larkin J, et al. 5-year survival update of cobimetinib plus vemurafenib BRAF V600 mutation-positive advanced melanoma: final analysis of the coBRIM study. Presented at: the 16th International Congress of the Society for Melanoma Research; November 20-23, 2019; Salt Lake City, UT.
32. Kim C, Lee CW, Kovacic L, Shah A, Klasa R, Savage KJ. Long-term survival in patients with metastatic melanoma treated with DTIC or temozolomide. Oncologist 2010; 15: 765–771.
33. Leung AM, Hari DM, Morton DL. Surgery for distant melanoma metastasis. Cancer J 2012; 18: 176–184.
34. Ollila DW, Hsueh EC, Stern SL, Morton DL. Metastasectomy for recurrent stage IV melanoma. J Surg Oncol 1999; 71: 209–213.
35. Finn RS, Dering J, Conklin D, Kalous O, Cohen DJ, Desai AJ, et al. PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. Breast Cancer Res 2009; 11: R77.
36. Finn RS, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol 2015; 16: 25–35.
37. Ihle MA, Fassunke J, Konig K, Grunewald I, Schlaak M, Kreuzberg N, et al. Comparison of high resolution melting analysis, pyrosequencing, next generation sequencing and immunohistochemistry to conventional Sanger sequencing for the detection of p.V600E and non-p.V600E BRAF mutations. BMC Cancer 2014; 14: 13.
38. Ugurel S, Rohmel J, Ascierto PA, Becker JC, Flaherty KT, Grob JJ, et al. Survival of patients with advanced metastatic melanoma: The impact of MAP kinase pathway inhibition and immune checkpoint inhibition - Update 2019. Eur J Cancer 2020; 130: 126-138.
39. Hamid O, Puzanov I, Dummer R, Schachter J, Daud A, Schadendorf D, et al. Final analysis of a randomised trial comparing pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory advanced melanoma. Eur J Cancer 2017; 86: 37–45.
40. Ascierto PA, Long GV, Robert C, Brady B, Dutriaux C, Di Giacomo AM, et al. Survival outcomes in patients with previously untreated braf wild-type advanced melanoma treated with nivolumab therapy: three-year follow-up of a randomized phase 3 trial. JAMA Oncol 2019; 5: 187–194.
41. Larkin J, Minor D, D'Angelo S, Neyns B, Smylie M, Miller WH, Jr., et al. Overall survival in patients with advanced melanoma who received nivolumab versus investigator's choice chemotherapy in checkmate 037: a randomized, controlled, open-label phase III trial. J Clin Oncol 2018; 36: 383–390.
42. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob J-J, Rutkowski P, Lao CD, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med 2019; 381: 1535–1546.
43. Tawbi HA, Forsyth PA, Algazi A, Hamid O, Hodi FS, Moschos SJ, et al. Combined Nivolumab and Ipilimumab in Melanoma Metastatic to the Brain. N Engl J Med 2018; 379: 722–730.
44. Delord JP, Robert C, Nyakas M, McArthur GA, Kudchakar R, Mahipal A, et al. Phase I dose-escalation and -expansion study of the BRAF inhibitor encorafenib (LGX818) in metastatic BRAF-mutant melanoma. Clin Cancer Res 2017; 23: 5339–5348.
45. Larkin J, Ascierto PA, Dreno B, Atkinson V, Liszkay G, Maio M, et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med 2014; 371: 1867–1876.
46. Long GV, Flaherty KT, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, et al. Dabrafenib plus trametinib versus dabrafenib monotherapy in patients with metastatic BRAF V600E/K-mutant melanoma: long-term survival and safety analysis of a phase 3 study. Ann Oncol 2017; 28: 1631–1639.
47. Hu-Lieskovan S, Mok S, Homet Moreno B, Tsoi J, Robert L, Goedert L, et al. Improved antitumor activity of immu-

notherapy with BRAF and MEK inhibitors in BRAF(V600E) melanoma. Sci Transl Med 2015; 7: 279ra41.
48. Ascierto PA, Dummer R. Immunological effects of BRAF+MEK inhibition. Oncoimmunol 2018; 7: e1468955.
49. Deken MA, Gadiot J, Jordanova ES, Lacroix R, van Gool M, Kroon P, et al. Targeting the MAPK and PI3K pathways in combination with PD1 blockade in melanoma. Oncoimmunol 2016; 5: e1238557.
50. Frederick DT, Piris A, Cogdill AP, Cooper ZA, Lezcano C, Ferrone CR, et al. BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. Clin Cancer Res 2013; 19: 1225–1231.
51. Ribas A, Hodi FS, Callahan M, Konto C, Wolchok J. Hepatotoxicity with combination of vemurafenib and ipilimumab. N Engl J Med 2013; 368: 1365–1366.
52. Vella LJ, Pasam A, Dimopoulos N, Andrews M, Knights A, Puaux AL, et al. MEK inhibition, alone or in combination with BRAF inhibition, affects multiple functions of isolated normal human lymphocytes and dendritic cells. Cancer Immunol Res 2014; 2: 351–360.
53. Ebert PJR, Cheung J, Yang Y, McNamara E, Hong R, Moskalenko M, et al. MAP kinase inhibition promotes T cell and anti-tumor activity in combination with PD-L1 checkpoint blockade. Immunity 2016; 44: 609–621.
54. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, et al. RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. N Engl J Med 2012; 366: 207–215.
55. Ascierto PA, Ferrucci PF, Fisher R, Del Vecchio M, Atkinson V, Schmidt H, et al. Dabrafenib, trametinib and pembrolizumab or placebo in BRAF-mutant melanoma. Nature Med 2019; 25: 941–946.
56. McArthur GA, Stroyakovskiy D, Gogas H, et al: Evaluation of atezolizumab, cobimetinib, and vemurafenib in previously untreated patients with BRAF V600 mutation-positive advanced melanoma: Primary results from the phase 3 IMspire150 trial. 2020 AACR Virtual Annual Meeting. Abstract CT012. Presented April 27, 2020.
57. Long GV, Lebbe C, Atkinson V, Mandalà M, Nathan PD, Arance Fernandez ANAM, et al. The anti–PD-1 antibody spartalizumab (S) in combination with dabrafenib (D) and trametinib (T) in previously untreated patients (pts) with advanced BRAF V600–mutant melanoma: Updated efficacy and safety from parts 1 and 2 of COMBI-i. J Clin Oncol 2019; 37: 9531.
58. Gershenwald JE, Scolyer RA, Hess KR, Sondak VK, Long GV, Ross MI, et al. Melanoma staging: Evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin 2017; 67: 472–492.
59. https://www.ascopost.com/news/october-2019/3-year-results-from-checkmate-238-adjuvant-nivolumab-vs-ipilimumab-in-advanced-melanoma/.
60. Eggermont AMM, Blank CU, Mandala M, Long GV, Atkinson V, Dalle S, et al. Adjuvant pembrolizumab versus placebo in resected stage III melanoma. N Engl J Med 2018; 378: 1789–1801.
61. Hauschild A, Dummer R, Schadendorf D, Santinami M, Atkinson V, Mandala M, et al. Longer follow-up confirms relapse-free survival benefit with adjuvant dabrafenib plus trametinib in patients with resected BRAF V600-mutant stage III melanoma. J Clin Oncol 2018; 36: 3441–3449.
62. Weber J, Mandala M, Del Vecchio M, Gogas HJ, Arance AM, Cowey CL, et al. Adjuvant nivolumab versus ipilimumab in resected stage III or IV melanoma. N Engl J Med 2017; 377: 1824–1835.
63. Kaufman HL, Kirkwood JM, Hodi FS, Agarwala S, Amatruda T, Bines SD, et al. The Society for Immunotherapy of Cancer consensus statement on tumour immunotherapy for the treatment of cutaneous melanoma. Nat Rev Clin Oncol 2013; 10: 588–598.
64. Long GV, Hauschild A, Santinami M, Atkinson V, Mandala M, Chiarion-Sileni V, et al. Adjuvant Dabrafenib plus Trametinib in Stage III BRAF-Mutated Melanoma. N Engl J Med 2017; 377: 1813–1823.
65. Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, et al. Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, phase 3 trial. Lancet Oncol 2015; 16: 522–530.
66. Eggermont AMM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, et al. Adjuvant ipilimumab versus placebo after complete resection of stage III melanoma: long-term follow-up results of the European Organisation for Research and Treatment of Cancer 18071 double-blind phase 3 randomised trial. Eur J Cancer 2019; 119: 1–10.
67. Weber JS, Mandalà M, Del Vecchio M, Gogas H, Arance AM, Cowey CL, et al. Adjuvant therapy with nivolumab (NIVO) versus ipilimumab (IPI) after complete resection of stage III/IV melanoma: Updated results from a phase III trial (CheckMate 238). J Clin Oncol 2018; 36: 9502.
68. Maio M, Lewis K, Demidov L, Mandala M, Bondarenko I, Ascierto PA, et al. Adjuvant vemurafenib in resected, BRAF(V600) mutation-positive melanoma (BRIM8): a randomised, double-blind, placebo-controlled, multicentre, phase 3 trial. Lancet Oncol 2018; 19: 510–520.
69. Rozeman EA, Menzies AM, van Akkooi ACJ, Adhikari C, Bierman C, van de Wiel BA, et al. Identification of the optimal combination dosing schedule of neoadjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma (OpACIN-neo): a multicentre, phase 2, randomised, controlled trial. Lancet Oncol 2019; 20: 948–960.
70. Retseck J, Nasr A, Lin Y, Lin H, Mendiratta P, Butterfield LH, et al. Long term impact of CTLA4 blockade immunotherapy on regulatory and effector immune responses in patients with melanoma. J Transl Med 2018; 16: 184.
71. Rozeman EA, Blank CU, Van Akkooi ACJ, Kvistborg P, Fanchi L, Van Thienen JV, et al. Neoadjuvant ipilimumab + nivolumab (IPI+NIVO) in palpable stage III melanoma: Updated data from the OpACIN trial and first immunological analyses. J Clin Oncol 2017; 35: 9586.
72. Amaria RN, Reddy SM, Tawbi HA, Davies MA, Ross MI, Glitza IC, et al. Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. Nat Med 2018; 24: 1649–1654.
73. Tarhini A, Lin Y, Lin H, Rahman Z, Vallabhaneni P, Mendiratta P, et al. Neoadjuvant ipilimumab (3 mg/kg or 10 mg/kg) and high dose IFN-α2b in locally/regionally advanced melanoma: safety, efficacy and impact on T-cell repertoire. J Immunother Cancer 2018; 6: 112.
74. Tarhini AA, Lin Y, Drabick JJ, Neves RI, Ernstoff MS, Puzanov I, et al. Neoadjuvant combination immunotherapy with pembrolizumab and high dose IFN-α2b in locally/regionally advanced melanoma. J Clin Oncol 2018; 36: 181.
75. Amaria RN, Prieto PA, Tetzlaff MT, Reuben A, Andrews MC, Ross MI, et al. Neoadjuvant plus adjuvant dabrafenib and trametinib versus standard of care in patients with high-risk, surgically resectable melanoma: a single-centre, open-label, randomised, phase 2 trial. Lancet Oncol 2018; 19: 181–193.
76. Long GV, Saw RPM, Lo S, Nieweg OE, Shannon KF, Gonzalez M, et al. Neoadjuvant dabrafenib combined with trametinib for resectable, stage IIIB-C, BRAF(V600) mutation-positive melanoma (NeoCombi): a single-arm, open-label, single-centre, phase 2 trial. Lancet Oncol 2019; 20: 961–971.
77. Sloot S, Zager JS, Kudchadkar RR, Messina JL, Benedict JJ, Gonzalez RJ, et al. BRAF inhibition for advanced locoregional BRAF V600E mutant melanoma: a potential neoadjuvant strategy. Melanoma Res 2016; 26: 83–87.
78. Zippel D, Markel G, Shapira-Frommer R, Ben-Betzalel G, Goitein D, Ben-Ami E, et al. Perioperative BRAF inhibitors in locally advanced stage III melanoma. J Surg Oncol 2017; 116: 856–861.
79. Eroglu Z, Khushalani NI, Rich J, Sarnaik A, Zager JS, Markowitz J, et al. Patterns of histologic response to neoadjuvant targeted therapy in patients with BRAF mutant melanoma. J Clin Oncol 2017; 35: 9584.

REVIEW ARTICLE

# Biomarkers Predicting for Response and Relapse with Melanoma Systemic Therapy

Sarah J. WELSH AND Pippa G. CORRIE
*Cambridge Cancer Centre, Cambridge University Hospitals NHS Foundation Trust, Cambridge CB2 0QQ, UK*

**Introduction of new systemic therapies in the last 10 years has radically improved outcomes for melanoma patients. Even so, not all patients benefit, so getting the right treatment to the right patient is a priority. These two major drug classes, small molecule targeted kinase inhibitors and immune checkpoint inhibitors, both come at significant cost, with sometimes serious side effects as well as high expense for health services. Almost half of melanomas harbour a *BRAF*$^{V600}$ mutation and virtually all patients receiving BRAF targeted therapy will experience some amount of response. However, duration of response with these agents is uncertain, due to acquired resistance, which means few patients remain in response long term. Most metastatic melanoma patients are potentially eligible for immune checkpoint inhibitors, irrespective of BRAF status. However, only about half of patients will respond to these agents, and only half again will benefit long term. Thus, both primary and acquired resistance limit response. In this era of personalized anti-cancer therapy, biomarkers offer a means to predict for both response and relapse to a particular treatment. To date, the only validated biomarker applied to selecting melanoma systemic therapy is the *BRAF* gene. However, modern technologies are now opening up a wide range of candidate genes, polypeptides and proteins which are being evaluated for their potential clinical application as predictive biomarkers of the future.**

*Key words:* melanoma; biomarkers; immunotherapy; BRAF targeted therapy; response.

Accepted Apr 27, 2020; Epub ahead of print Apr 28, 2020

Acta Derm Venereol 2020; 100: adv00142.

*Corr:* Dr Pippa Corrie, Cambridge Cancer Centre, Cambridge University Hospitals NHS Foundation Trust, Cambridge CB2 0QQ UK. E-mail: pippa.corrie@addenbrookes.nhs.uk

## SIGNIFICANCE

Systemic therapy options for melanoma patients are rapidly increasing. They offer life extension for many, but not all patients benefit. These high cost drugs also have complex, life-changing and potentially life-threatening side effects. Modern 'Precision Medicine' aims to personalize therapy for individuals and hence offer the opportunity to selectively treat only those expected to benefit from a particular therapy, while avoiding exposure to ineffective treatment in others. To date, the only validated predictive melanoma biomarker guiding treatment decisions is the BRAF gene mutation, although emerging modern technologies are identifying many more candidates whose clinical application have yet to be ascertained.

In the last decade, treatment of metastatic melanoma has undergone unprecedented transformation, with two new classes of anticancer drugs entering routine clinical practice, tripling overall survival of people whose life expectancy previously was limited to under one year. Both sets of drugs – BRAF targeted therapies and immune checkpoint inhibitors – are now being offered earlier in the disease pathway, to people who have undergone surgery for locoregional melanoma, based on evidence that adjuvant therapy halves the rate of recurrence (1–3). Despite this positive outlook, there are serious limitations yet to be overcome: little more than half of metastatic melanoma patients embarking on systemic therapy will achieve durable response, drug-induced toxicity can be life-threatening and certainly life-changing, while the cost of chronic drug prescribing is crippling many healthcare systems.

This same decade has seen a massive step change in our understanding of cancer biology. We are now in the era of 'Precision medicine', which aims to personalize treatment based on specific biological characteristics of an individual and their cancer. So-called biomarkers should, in theory, enable preferential selection of effective treatment, while avoiding exposure to inactive drugs causing unnecessary side-effects, thus also contributing to more cost-effective healthcare. Primary and acquired resistance to both molecularly targeted agents and immunotherapy limit treatment response. Therefore, biomarkers may be valuable adjuncts to clinical decision-making both prior to initiation of treatment, as well as during treatment, to predict the likelihood of treatment failure and disease relapse (**Fig. 1**). In practice, despite an explosion of research in this field, the role of predictive biomarkers in the clinic currently remains limited. The case of modern melanoma therapeutics well illustrates both the successes and challenges of biomarker discovery and their application.

doi: 10.2340/00015555-3497
Acta Derm Venereol 2020; 100: adv00142

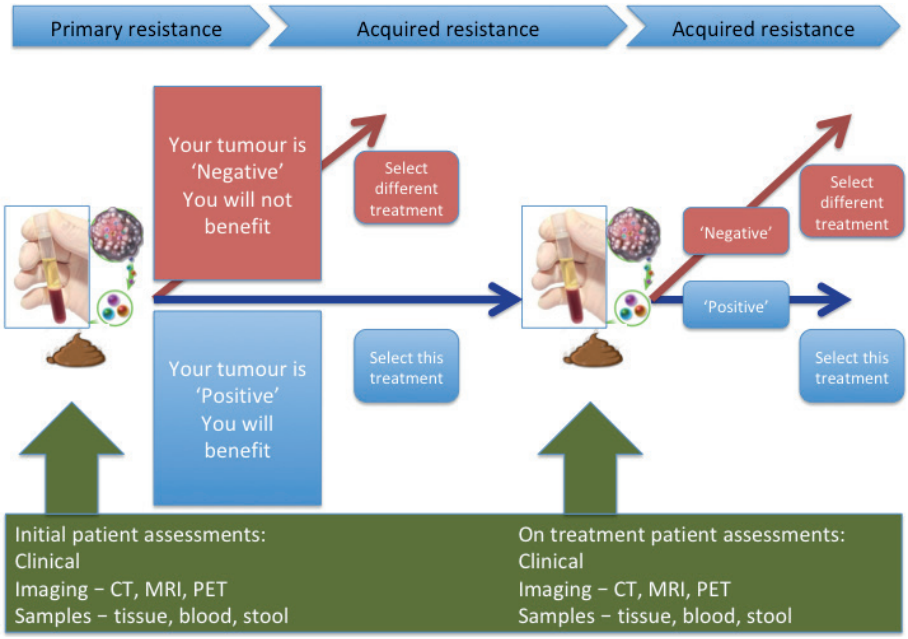


**Fig. 1. Integrating biomarkers into routine clinical practice.**

## BRAF – THE PERFECT BIOMARKER?

In the whole of modern drug development, the mutant *BRAF* gene stands out as a massive success story in biomarker discovery. In 2002, a team at the Wellcome Sanger Institute reported *BRAF* mutations in 66% of melanoma cell lines tested and these findings were subsequently corroborated in melanoma patients (4). Its success as a treatment response biomarker is thanks to a talented biochemist who designed a drug to specifically block the active kinase domain of the mutant BRAF protein. This 'lock and key' approach generated groundbreaking responses in *BRAF* mutant metastatic melanoma patients treated in the phase 1 trial of the first specific BRAF kinase inhibitor, vemurafenib (5). In subsequent large-scale randomised trials, BRAF-targeted kinase inhibitors have generated objective response rates of up to 70% with virtually all treated patients experiencing some degree of response (6). The limitation of BRAF inhibition, however, is duration of response, due to onset of secondary resistance in most cases within a year of starting treatment.

Molecular characterization of tumours biopsied at the time of disease progression showed that reactivation of MEK downstream of BRAF was a consistent feature. Dual blockade with BRAF and MEK inhibitor combination regimens delay onset of secondary resistance, significantly extending duration of response (6). Unequivocal evidence that mutant *BRAF* drives malignancy in some 45% of melanomas led rapidly to adoption of *BRAF* testing of patient's tumour tissue into routine clinical practice worldwide. Progression biopsies identified emergence of new mutations associated with loss of treatment response, some of which might be actionable and offer options for subsequent treatment.

However, accessing tumour is not always practical and is fraught with issues, particularly around tumour heterogeneity. Measuring circulating tumour DNA (ctDNA) in plasma as a 'liquid' biopsy offers an attractive, less-invasive alternative surrogate for disease burden. Preliminary studies support mutant *BRAF* ctDNA as a biomarker predicting for minimal residual disease and recurrence after surgical resection of locoregional melanoma (7) as well as lending value to monitor metastatic melanoma patients on treatment (6, 8), for early signs of both response and disease progression. Although a significant step change in patient management, work is still needed to optimize and standardize liquid biopsy methodologies, while larger scale prospective trials are essential to fully determine the clinical application of ctDNA before being introduced into routine clinical practice.

Other less common driver mutations occurring in melanoma include *NRAS*, *PTEN* loss and *CKIT*. Despite attempts to block signalling from these aberrant pathways, clinical benefits have been modest and no targeted agents have yet been approved for patients with these molecular characteristics. Currently, therefore, their significance as biomarkers is confined to research studies.

## CLINICAL BIOMARKERS OF RESPONSE

In contrast to molecular targeted agents, and also to some other cancers for whom they are approved, access to immune checkpoint inhibitors is not limited by any biomarker-determined subgroup of melanoma patients. Since first tested in melanoma patient trials, eligibility has been primarily determined by concerns for patient safety, as well as enrichment for better prognostic groups. Outside of clinical trials, real world experience has widened access and together with increasing understanding of how checkpoint inhibitors work, some clinical features have emerged that may help predict for benefit. This is particularly pertinent for *BRAF* mutant melanoma patients, who must choose which order to access the two drug classes available to them.

Immune checkpoint inhibitors rely on activating cytotoxic ($CD8^+$) T-cell function, which can take a few weeks to kick in after initiating therapy. Evidence suggests that patients with slowly progressing, low disease

burden (reflected in routine clinical and laboratory parameters including good performance status, normal serum lactate dehydrogenase, few organs involved, non-visceral disease) tend to respond to checkpoint inhibitors better than patients with high burden, rapidly progressing disease. These factors are readily identifiable in the clinic, but mainly reflect overall disease prognosis. Similarly, they predict for better outcomes with BRAF-targeted therapy (9) (**Fig. 2**). A recent meta-analysis of advanced melanoma interventional registration trials of systemic targeted therapies and checkpoint inhibitors demonstrated that BRAF-targeted therapies offer superior overall survival in the short term, which may be the priority for those patients with more aggressive disease and poorer prognosis, but checkpoint inhibition offers longer term survival gains for those who respond (10). However, given complex toxicities, high drug cost and limited overall survival benefits, there is a pressing need to utilise modern scientific capability to select the right treatment for the right patient based on their individual disease biology.

## CHALLENGES OF CHECKPOINT INHIBITORS

Increasing numbers of melanoma patients are receiving immune checkpoint inhibitors as their first line of treatment both in the adjuvant and advanced setting, striving for long term survival benefits. The dominant agents in clinical use are the anti-PD-1 antibodies, nivolumab and pembrolizumab (6). Both are generally well tolerated in all age groups, so in this modern age, advancing years is not a barrier to access and the numbers of melanoma patients being treated worldwide is rising exponentially, despite relatively modest benefits: response rate in metastatic melanoma is around 40%, while only the minority of those patients receiving adjuvant anti-PD-1 monotherapy are likely to benefit (1,2). Identifying the subgroup of patients expected to respond is a major research priority. Anti-PD-1 agents are licensed to be administered until disease progression, but chronic drug administration is driven by Pharma, not by biology. Can biomarkers also help determine treatment duration for an individual patient?

As a strategy to enhance activity, nivolumab (nivo) was combined with the anti-CTLA-4 antibody, ipilimumab (ipi) and the combination (ipi+nivo) regimen was compared to both monotherapies in the CheckMate 067 international registration trial. Response rates with the combination regimen were higher, reaching 58% for ipi+nivo compared with 45% for nivo and 19% for ipi, but the overall survival gain with ipi+nivo compared with nivo alone was marginal: 4-year overall survival 53% versus 46% (11). On the other hand, ipi+nivo was associated with a three-fold increase (59% versus 22%) in severe or life-threatening adverse events, compared to nivo alone, while 40% and 12% of patients discontinued treatment due to adverse events in these 2 trial arms. There is therefore a pressing need to identify those patients unlikely to benefit from the combination regimen to avoid unnecessary treatment-related toxicity.

In the last 5 years, a huge amount of resource has been invested in better understanding tumour immunology with significant focus on identification of biomarkers to address the questions posed here. As summarised by

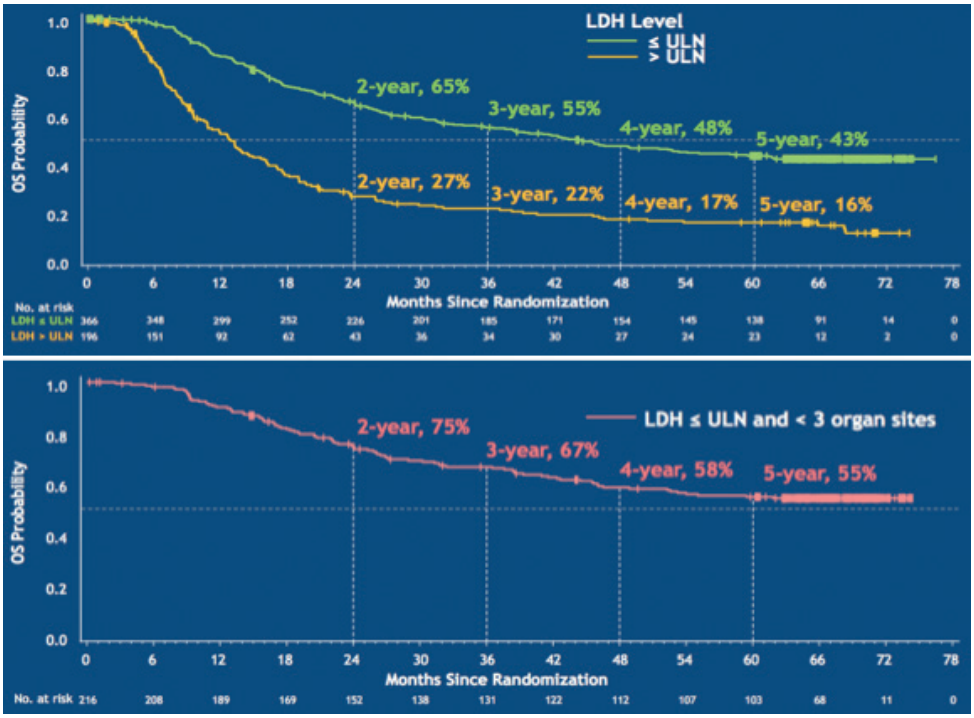


**Fig. 2. Impact of tumour burden (as defined by lactate dehydrogenase (LDH) and number of body organ sites affected) on overall survival (OS) following treatment with dabrafenib+trametinib.** ULN: upper limit of normal. (Reprinted with permission from The New England Journal of Medicine, Caroline Robert et al., Five-Year Outcomes with Dabrafenib plus Trametinib in Metastatic Melanoma, 381:626-636. Copyright © (2019) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society).

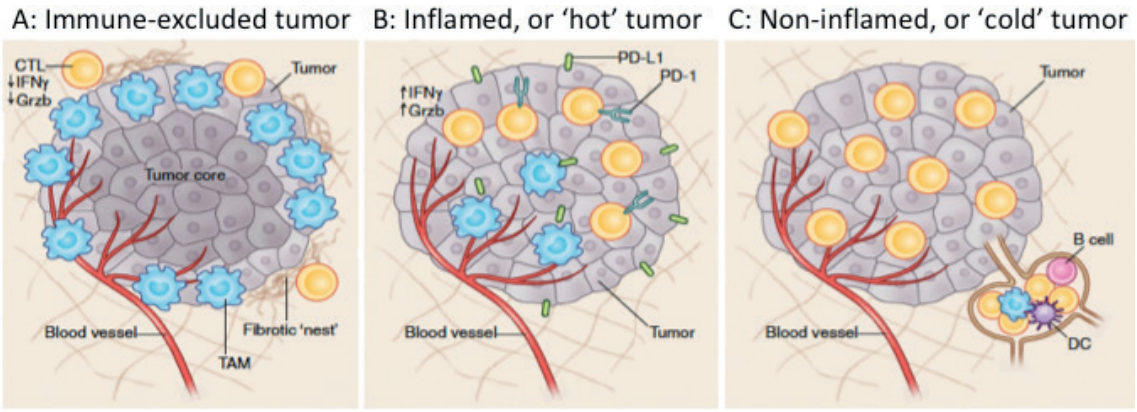


**Fig. 3. The tumour immune-microenvironment can be classified as being either (A) immune-excluded, (B) inflamed, or (C) non-inflamed.** (Reprinted with permission from Springer Nature: Nature Medicine (Understanding the tumor immune microenvironment (TIME) for effective therapy, Mikhail Binnewies et al. (63), COPYRIGHT (2018)).

Chen & Mellman (12), cancers can be categorized into 3 groups: 1) 'hot' or inflamed tumours, characterized by a high T-cell infiltrate, 2) 'cold' or non-inflamed tumours, devoid of any T-cell infiltrate, and 3) cancers that have T cells and other immune cells present, but only at the periphery or within the stromal tissue and not within the tumour itself (**Fig. 3**). 'Hot' tumours are most likely to respond to checkpoint blockade, and melanomas fall in to this category. However, overall, the minority of melanoma patients respond to checkpoint blockade, demonstrating that the relationship between the tumour, host and microenvironment is hugely complex and no perfect biomarker of response or toxicity is yet available for clinical application. Highlights of expansive research in biomarker identification have been reviewed in various recent publications (for example, see 13–15). While not meant to be an exhaustive list, the role of the most promising biomarkers is summarized here (**Table I**) under these 3 headings.

## TUMOUR FACTORS

### *Programmed death ligand 1 expression*

Programmed death ligand 1 (PD-L1) is a protein expressed on cancer cells, tumour infiltrating lymphocytes (TILs) and myeloid cells which, through engagement with its receptor, PD-1, attenuates T-cell responses, thereby helping cancer cells evade immune surveillance. Anti-PD-1 antibodies disrupt PD-1:PD-L1 interactions to reinvigorate T-cell cytotoxicity. PD-L1 expression was therefore the first tumour-associated protein to be explored as a putative biomarker of response to anti-PD-1 antibodies. Initial analysis in the CheckMate 067 trial suggested that patients with high levels of PD-L1 had higher response rates compared with those whose tumours had low, or no expression (16). However, responses still occurred among these patients with low/no expression and the predictive value of PD-L1 expression was not borne out with longer follow-up (11). Since CheckMate 067 was initiated, the limitations of PD-L1 testing have received much attention: which antibody, which cells to count (tumour, immune cells, or both), which cut-off to use (cell count is linear, not binary) and all lack clarity. While in some other cancers PD-L1 expression does appear predictive, currently there is no place for routine testing in melanoma clinical practice.

### *Tumour mutational burden*

Response to immune checkpoint inhibitors is highest among tumour types with a high mutation load and melanomas generally have high levels of mutations (17). This may be attributable, at least in part, to the production of tumour-specific neoantigens. Mutations within a tumour may lead to the formation of peptides unique to tumour cells that have the potential to be antigenic. Therefore, an increase in the tumour mutational burden (TMB) of a tumour could increase the likelihood of production of antigenic tumour-specific peptides, in turn leading to a

**Table I. Summary of potential melanoma predictive biomarkers**

| Tumour | Host | Microenvironment |
|---|---|---|
| Tumour mutation burden and neoantigen expression | CD8 T cells | Microbiome |
| Driver mutations | T-cell receptor | Immunosuppressive stroma/immune cell |
| Aberrant signaling pathways (including WNT/bcatenin, JAK1/2, VEGF) | Immunoscore | environment including TGFb pathway |
| MHC | Neutrophil:lymphocyte ratio | PD-L1 expression |
| B2Microglobulin | Cytokines eg. IL17 | |
| PD-L1 expression | Immune-related gene expression profiles | |
| Imaging (eg. FDG-PET) | IFN γ signature | |
| | Inflammatory markers eg. IL6, CRP | |

larger pool of tumour-specific T cells. This larger pool of tumour-specific T cells would theoretically produce a greater antitumor response on inhibition of immune checkpoints that may be mediating tumour immune tolerance.

The first confirmatory human data came from whole-exome sequencing of DNA from tumours and matching blood from 25 metastatic melanoma patients treated with ipilimumab (18). There was a significant difference in TMB between patients with a long-term clinical benefit and those with minimal or no benefit, which was then reproduced in a subsequent validation set. High TMB was subsequently shown to correlate with survival following anti-PD1 blockade (19). Even so, as with PD-L1, measuring TMB is not straightforward. Gene sequencing methodology – which platform to use, which cut-off for a non-binary measure – is still evolving. Tumour heterogeneity will influence any measure of TMB in a discrete tumour sample, although some early research suggests this could be overcome by measuring TMB in a blood sample. Therefore, TMB remains an exploratory biomarker for the time being.

*Aberrant signaling pathways driven by tumour mutations*

Genetic mutations within melanoma cells have downstream effects on signalling pathways, which influence response to immunotherapy. A key pathway implicated in resistance to both anti-PD-1 and anti-CTLA-4 antibodies is the WNT/β-catenin-signalling pathway (20) which induces T-cell exclusion. Studies have demonstrated that loss of *PTEN* correlates with decreased T-cell infiltration at tumour sites, reduced likelihood of successful T-cell expansion from resected tumours, and inferior outcomes with anti-PD-1 antibodies (21). Mutations in several components of the Janus kinase (JAK1/JAK2) pathway have been implicated in both acquired (22) and primary (23) immune resistance in melanoma, by impairing interferon gamma (IFN-γ) signalling. Thus, screening for JAK1/2 mutations has been proposed as a mechanism to identify patients unlikely to respond to immune checkpoint inhibitors.

Recent studies have implicated loss of antigen presentation as a key mechanism of resistance to immune checkpoint inhibitors. β2microglobulin (β2M) is an essential component of MHC class I antigen presentation in which point mutations, deletions or loss of heterozygosity (LOH) have been identified in 30% of melanoma patients with progressing disease (24). In metastatic melanoma patients treated with anti-CTLA-4 and anti-PD-1 agents, β2M LOH was enriched threefold in non-responders compared to responders and was associated with poorer overall survival. Loss of both copies of β2M was found only in non-responders.

A further factor implicated in driving resistance to immune checkpoint inhibitors is transforming growth factor beta (TGF-β) (25). TGF-β is a multi-functional cytokine involved in the regulation of many cellular processes including cell proliferation, differentiation and survival. Melanoma produces increasing amounts of TGF-β with disease progression, inhibiting immune responses and providing an optimal microenvironment for undisturbed tumour growth. Its role as a response biomarker needs further investigation.

## HOST IMMUNE-BASED BIOMARKERS

Many immune-based biomarker candidates have been identified to date in retrospective datasets, or preclinical models. The majority of these studies have focused on immune cells, either within the tumour, or circulating in blood.

*Tumour-based immune-related biomarkers*

The inflamed tumour microenvironment is characterized by the presence of T-cell markers and chemokines that mediate effector T-cell recruitment, with enhanced numbers of $CD8^+$ T cells, macrophages, as well as some B cells and plasma cells. Therefore, it is perhaps not surprising that one of the most reproducible factors predicting response to immunotherapy in melanoma patients has been the presence of tumour-infiltrating lymphocytes (TILs) within tumours: increased numbers of TILs generally correlates with improved response and survival (26). Tumour infiltrating immune cells include T cells, macrophages and various types of immune suppressive cells, all of which contribute to the balance of a pro-immunogenic versus immunosuppressive microenvironment. Thus, low intratumoral CD8:CD4 ratios correlate with lack of response to treatment, while response rates as high as 80% have been reported to be associated with high intratumoral CD8:CD4 in metastatic melanoma patients treated with anti-PD-1 monotherapy (27). Because the nature of the immune microenvironment of a tumour at baseline is associated with efficacy of immune checkpoint inhibition, the assessment of an individual's immune signature to predict treatment outcome is an area of active investigation. This emerging concept, known as immunoprofiling, relies on the 'immunoscore': an assessment of the type, density, and location of immune cells (28). Absolute numbers is a gross oversimplification of a highly complex microenvironment influencing T-cell function. It is likely that multiple markers may need to be combined to fully encompass the heterogeneity of immune cell responses in individual patients receiving specific therapies.

One way of combining multiple factors affecting response to immunotherapy is by gene expression profiling of tumour tissue. A T-cell inflamed tumour microenvironment rich in pro-inflammatory chemokines with an IFN-γ signature has been shown to correlate with the

clinical efficacy of immune checkpoint inhibitors in melanoma patients (29–31). Several multi-gene expression profiles have been proposed as having predictive value, although results are not always consistent across studies. However, evidence from a large cohort of > 300 tumours from multiple cancers including melanoma reported that integrated analysis of an immune gene signature combined with TMB enriches for anti-PD1 responders (32) (**Fig. 4**). This novel approach may provide a precision medicine framework for stratifying patient therapy in the future.

*Blood-based biomarkers*

Multiple blood-based biomarkers have been identified in retrospective studies and show promise to predict both response, and, potentially, toxicity, and have been extensively reviewed elsewhere (33–35). They include absolute neutrophil count, absolute lymphocyte count, neutrophil:lymphocyte ratio, absolute eosinophil count, relative lymphocyte count (RLC), absolute monocyte count, antibodies against NY-ESO1, T-regulatory cell count, and myeloid-derived suppressor cell (MDSC) count. Recent analysis of patients recruited to the CheckMate 064, 066 and 067 trials identified serum IL6 and CRP as predictors of improved response and survival after checkpoint blockade (36). Even so, most studies have been undertaken on small cohorts using a variety of different evaluation criteria (37) and all require validation in larger prospective trials.

The most extensive analysis of the effects of immune checkpoint inhibitors on peripheral blood was performed in metastatic melanoma patients treated with pembrolizumab (38). The study showed that 1) PD1 inhibition leads to an on-target immunological effect on CD8 T cells and this effect can be detected, longitudinally monitored and mechanistically interrogated in the peripheral blood with the major cell type affected being the $Ki67^+$ CD8 T-cell population, characteristic of exhausted T cells ($T_{ex}$). 2) Most patients had a single peak of anti-PD-1-induced immune reinvigoration, despite on-going treatment which occurred early during treatment (within 3–6 weeks). 3) Since the $T_{ex}$ cells were the major target of PD-1 blockade in most patients, the authors were able to develop a 'reinvigoration score' by relating changes in circulating $T_{ex}$ cells to tumour burden. 4) Responding $T_{ex}$ cells in the blood contained T-cell receptor clones shared with tumour-infiltrating T cells, and 5) The ratio of $T_{ex}$-cell reinvigoration to tumour burden distinguished clinical outcomes and predicted for response. The relationship between $T_{ex}$-cell reinvigoration and tumour

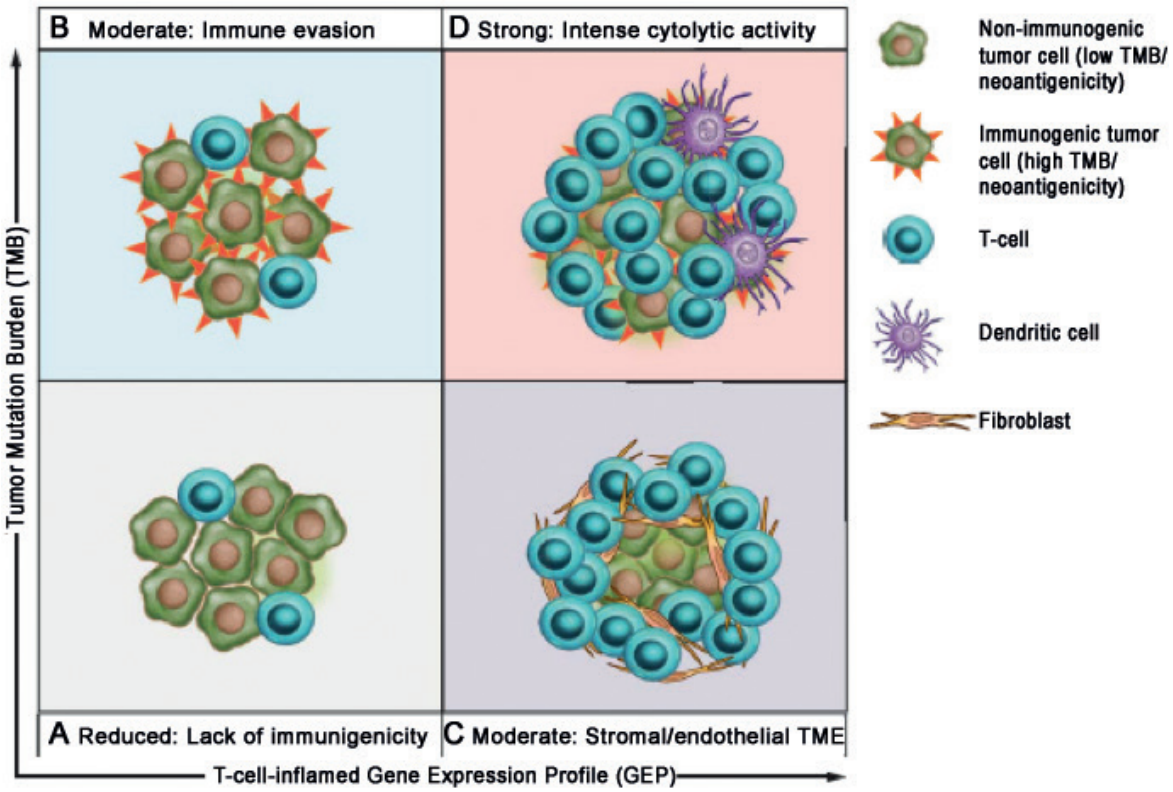


**Fig. 4. Biomarker-defined responses to pembrolizumab monotherapy identify targetable resistance biology.** (A) Tumours have low TMB and low neoantigenicity and lack a T cell-inflamed TME. (B) Tumours can evade the immune response despite high TMB and high neoantigenicity. (C) Although T cells are present, stromal and/or endothelial factors in the TME, low TMB and low neoantigenicity impede their activity. (D) Tumours have high TMB, high neoantigenicity and a T cell-inflamed TME, typified by activated T cells and other immune cells with cytolytic roles. (From Cristescu R et al., Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. Science. 2018 Oct 12;362(6411). pii: eaar3593. doi: 10.1126/science.aar3593. Reprinted with permission from The American Association for the Advancement of Science).

burden suggests a 'calibration' of immune responses to antigen burden and raises the possibility that even robust reinvigoration by anti-PD-1 therapy may be clinically ineffective if the tumour burden is high. This study provides a clinically accessible potential on-treatment predictor of response to PD-1 blockade which now needs validating prospectively.

There are now several mature technologies available for plasma and serum protein identification and quantification, including mass spectrometry proteome profiling and affinity-based methods (37), which offer the opportunity for larger scale analyses and have identified several potential protein-based biomarkers. They include vascular endothelial growth factor (VEGF). Since an early observation that high serum VEGF were associated with decreased overall survival in metastatic melanoma patients treated with ipi (39), angiogenesis is increasingly appreciated as an immune modulator with therapeutic potential combined with checkpoint blockade. Markers of angiogenesis are now receiving increasing attention for their potential clinical application.

## BIOMARKERS PREDICTING FOR IMMUNE CHECKPOINT INHIBITOR TOXICITY

Changes in IL-17, CD8 T-cell clonal expansion, eosinophil counts, and markers of neutrophil activation have been associated with specific immune-related adverse events (irAEs) after treatment induction, but did not predict toxicity development when tested at baseline (40–42). Several other potential baseline risk factors for development of irAEs from ICPIs have been suggested, including a family history of autoimmune diseases (43, 44), but these require further validation. It is intriguing to suggest that similar genetic loci that predispose to autoimmune conditions also contribute towards development of irAES but, to date, no germline factors have been associated with development of irAEs (45). Similarly, preliminary studies suggest the microbiome (discussed in more detail below) may influence risk of irAEs, particularly colitis (46).

A recent study implicated a group of cytokines in predicting immune checkpoint mediated toxicity (47). Eleven cytokines (including pro-inflammatory cytokines such as IL-1a, IL-2 and IFNa2; developed into a score called the 'CYTOX score') measured both pre- and early during treatment were found to be significantly up-regulated in patients with severe immune-related toxicities in 98 melanoma patients treated with PD-1 inhibitors, alone or in combination with anti-CTLA-4. The findings were then validated in an independent validation cohort of 49 patients treated with combination anti-PD-1 and anti-CTLA-4. If validated in larger prospective studies, the CYTOX score could identify toxicity-prone patients to either avoid harmful treatment or consider prophylactic interventions to mitigate side effects.

## THE MICROENVIRONMENT

*The microbiome*

The gut microbiome influences host immunity and has been implicated in multiple diseases including cancer. The presence of certain gut bacteria, including Akkermansia muciniphila and Bifidobacterium, was reported to improve efficacy of PD-1 blockade in animal models. In melanoma patients, significant differences have been reported in the composition and diversity of the gut microbiome between responders and non-responders to anti–PD-1 immunotherapy. However, the reported findings have so far been inconsistent (48–52), which may say more about the limitations of the sequencing technology being used. Even so, the significance of the microbiome is further implicated by preliminary studies suggesting that antibiotic (53, 54), probiotic and prebiotic (ie. dietary fibre) intake all can all influence response to checkpoint inhibition.

## IS TOXICITY A BIOMARKER OF RESPONSE?

A key element of drug development is understanding drug-induced toxicity, whether on-target or off-target effects, and whether toxicity has any correlation with predicting efficacy. In the context of BRAF-targeted agents, there is no evidence that the two are connected. With checkpoint inhibitors, the data is far more intriguing, although not at all clear cut. For ipilimumab, immune-related adverse events do not correlate with response, or survival (55, 56). For anti-PD-1 monotherapy, results are conflicting, both in the advanced (57, 58), and most recently in the adjuvant setting (59, 60). The most compelling data comes from the CheckMate 067 trial, when it was observed that 68% of patients receiving combination ipi+nivo who stopped treatment early due to unacceptable toxicity continued to maintain a response over time (15). Thus, at least for metastatic melanoma patients receiving ipi+nivo, it is reasonable to reassure patients experiencing severe, sometimes life-threatening toxicity, that this may predict for good outcome, although the converse is not necessarily true. Understanding the mechanisms that underlie irAEs and their optimal management are key areas requiring active research.

## WHEN TO STOP ANTI-PD1 ANTIBODY TREATMENT?

Anti-PD-1 antibodies are licensed to be administered to metastatic melanoma patients for as long as there is evidence of clinical benefit. For those patients who respond, they may be consigned to treatment for many years, risking toxicity, impacting quality of life, and requiring significant healthcare resources. Adjuvant therapy has been approved for a duration of one year. The biological

necessity for long term therapy in either setting is not determined and in fact, there is accumulating evidence arguing against the need. Evidence from following-up advanced melanoma patients stopping treatment due to toxicity suggest that response can be maintained in the absence of drug being administered. Long term follow-up of melanoma patients recruited to the KEYNOTE 006 trial who stopped treatment after 2 years reported durable complete remissions after discontinuation and low incidence of relapse (61). The mechanisms underlying this observation clearly need to be studied, but functional imaging may be a useful adjuvant to clinical decision-making.

Retrospective data from 104 metastatic melanoma patients treated with anti-PD1 antibodies suggests that performing $^{18}$F-2-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) at one year accurately predicts long-term outcome: PFS of complete metabolic response (CMR) was 96%, compared with 49% without CMR (HR 0.06, p<0.06) (62). The UK DANTE study is randomizing melanoma patients who are progression-free after one year of anti-PD1 antibody therapy to either stop or continue treatment. A sub-study has been proposed to evaluate prospectively the value of performing PET at one year and will also determine the value of earlier PET scanning performed at or before the first routine 12 week CT response assessment. The rationale for shorter duration of adjuvant therapy also warrants evaluation in randomised trials.

## SUMMARY

Now that systemic therapy is established for treatment of both metastatic and high-risk resected melanoma, a key next phase of research is to optimize selection of treatment by identifying biomarkers which can reliably predict both response to and relapse on therapy. This rapidly evolving and expanding personalized approach, offers the opportunity safer, more cost-effective healthcare in years to come.

## REFERENCES


1. Weber J, Mandala M, Del Vecchio M, Gogas HJ, Arance AM, Cowey CL, et al. Adjuvant nivolumab versus ipilimumab in resected stage III or IV melanoma. N Engl J Med 2017; 377: 1824–1835.
2. Long GV, Hauschild A, Santinami M, Atkinson V, Mandala M, Shiarion-Sileni V, et al. Adjuvant dabrafenib plus trametinib in stage III BRAF-mutated melanoma. N Engl J Med 2017; 377: 1813–1823.
3. Eggermont AMM, Blank CU, Mandala M, Long GV, Atkinson V, Dalle S, et al. Adjuvant pembrolizumab versus placebo in resected stage III melanoma. N Engl J Med 2018; 378: 1789–1801.
4. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature 2002; 417: 949–954.
5. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of Mutated, Activated BRAF in Metastatic Melanoma. N Engl J Med 2010; 363: 809–819.
6. Luke JJ, Flaherty KT, Ribas A, Long GV. Targeted agents and immunotherapies: optimizing outcomes in melanoma. Nat Rev Clin Oncol 2017; 14: 463–482.
7. Lee RJ, Gremel G, Marshall A, Myers KA, Fisher N, Dunn J, et al. Circulating tumor DNA predicts survival in patients with resected high risk stage II/III melanoma. Annals Oncol 2018; 29: 490–496.
8. Lee JH, Long GV, Boyd S, Lo S, Menzies AM, Tembe V, et al. Circulating tumour DNA predicts response to anti-PD1 antibodies in metastatic melanoma. Ann Oncol 2017; 28: 1130–1136.
9. Robert C, Grob JJ, Stroyakovskiy D, Karaszewska B, Hauschild A, Levchenko E, et al. Five-year outcomes with dabrafenib plus trametinib in metastatic melanoma. N Engl J Med 2019; 381: 626–636.
10. Ugurel S, Rohmel J, Ascierto PA, Flaherty KT, Grob JJ, Hauschild A, et al. Survival of patients with advanced metastatic melanoma: the impact of novel therapies-update 2017. Eur J Cancer 2017; 83: 247–257.
11. Hodi FS, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Cowey CL, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. Lancet Oncol 2018; 19: 1480–1492.
12. Chen DS and Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature 2017; 541: 321–330.
13. Tarhini A, Kudchadkar RR. Predictive and on-treatment monitoring biomarkers in advanced melanoma: Moving toward personalized medicine. Cancer Treat Rev 2018; 71: 8–18.
14. Carmona J. Biomarkers for checkpoint blockade. Nature Medicine 2018; 24: 1637.
15. Buder-Bakhaya K, Hassel JC. Biomarkers for clinical benefit of immune checkpoing inhibitor treatment – a review from the melanoma perspective and beyond. Front Immunol 2018; 9: 1474.
16. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lau CD. Combined Nivolumab and Ipilimumab or Monotherapy in previously Untreated Melanoma. N Engl J Med 2015; 373, 1270–1271.
17. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013; 500: 415–421.
18. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blocaked in melanoma. N Engl J Med 2014: 371: 2189–2199.
19. Hugo W, Zaretsky JM, Sun L, et al. Genomic and transcriptomic features of resposne to anti-PD-1 therapy in metastatic melanoma. Cell 2016; 165: 35–44.
20. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature 2015; 523: 231–235.
21. Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, et al. Loss of PTEN promotes resistance to T cell-mediated immunotherapy. Cancer Discov 2016; 6: 202–216.
22. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H,, Hugo W, Hu-Lieskovan S, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. N Engl J Med 2016; 375: 819–829.
23. Shin DS, Zaretsky JM, Escuin-Ordinas H, Garcia-Diaz A, Hu-Lieskovan S, Kalbasi A, et al. Primary Resistance to PD-1 Blockade Mediated by JAK1/2 Mutations. Cancer Discov 2017; 2: 188–201.
24. Sade-Feldman M, Jiao YJ, Chen JH, Rooney MS, Barzily-Rokni M, Eliane JP, et al. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. Nat Commun 2017; 8: 1136.
25. Busse A, Keilholz U. Role of TGF-β in melanoma. Curr Pharm Biotechnol 2011; 12: 2165-2175.
26. Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 2012; 12: 298–306.
27. Uryvaev A, Passhak M, Hershkovits D, Sabo E, Bar-Sela G.

The role of tumor-infiltrating lymphocytes (TILs) as a predictive biomarker of response to anti-PD1 therapy in patients with metastatic non-small cell lung cancer or metastatic melanoma. Med Oncol 2018; 35: 25.
28. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, et al. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. J Pathol 2014; 232: 199–209.
29. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol 2013; 14: 1014–1022.
30. Ribas A, Robert C, Hodi FS, Wolchock JD, Joshua AM, Hwu W-J, et al. Association of response to programmed death receptor 1 (PD-1) blockade with pembrolizumab (MK-3475) with an interferon-inflammatory immune gene signature. J Clin Oncol 2015; 33 suppl. 15: 3001.
31. Tarhini AA, Lin Y, Lin HM, Vallabhaneni P, Sander C, LaFramboise W, et al. Expression profiles of immune-related genes are associated with neoadjuvant Ipilimumab clinical benefit. Oncoimmunology 2016; 6: e1231291.
32. Critescu R, Mogg, R, Ayers M, Albright A, Murphy E, Yearley J, et al. Pan-tumour genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. Science 2018; 362: 6411.
33. Jessurun CAC, Vos JAM, Limpens J, Luiten RM. Biomarkers for response of melanoma patients to immune checkpoint inhibitors: a systematic review. Front Oncol 2017: 7: 233.
34. Hogan SA, Levesque MP and Cheng PF. Melanoma immunotherapy: next-generation biomarkers. Front Oncol 2018; 8: 178.
35. Lim SY, Lee JH, Diefenbach RJ, Kefford RF, Rizos H. Liquid biomarkers in melanoma: detection and discovery. Mol Cancer 2018; 17: 8.
36. Yuan J, Hegde PS, Clynes R, Foukas PG, Harari A, Kleen TO, et al. Novel technologies and emerging biomarkers for personalized cancer immunotherapy. J Immunother Cancer 2016; 4: 3.
37. Weber JS, Tang H, Hippeli L, Qian M, Wind-Rotolo M, Larkin JMG, et al. Serum IL-6 and CRP as prognostic factors in melanoma patients receiving single agent and combination checkpoint inhibition. J Clin Oncol 2019; 37(suppl): abstr 100.
38. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, et al.T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. Nature 2017 May 4; 545: 60–65.
39. Yuan J, Zhou J, Dong Z, Tandon S, Kuk D, Panageas KS, et al. Pretreatment serum VEGF is associated with clinical response and overall survival in advanced melanoma patients treated with ipilimumab. Cancer Immunol Res 2014; 2: 127–132.
40. Shahabi V, Berman D, Chasalow SD, Wang L, Tsuchihashi Z, Hu B, et al. Gene expression profiling of whole blood in ipilimumab-treated patients for identification of potential biomarkers of immune-related gastrointestinal adverse events. J Transl Med 2013; 11: 75.
41. Subudhi SK, Aparicio A, Gao J, Zurita AJ, Araujo JC, Logothetis CJ, et al. Clonal expansion of CD8 T cells in the systemic circulation precedes development of ipilimumab-induced toxicities. Proc Natl Acad Sci USA 2016; 113: 11919–11924.
42. Tarhini AA, Zahoor H, Lin Y, Malhotra U, Sander C, Butterfield LH, et al. Baseline circulating IL-17 predicts toxicity while TGF-beta1 and IL-10 are prognostic of relapse in ipilimumab neoadjuvant therapy of melanoma. J Immunother Cancer 2015; 3: 39.
43. Champiat S, Lambotte O, Barreau E, Belkhir R, Berdelou A, Carbonnel F, et al. Management of immune checkpoint blockade dysimmune toxicities: a collaborative position paper. Ann Oncol 2016; 27: 559–574.
44. Manson G, Norwood J, Marabelle A, Kohrt H, Houot R. Biomarkers associated with checkpoint inhibitors. Ann Oncol 2016; 27: 1199–1206.
45. Wolchok JD, Weber JS, Hamid O, et al. Ipilimumab efficacy and safety in patients with advanced melanoma: a retrospective analysis of HLA subtype from four trials. Cancer Immun 2010; 10: 9.
46. Dubin K, Callahan MK, Ren B, et al. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint blockade-induced colitis. Nat Commun 2016; 7: 10391.
47. Lim SY, Lee JH, Gide TN, Menzies AM, Guminski A, Carlino MS, et al. Circulating cytokines predict immune-related toxicity in melanoma patients receiving anti-PD-1-based immunotherapy. Clin Cancer Res 2019; 25: 1557–1563.
48. Vétizou M, Pitt, JM, Daillere R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015; 350: 1079–1084.
49. Sivan A, Corrales L, Hubert, N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti–PD-L1 efficacy. Science 2015; 350: 1084-1089.
50. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients. Science 2018: 359: 97–103.
51. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Dailiere R, et al. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. Science 2018; 359: 91–97.
52. Matson V, Fesser J, Bao RChongsuwat T, Zha Y, Alegre ML, et al. The commensal microbiome is associated with anti–PD-1 efficacy in metastatic melanoma patients. Science 2018; 359: 104–108.
53. Derosa L, Hellmann MD, Spaziano M, Halpenny D, Fidelle M, RizviH, et al. Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. Annals Oncol 2018; 29: 1437–1344.
54. Tinsley N, Zhou C, Villa S, Tan G, Lorigan P, FH, et al. Cumulative antibiotic use and efficacy of immune checkpoint inhibitors in patients with advanced cancer. J Clin Oncol 2018; 36(suppl): 3010.
55. Weber JS, O'Day S, Urba W, Powderly J, Nichol G, Yellin M, et al, Phase I/II study of ipilimumab for patients with metastatic melanoma. J Clin Oncol 2008; 26: 5950–5956.
56. Horvat TZ, Adel NG, Dang TO, Momtaz P, Postow MA, Callaghan MK, et al, Immune-related adverse events, need for systemic immunosuppression, and effects on survival and time to treatment failure in patients with melanoma treated with ipilimumab at Memorial Sloan Kettering Cancer Center. J Clin Oncol 2015; 33: 3193–3198.
57. Rogado J, Sanchez-Torres JM, Romero-Laorden N, Ballesteros AI, Pacheco-Barcia V, Ramos-Levı A, et al. Immune-related adverse events predict the therapeutic efficacy of anti PD-1 antibodies in cancer patients. Eur J Cancer 2019; 109: 20–27.
58. Saleh K, Khalife-Saleh N and Kourie HR. Do immune-related adverse events correlate with response to immune checkpoint inhibitors? Immunotherapy 2019; 11: 257–259.
59. Eggermont AMM, Kicinski M, Blank CU, Mandalà M, Long GV, Atkinson V, et al. Prognostic and predictive value of an immune-related adverse event among stage III melanoma patients included in the EORTC 1325/KEYNOTE-054 pembrolizumab versus placebo trial. J Clin Oncol 2019; 37(suppl): abstr 2517.
60. Mandalà M, Larkin JMG, Ascierto PA, Del Vecchio M, Gogas H, Cowey CL, et al. An analysis of nivolumab-mediated adverse events and association with clinical efficacy in resected stage III or IV melanoma (CheckMate 238). J Clin Oncol 2019; 37(suppl): abstr 9584.
61. Robert C, Ribas A, Hamid O, Daud A, Wolchok JD, Joshua AM, et al, Durable complete response after discontinuation of pembrolizumab in patients with metastatic melanoma. J Clin Oncol 2018; 36: 1668–1674.
62. Tan AC, Emmett L, Lo S, Liu V, Kapoor R, Carlino MS, et al. FDG-PET response and outcome from anti-PD-1 therapy in metastatic melanoma. Ann Oncol 2018; 29: 2115–2120.
63. Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Mered M, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med 2018; 24: 541–550.

Centenary theme section: SKIN MALIGNANCIES

REVIEW ARTICLE

# Update on the Management of Cutaneous Squamous Cell Carcinoma

Eve MAUBEC
*Department of Dermatology, AP-HP, Hôpital Avicenne, University Paris 13, Bobigny, France*

**For all primary cutaneous squamous cell carcinomas (cSCCs), physical examination should include full skin examination, recording of tumour diameter and regional lymph-node–basin status. Surgery is the treatment of choice, with a minimal 5-mm margin. For elderly patients with well-differentiated tumours, other surgical modalities can be explored. Surgery for organ-transplant recipients should not be delayed. The issue with cSCC is identifying high-risk tumours with staging, as this may alter treatment and follow-up schedules. Adjuvant radiation therapy should be considered for incomplete resection, when re-excision is impossible or there are poor-prognosis histological findings. Recommendations are at least biannual dermatological surveillance for 2 years, but in elderly patients with small, well-differentiated tumours long-term follow-up is not always necessary. In case of positive lymph nodes, radical dissection is needed, with regional postoperative adjuvant radiation. Advanced cSCCs are defined as unresectable local, regional or distant disease requiring systemic treatment. Their only approved treatment is the PD-1 inhibitor, cemiplimab. Trials evaluating adjuvant or neo-adjuvant anti-PD-1 are ongoing. Platin-based chemo or anti-epidermal growth-factor–receptor therapies are possible second-line treatments. For transplant patients, minimizing immunosuppression and switching to sirolimus must be considered at first appearance of cSCC.**

*Key words:* cutaneous squamous cell carcinoma; anti-PD-1; adjuvant treatment.

Accepted Apr 27, 2020; Epub ahead of print Apr 28, 2020

Acta Derm Venereol 2020; 100: adv00143.

*Corr:* Eve Maubec, Department of Dermatology, AP-HP, Hôpital Avicenne, 125, route de Stalingrad, FR-93000 Bobigny, France. E-mail: eve.maubec@aphp.fr

## SIGNIFICANCE

This review updates the management of primary resectable cutaneous and advanced cutaneous squamous cell carcinomas. It is important for physicians treating cutaneous squamous cell carcinoma to know that currently available staging systems can help identify high-risk tumours and should guide work-up and treatment. This article describes risk factors and staging methods, along with an overview of current treatments according to disease stage.

Historically, cutaneous squamous cell carcinoma (cSCC) was the second most common skin cancer after basal cell carcinoma (BCC), but several recent reports on the Australian and US populations have shown a shift in the numbers of cSCCs compared with BCCs. A study of Medicare patients shows a 1:1 ratio of cSCC to BCC (1). The incidence of cSCC has increased markedly over recent decades worldwide, probably because very early cSCC are being resected more often, but also because of increased exposure to the sun (1). cSCC frequency quadrupled for both sexes in Sweden between 1960 and 2004 (2). cSCCs often occur in elderly and male patients. The main risk factors for developing cSCCs are chronic cumulative exposure to ultraviolet (UV), including sunbed use and psoralen and ultraviolet A (UVA), having fair skin or hair, and taking immunosuppressive medication for $\geq 1$ month (3–5). Immunocompromised patients, including organ-transplant recipients and human immunodeficiency virus (HIV)-positive patients, are at increased risk of cSCC (6, 7). cSCCs are the most common cancers following organ transplantation, with their risk increasing 100-fold for transplantees (6, 8–10). Oncogenic human papillomavirus, chronic scarring conditions, exposure to arsenic or ionizing radiation, recessive dystrophic epidermolysis bullosa and rare familial syndromes (e.g. xeroderma pigmentosum, albinism and Lynch syndrome) have also been associated with increased risk of cSCCs. Ageing of the population, more organ-transplant recipients, change in attitude toward UV exposure, and increased ascertainment contribute to the increase in incidence of cSCC.

Although initial surgical excision cures 95% of patients, a minority of cSCCs recur locally (3–4%) or metastasize (2–4%), usually to regional lymph nodes or, rarely, to distant locations (11, 12). In addition, 1–4% of cSCCs are fatal (13, 14). cSCC-attributed mortality is increasing in Australia. The mortality rate in the southern and central USA approached that of melanoma, emphasizing that cSCC is a critical public health concern (15).

Awareness of risk factors for cSCC is essential to improve primary prevention with the objective of containing, and hopefully lowering, the increasing incidence of cSCC. Thus, because sunscreens can prevent cSCC (16), its use should be strongly encouraged, and use of sunbeds should be strongly discouraged. Moreover, high-risk patients, i.e. immunocompromised patients, should undergo regular dermatological monitoring and

education about skin self-examination and safe behaviour in the sun.

Application of the currently available staging systems helps to identify patients at high risk of recurrence. The American Joint Committee on Cancer staging 8th edition (AJCC-8) (11) tumour-staging items include tumour diameter, as summarized in **Fig. 1**a. Lymph-node size, number of positive lymph nodes and their location(s) (ipsilateral, contralateral, bilateral) and extranodal extension. However, the AJCC-8 is relevant only for head-and-neck cSCCs, which might limit its usefulness. The Brigham and Women's Hospital (BWH)-staging system (17) is based on the presence of 4 risk factors, summarized in Fig. 1b. BWH stage T3 represents only 5% of tumours, but 70% of nodal metastases and 83% of disease-specific deaths. A recent monocentre retrospective study on 186 head-and-neck cSCCs (18) compared the 2 systems and found an overlapping of poor-prognosis predictions.

Several other poor-prognosis risk factors are not included in these classifications: high-risk locations (lip, ear), histological thickness or Clark level ≥IV, desmoplastic and adenosquamous histological subtypes or immunosuppression. Organ-transplant recipients' cSCCs are often aggressive tumours and in view of the presence of multiple viral warts in these patients, which may be difficult to differentiate from early SCC, it is recommended that dedicated dermatology clinics look after these high-risk patients, if possible.

High-risk cSCCs have a higher recurrence, estimated at 16%. Recurrences occur mainly during the first 2 years post-diagnosis (19). However, a review of the literature showed that, for patients with high-risk cSCCs and clearly documented surgical margins, risks of local recurrence, regional metastasis, distant metastasis and disease-specific death were 5%, 5%, 1% and 1%, respectively (20).

Advanced cSCCs are defined as either locally unresectable, deeply invasive involving muscle, nerve or bone structures, unresectable regional lymph-node disease or multiple distant metastases requiring systemic curative treatment (**Fig. 2**).

A **Tx Primary tumor cannot be assessed**
**T0 No evidence of primary tumor**
**Tis Carcinoma *in situ***
**T1 Tumor diameter ≤2 cm**
**T2 Tumor diameter >2 cm but ≤4 cm**
**T3 Tumor diameter >4 cm, minor bone invasion, perineural invasion# or deep invasion***
**T4 Tumor with gross cortical bone/marrow, skull base and/or its foramen invasion**

**#Defined as tumor cells within a nerve sheath lying deeper below the dermis, ≥0.1 mm in caliber, with clinical or radiographic involvement of named nerves without skull base invasion or transgression.**
***Defined as that going beyond the subcutaneous fat or >6 mm.**

B

| | |
|---|---|
| T1 | 0 risk factor |
| T2a | 1 risk factor |
| **T2b** | **2–3 risk factors** |
| **T3** | **≥4 risk factors or bone invasion** |

**High-risk patients**

**Risk factors**

- Tumor diameter ≥2 cm
- Tumor invasion beyond subcutaneous fat (excluding bone invasion, which automatically upgrades tumor to T3)
- Perineural invasion ≥0.1 mm
- Poorly differentiated

**Fig. 1. Cutaneous squamous cell carcinoma – staging criteria.** (a) American Joint Committee on Cancer 8th edition staging of head-and-neck tumours (adapted from (11). (b) Brigham and Women's Hospital tumour-staging items (adapted from (17)).

## MANAGEMENT OF PRIMARY RESECTABLE CUTANEOUS SQUAMOUS CELL CARCINOMAS

### *Physical examination and biological staging*

Staging should systematically include primary tumour diameter, regional lymph-node–basin status, and search for other skin cancers and chronic inflammatory disorders and previous or current immunosuppression. Rare genetic syndromes, such as xeroderma pigmentosum, albinism and Lynch syndrome have to be ruled out in patients who have early onset and/or multiple cSCC without obvious risk factors.

### *Imaging studies for staging*

Because few studies have addressed cSCC imaging, its value for regional and distant staging is uncertain, even for high-risk cSCCs (21). A meta-analysis of head-and neck tumours evaluating the contributions of computed tomography (CT) scans, magnetic resonance imaging (MRI), ultrasonography (US) and US-guided fine-needle aspiration showed that the last accuracy was the best (22). Ultrasound scanning with fine needle aspiration cytology was found superior to CT in assessing primary SCC of the vulva regional disease status (23). Based on a retrospective series of 98 high-risk patients with BWH-stage T2b or T3 cSCCs, with imaging staging (CT, positron-emission tomography (PET–CT scans or MRI) or without, imaging impacted cSCC management for one-third of them; moreover, patients without imaging staging tended to develop nodal metastases more frequently ($p=0.046$) (24). Prospective studies are needed to confirm that an initial imaging work-up can impact management and outcomes, and that imaging should be considered for regional staging in high-risk patients. In 2020, the European Dermatology Forum (EDF), European Association of Dermato-Oncology (EADO) and the European Organization for Research and Treatment of Cancer (EORTC) (EDF–EADO–EORTC) consensus group recommended lymph-node US for high-risk patients (25).

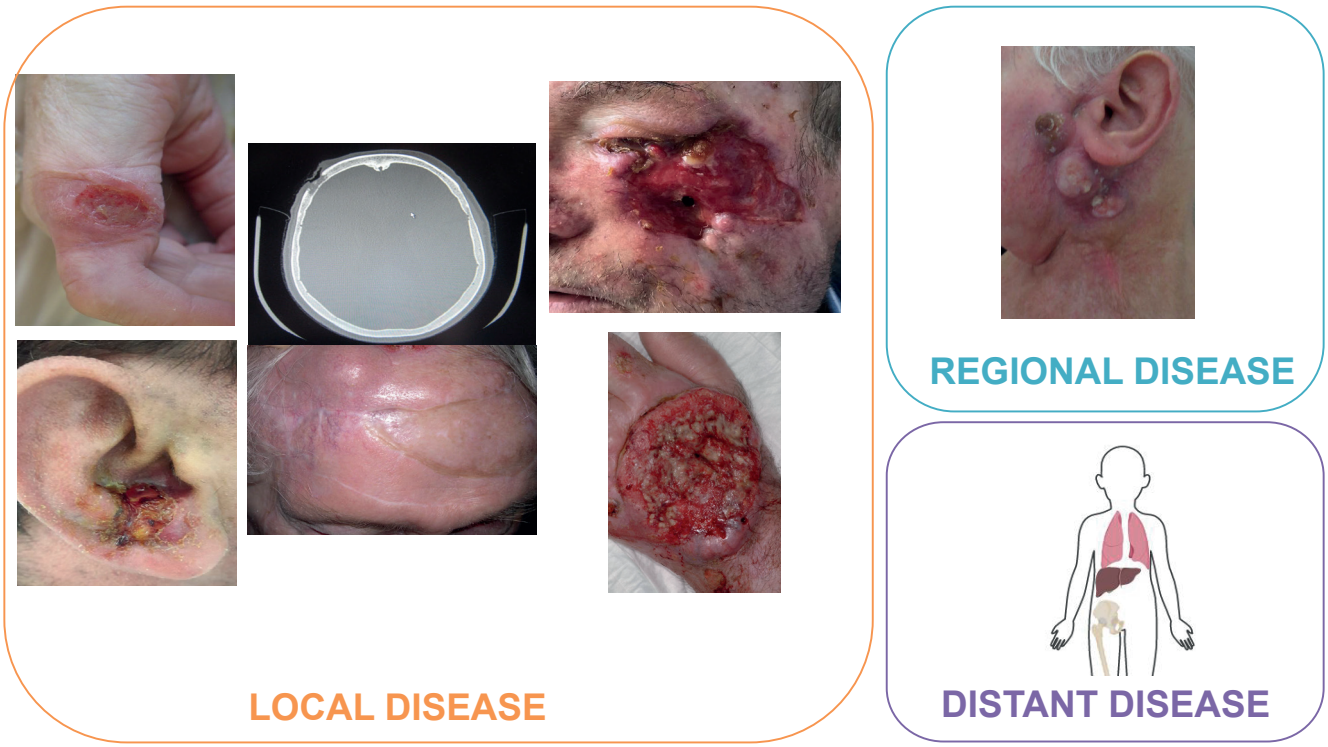


**Fig. 2. The different types of advanced cutaneous squamous cell carcinomas.** Local disease (*left*): local unresectable disease without regional or distant disease. Regional disease (*top right*): at least regional unresectable disease without distant disease. Distant disease (*bottom right*): at least one unresectable distant metastasis.

### *Surgery*

Biopsy or limited excision of the tumour is usually performed to confirm a clinically suspected cSCC, but if the tumour is small, a single definitive excision is often performed outright with various margins. Surgery is the treatment of choice. Most primary resectable cSCCs are usually cured by conventional excision. Mohs surgery may be needed for high-risk tumours and/or difficult anatomical sites. Randomized controlled trials on resection-margin widths are lacking, therefore excision margins for SCC are controversial.

Excellent cure rates have been reported in several series. Experience suggests that small well-differentiated tumours, which are slow-growing in elderly patients on sun-exposed sites can be removed by experienced physicians with curettage (http://www.bad.org.uk/healthcare-professionals/clinical-standards/clinical-guidelines). Recurrences were rare in a study on 1,174 cSCC patients and did not differ significantly among tumours treated with electrodessication/curettage destruction, excision or Mohs surgery, respectively: 24.3% of 361 vs. 38.3% of 571, or 37.4% of 556 (26).

The EDF–EADO–EORTC consensus group has recommended surgical resection with a minimal 5-mm margin, even for low-risk tumours, which should be extended to 10 mm for high-risk tumours (**Table I**) when additional clinical or histological risk factors are present (25). When technically feasible, 1-step resection and

**Table I. Summary of treatment options**

| Treatment options |
|---|
| *Primary resectable cSCCs* |
| Surgical resection (5–10 mm margin) |
| Alternative: curative radiation therapy |
| Alternative for low risk small tumours on sun exposed sites: 2 cycles curettage and cautery |
| *Adjuvant treatment for primary high-risk cSCCs** |
| Radiation therapy |
| Ongoing immunotherapy trials |
| *Neoadjuvant treatment* |
| Ongoing immunotherapy trials |
| *cSCCs with regional lymph node involvement* |
| Radical lymph-nodes dissection |
| Adjuvant radiation therapy |
| *Advanced cSCCs* |
| *First line:* |
| • Cemiplimab (350 mg infused intravenously over 30 min every 3 weeks) |
| *Second line:* |
| Cisplatin-based chemotherapies |
| • or Carboplatin-based chemotherapies (better tolerated in patients with comorbidities) |
| • or epidermal growth-factor receptor (EGFR)-targeted therapies (cetuximab) |
| • or hyperthermic isolated-limb perfusion |
| - or ongoing combined immunotherapy trials |
| Prevention |
| *Topical treatments* |
| 5% 5-FU cream |
| Alternatives: imiquimod, diclofenac and photodynamic therapy |
| *Oral treatments* |
| Acitretin, nicotinamide |
| *Primary cSCCs in transplant recipients* |
| Minimizing immunosuppression and switching to sirolimus |

*Incomplete resection, poor-prognosis histological findings.
cSSC: cutaneous squamous cell carcinoma.

closure is preferred; 2-step resection is recommended when a graft or flap reconstruction is planned. If the resection is incomplete, then surgical re-excision should be performed.

In an earlier prospective, multicentre Australian case series of 1,263 cSCC patients, characterized by an elevated percentage of high-risk tumours treated with Mohs micrographic surgery, 5-year recurrence rates were low: 2.6% in patients with primary cSCCs and 5.9% in patients with locally recurrent cSCC, suggesting that this technique achieves a high cure rate for these high-risk cSCCs (12). However, randomized studies comparing Mohs surgery with conventional surgery are lacking.

The pathologist's report should specify histological differentiation grade, histological subtype, maximum tumour thickness and Clark level, invasion of muscle, cartilage, bone and/or fascia, perineural or lymphatic/vascular invasion, whether or not the resection was complete with minimal lateral and deep margins.

For high-risk cSCCs with negative regional staging on imaging, a sentinel lymph-node biopsy might be considered an option, but is not standard of care, depending on its potential comorbidities. Indeed, sentinel lymph-node biopsies are positive for one-third of the patients with BWH stage-T2b or -T3 cancers (27). However, the authors of a recent prospective German study found that 6% of a series of sentinel lymph-node-negative patients had distant metastases, suggesting the limited prognostic value of the procedure (28).

### *Curative radiation therapy*

Radiotherapy represents an alternative to primary surgical resection for SCC of the lip and when surgery is not appropriate for cSCCs. However, the risk of cSCC recurrence is higher after radiation therapy compared with surgery. For patients with comorbidities that predispose them to radiation-induced cancers, such as basal cell naevus syndrome or xeroderma pigmentosum, radiotherapy must be avoided. Radiation therapy can cause reversible dermatitis or mucositis. Late side-effects include skin atrophy with loss of hair, reduced sweating and sebaceous secretions, discoloration, telangiectasia, hypodermic sclerosis and/or skin carcinomas so should be avoided in younger patients (29).

### *Adjuvant radiation therapy for primary high-risk cutaneous squamous cell carcinoma*

According to a literature review on cSCCs with perineural invasion treated with surgery ($n=30$) or surgery plus adjuvant radiation therapy ($n=44$ cases), outcomes were comparable (20). The role of adjuvant radiation therapy for high-risk cSCCs, including those with perineural invasion, remains controversial. However, authors of a recent retrospective study on adjuvant radiation therapy for cSCCs with perineural invasion found it to be associated with prolonged survival (30), suggesting that such patients might benefit from adding radiation to surgery and decisions have to be made on a case-by-case basis.

### *Other adjuvant or neoadjuvant strategies for primary high-risk cutaneous squamous cell carcinoma*

No significant differences were found for retinoic acid and interferon vs. placebo for the time to recurrence or occurrence of second primary cSCCs in patients with high-risk cSCCs enrolled in a randomized phase-3 trial (31).

O'Bryan et al. prescribed adjuvant cetuximab for 7 patients with high-risk cSCCs (32); only 3 experienced disease recurrence. Neoadjuvant gefitinib therapy in a phase-2 study on 22 patients achieved a 45% response rate, including 3 histological complete responses (CRs) (33). However, disease progressed for 32% and the lack of known biomarkers of response highlights the need for further larger studies, including randomized trials. Jenni et al. (34) more recently reported size reduction after 14 days of lapatinib in 2 out of 8 assessable patients, among 10 with resectable cSCCs.

A recent phase-2 study (35), presented at European Society for Medical Oncology (ESMO) 2019, showed that cemiplimab neoadjuvant therapy given to 20 patients induced histological partial responses (PRs) or CRs in 70% of the patients. Moreover, it was well-tolerated. Ongoing trials are evaluating the potential contribution of anti-programmed cell-death protein-1 (PD-1) agents as adjuvant therapy for high-risk cSCCs.

### *Monitoring*

The majority of all recurrences of cSCC occur within 2 years of the initial diagnosis. In high-risk cSCCs the follow up should be at least 2 years and should include palpation of the primary excision site and of the regional lymph node area every 3 or 6 months depending on the initial stage and medical history. Moreover, the entire skin of all patients should be examined once annually or every 6 months in high-risk cSCCs patients (immunosuppression, multiple primary cSCCs, genetic predisposition) as recommended by the current European guidelines (25). However, in elderly patients with small well-differentiated SCC on sun-exposed sites (excluding high-risk sites, such as lips, ears, digits and mucosa), discharge after 3 months is possible.

## MANAGEMENT OF CUTANEOUS SQUAMOUS CELL CARCINOMAS WITH REGIONAL LYMPH-NODE INVOLVEMENT

Histological examination of fine-needle aspirates or resections of any enlarged nodes is mandatory. Available results of studies on lymph-node involvement of head-and-neck cSCCs indicated positive lymph nodes

as a negative factor for survival (36, 37). Extracapsular lymph-node spread is a significant risk factor for recurrence. The most frequently involved lymph-node region is around the parotid. Disease stage should be assessed by imaging studies, including CT or PET–CT scan(s) or MRI. When lymph nodes are histologically positive, they should be subjected to radical dissection. Postoperative adjuvant radiation delivered to the affected lymph-node region is required for head and neck tumours, as it enhances local–regional control and disease-free survival (DFS) and overall survival (OS) of those patients (30).

## MANAGEMENT OF ADVANCED CUTANEOUS SQUAMOUS-CELL CARCINOMAS

The PD-1 inhibitor, cemiplimab, is the only approved agent for locally advanced and metastatic cSCCs. Prior conventional treatment for advanced cSCCs, such as cisplatin-based chemotherapies or epidermal growth-factor receptor (EGFR)-targeted therapies, can be used as second-line treatments. Trials evaluating other anti-PD-1 molecules and combinations of anti-PD-1 with other drugs are currently ongoing.

A retrospective study in Europe, completed just before anti-PD-1 became available, described various treatments for patients with advanced cSCCs (38). Among 190 patients (median age 79 years) with locally advanced or metastatic disease, 32% received systemic anti-tumour therapies (excluding anti-PD1), mostly anti-EGFR tyrosine-kinase inhibitors. Half of the patients did not complete systemic therapy as planned. The objective response rate (ORR) was 26% and the mean response duration was 5 months. Among the 152 patients whose survival status was known, 49% had died. The availability of anti-PD-1 agents might allow access to treatment for more patients with cSCC.

### *Anti-programmed cell-death protein-1*

The immune system is important for cSCC, as suggested by the increased risk of cSCCs in transplant recipients (39), the rapid regression of keratoacanthoma, which is characterized by a more active immune response than generally seen in cSCCs (40), and activity of immunotherapy in advanced SCC as combination of interferon and retinoic acid (41). The PD-1 receptor is expressed on T cells, and T cells binding to its ligand (PD-L1) inhibit T-lymphocyte functions. PD-L1 is expressed in 30–50% of cSCCs and its expression was found to correlate with risk of metastases (42). The high mutation rate in cSCCs, as in other UV-induced tumours, is usually a predictor of responsiveness to anti-PD-1 (43).

Cemiplimab (3 mg/kg every 2 weeks) induced a response in approximately half of the 85 patients enrolled in a phase-2 study with locally, regional or distant disease and a phase-1 study with regional or distant disease (44). Those patients were treated, respectively, for up to 48 weeks and up to 96 weeks. Fifty-six to 58% of the patients had received systemic treatment before cemiplimab. Median phase-1 and phase-2 follow-ups were: 11 and 8 months, respectively. Their respective ORRs were 50% and 47%. Median time to response was 2 months for both. In the phase-2 trial, 7% were CRs; median progression-free survival (PFS) and OS had not been reached and median duration of response exceeded 6 months for 16/28 (57%) responders. The most common adverse reactions were fatigue, rash and diarrhoea. Serious adverse events were immune-mediated, such as pneumonitis, hepatitis, colitis, adrenal insufficiency, dysthyroidism, diabetes mellitus and/or nephritis, and, unlike other anti-PD-1 inhibitors, infusion reactions. Treatment was stopped for 7% of patients because of adverse events. Three cemiplimab-related deaths were reported (44). Cemiplimab was approved by the US Food and Drug Administration (FDA) in September 2018 and European Medicines Agency (EMA) in July 2019 for patients with metastatic or locally advanced cSCCs who were not candidates for curative surgery or radiation. The recommended cemiplimab dose and schedule is now 350 mg, infused intravenously over 30 min every 3 weeks. Factors predictive of response are still unknown. Treatment duration needs to be better defined.

Several trials have also assessed pembrolizumab in cSCCs. Interim results of the Keynote 629 study evaluating pembrolizumab (200 mg/3 weeks IV) in advanced cSCC have been presented at the ESMO meeting in 2019 (45). Response rate was 32% in 91 patients receiving pembrolizumab as a second-line treatment and 50% in 14 naïve patients. The median duration of response was not reached. The safety profile was consistent with that of other pembrolizumab monotherapy studies. Interim analysis of the CARSKIN study presented at the ASCO 2019 meeting, showed a response rate of 38.5% in 39 previously untreated patients with advanced cSCC with sustained responses to pembroluzimab (46).

### *Platin-based chemotherapies*

Few prospective trials are available and no treatment regimen has been recommended by health authorities. Because their ORRs are high, platin-based chemotherapies were the first-choice treatment before the anti-PD-1 era, but their administration can be limited by cisplatin toxicity or disease recurrence during treatment. Sadek et al. (47) treated 14 advanced cSCC patients with 1–4 cycles, repeated every 3–4 weeks, of neoadjuvant combination chemotherapy (bolus cisplatin injection, 5-fluorouracil (5-FU) and continuous 5-day bleomycin infusion). The ORR was 78% (4 CRs, 7 PRs). Local control after adjuvant radiation and/or surgery was achieved in 7 (50%) patients. CR lasted >10 months. All patients experienced major toxicities, including grade-3/4

nausea and vomiting; 4 patients had grade-3/4 haematological toxicities and one developed pulmonary fibrosis. In their prospective phase-2 trial, Guthrie et al. treated advanced BCC or locally advanced cSCC patients with cisplatin (75 mg/m$^2$ and doxorubicin 50 mg/m$^2$, every 3 weeks) (48). Among the 12 advanced-cSCC patients, 7 responded (4 CRs and 3 PRs). Based on 7 patients with advanced local-regional or metastatic cSCCs, Khansur et al. reported the activity of cisplatin (100 mg/m$^2$ on day 1) and 5-FU (1 g/m$^2$/day, days 1–4), given every 3 weeks. Six of 7 patients were responders: 3 PRs and 3 CRs (49). The mean duration for CR was one year. Toxicities included grade-1/2 nausea and vomiting. Carboplatin-combination therapy is better tolerated and can be administered as an alternative to patients with comorbidities. Hyperthermic isolated-limb perfusion can be a second-line limb-saving therapy for patients with unresectable disease located on the extremities (50).

*Epidermal growth-factor receptor-targeted therapies*

EGFR represents a family of proteins, including EGFR and human epidermal growth factor receptor (HER)-2, 3 and 4. Activation of EGFR tyrosine kinase results in autophosphorylation and activation of RAS serine/threonine kinase, murine sarcoma viral oncogene (RAF), mitogen-activated protein (MAP) kinase and phosphatidylinositol 3-kinase (PI3K), AKT protein kinase and mammalian target of rapamycin (mTOR) pathways leading to tumour growth. EGFR is strongly expressed in metastatic cSCCs and its overexpression in primary cSCCs is associated with poor outcome (18). Anti-EGFR therapy consists of monoclonal antibodies, such as cetuximab or panitumumab, which competitively inhibit EGFR, or small molecules, e.g. gefitinib or erlotinib, targeting the intracellular domain of the receptor. EGFR-targeted therapies have been developed and obtained promising ORRs in several clinical trials and retrospective studies on patients with unresectable cSCCs. So far, phase-3 trial results have not yet confirmed their efficacy against cSCCs. Anti-EGFR tyrosine-kinase inhibitors are not approved to treat advanced cSCCs, but cetuximab is listed in the National Comprehensive Cancer Network (NCCN) compendium as a therapy for recurrent and metastatic CSSCs. No biomarker predictive of a cSCC response has been identified.

Cetuximab was evaluated prospectively as first-line monotherapy in a French phase-3 study on 36 patients with metastatic ($n=3$), regional ($n=16$) or locally advanced ($n=17$) cSCCs. The ORR was 28%, including 2 CRs and 8 PRs, and the overall disease-control rate was 69% (25/36 patients). Median PFS lasted 4 months. The median duration of response was 7 months and the mean OS was 8 months. The more frequent severe adverse events were infections (22%) and tumour bleeding (11%). Cetuximab-related adverse events included 2 grade-4 infusion reactions and 1 grade-3 interstitial pneumopathy (51). Cetuximab can be combined with platin-based chemotherapies and this combination might prolong PFS (9.03 vs. 3.55 months), according to a retrospective series of 14 patients treated with cetuximab monotherapy or cetuximab combined with carboplatin (52). Low-grade specific acne-like rash, pruritus and nail changes have been observed. Severe infusion reactions occurred in 3% of patients.

Panitumumab efficacy (6 mg/kg, repeated every 2 weeks) was of the same order of magnitude for 11 Italian patients with advanced penile SCC (53) and 16 Australian patients with advanced cSCC enrolled in a phase-2 study (54). Median PFS and OS, respectively, were 8 and 11 months for cSCC patients, and 2 and 9 months for those with penile SCC. Severe skin rash, mucositis and diarrhoea occurred.

Efficacy of oral small molecules against advanced cSCCs was variable, with ORR of 10–32%. Based on available phase-2 studies, gefitinib or erlotinib alone obtained only poor ORRs of 15% (6/40 patients) and 7% (3/39 patients), respectively (55, 56). Higher ORRs, of the same order of magnitude as those achieved with monoclonal antibodies, were obtained with second-generation irreversible pan-HER tyrosine-kinase inhibitors, such as dacomitinib: in 28% of cSCCs and 32% (9/28 patients) of penile SCC (57, 58). The tolerance profile of small molecules differed, with more diarrhoea and mucositis than with antibodies.

Concurrent radiotherapy with cetuximab did not significantly prolong PFS and OS compared with concurrent radiotherapy and cisplatin-based chemotherapy in a retrospective series of 23 patients with head-and-neck cSCCs (59).

Further prospective studies are needed to determine the characteristics of patients who would benefit from anti-EGFR and to evaluate combinations of anti-EGFR and other drugs to improve outcomes.

## PREVENTION

Available topical agents to treat actinic keratosis and cSCC *in situ* field of cancerization include mainly 5-FU cream, imiquimod, diclofenac and photodynamic therapy. Ingenol metubate (Picato) is now withdrawn because of safety issues. A recent randomized Dutch trial evaluating efficacy of 5% 5-FU cream, 5% imiquimod cream, methyl aminolevulinate photodynamic therapy or 0.015% ingenol mebutate gel in 624 patients with ≥5 actinic keratosis lesions on the head and neck showed that 5% 5-FU cream was the most effective in controlling solar keratoses (60). However, it has not been confirmed that it does, in turn, reduce the risk of SCC.

Oral acitretin can prevent the occurrence of new cSCCs in patients with multiple tumours; for example,

xeroderma pigmentosum patients or transplant recipients. However, cutaneous adverse events often led patients to discontinuation, which, in turn, allowed quick appearance of new cSCCs.

Oral nicotinamide can be prescribed off-label. Indeed, it was evaluated in a randomized study on 386 patients with a history of 2 or more non melanoma skin cancers. Patients received either nicotinamide (500 mg, 2 times per day) or placebo for one year. The nicotinamide group had 30% significantly fewer new cSCCs (61). However, the long-term benefit remains unknown. Liver toxicity can sometimes occur.

## TRANSPLANT RECIPIENTS

All transplant recipients are at high risk of developing cSCCs. These cSCCs are more aggressive, with a 5–10-fold higher risk of metastasis (62, 63). Immunosuppression duration and drug types and doses are involved. Surgery must not be delayed in transplant recipients with resectable tumours.

For transplantees, minimizing immunosuppression and switching to sirolimus should be considered as soon as the first cSCC appears. The benefit of switching to sirolimus is maintained for 5 years, with no negative effect on the graft and patient survival (64). However, administration of mTOR inhibitors remains limited because of poor tolerance. Indeed, 25–40% of patients discontinue sirolimus because of adverse events, e.g. hyperlipidaemia, glucose intolerance, interstitial pneumonia and/or lymphoedema. For transplantees with advanced cSCCs, currently available drugs should be used with caution, as anti-PD-1 agents are associated with a high rate of irreversible allograft rejection, while anti-cutaneous T-lymphocyte antigen-4 (CTLA-4) seems to be better tolerated (65). Moreover, the risk of infections with conventional chemotherapy is higher in immunosuppressed patients. Notably, 2 lung-transplant recipients with metastatic cSCCs died 1–3 weeks after their first infusions of cetuximab due to diffuse alveolar damage (66).

## CONCLUSION

Due to the increasing incidence of cSCC, it has become a serious public health concern. All tumours should systematically be staged with AJCC-8 or BWH systems, in order to adapt treatment according to the risk of recurrence. Surgery is the treatment of choice whenever the tumour is resectable. Adjuvant radiation therapy must be considered for high-risk cSCCs. PD-1 inhibition is now the standard-of-care for advanced cSCCs. Platin-based chemotherapy or anti-EGFR can be prescribed in the second-line setting. Factors predictive of cSCC response to anti-PD-1 or anti-EGFR remain to be elucidated. Due to the high rate of irreversible allograft rejection associated with anti-PD-1 in organ-transplant recipients, other, less toxic, anti-CTLA-4 or other approaches warrant investigation. Switching from calcineurin inhibitors to sirolimus, or de-escalating immunosuppression, should always be considered. Because most advanced tumours may not respond to various current treatments, the search for new approaches is warranted. Prevention should not be forgotten. SCC incidence is increasing rapidly because of better screening, therefore most cSCC seen in dermatology or plastic surgery clinics are now detected earlier with better prognosis. Only 1–4% of cSCC are fatal; hence patients with cSCC must be accurately staged, to ensure that they are not over-investigated and do not undergo unnecessary surgical procedures or systemic treatments.

## ACKNOWLEDGEMENTS

The authors thank Margot Denis for technical assistance in the preparation of this manuscript and Janet Jacobson for editorial assistance.

EM served as a principal investigator for SANOFI and principal investigator and coordinator for MSD investigations, and received research funding from MSD.

## REFERENCES

1. Rogers HW, Weinstock MA, Feldman SR, Coldiron BM. Incidence estimate of nonmelanoma skin cancer (keratinocyte carcinomas) in the U.S. population, 2012. JAMA Dermatol 2015; 151: 1081–1086.
2. Wassberg C, Thorn M, Johansson AM, Bergstrom R, Berne B, Ringborg U. Increasing incidence rates of squamous cell carcinoma of the skin in Sweden. Acta Derm Venereol 2001; 81: 268–272.
3. Dal H, Boldemann C, Lindelof B. Trends during a half century in relative squamous cell carcinoma distribution by body site in the Swedish population: support for accumulated sun exposure as the main risk factor. J Dermatol 2008; 35: 55–62.
4. Schmitt J, Seidler A, Diepgen TL, Bauer A. Occupational ultraviolet light exposure increases the risk for the development of cutaneous squamous cell carcinoma: a systematic review and meta-analysis. Br J Dermatol 2011; 164: 291–307.
5. Christensen GB, Ingvar C, Hartman LW, Olsson H, Nielsen K. Sunbed use increases cutaneous squamous cell carcinoma risk in women: a large-scale, prospective study in Sweden. Acta Derm Venereol 2019; 99: 878–883.
6. Jensen P, Hansen S, Moller B, Leivestad T, Pfeffer P, Geiran O, et al. Skin cancer in kidney and heart transplant recipients and different long-term immunosuppressive therapy regimens. J Am Acad Dermatol 1999; 40: 177–186.
7. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet 2007; 370: 59–67.
8. Garrett GL, Blanc PD, Boscardin J, Lloyd AA, Ahmed RL, Anthony T, et al. Incidence of and risk factors for skin cancer in organ transplant recipients in the United States. JAMA Dermatol 2017; 153: 296–303.
9. Rizvi SMH, Aagnes B, Holdaas H, Gude E, Boberg KM, Bjortuft O, et al. Long-term change in the risk of skin cancer after organ transplantation: a population-based nationwide cohort study. JAMA Dermatol 2017; 153: 1270–1277.
10. Lindelöf B, Sigurgeirsson B, Gabel H, Stern RS. Incidence of skin cancer in 5356 patients following organ transplantation. Br J Dermatol 2000; 143: 513–519.
11. Karia PS, Jambusaria-Pahlajani A, Harrington DP, Murphy GF,

Qureshi AA, Schmults CD. Evaluation of American Joint Committee on Cancer, International Union Against Cancer, and Brigham and Women's Hospital tumor staging for cutaneous squamous cell carcinoma. J Clin Oncol 2014; 32: 327–334.
12. Leibovitch I, Huilgol SC, Selva D, Hill D, Richards S, Paver R. Cutaneous squamous cell carcinoma treated with Mohs micrographic surgery in Australia I. Experience over 10 years. J Am Acad Dermatol 2005; 53: 253–260.
13. Czarnecki D. Non-melanoma skin cancer mortality rising in susceptible Australians. J Eur Acad Dermatol Venereol 2017; 31: e286–e287.
14. Osterlind A, Hjalgrim H, Kulinsky B, Frentz G. Skin cancer as a cause of death in Denmark. Br J Dermatol 1991; 125: 580–582.
15. Karia PS, Han J, Schmults CD. Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. J Am Acad Dermatol 2013; 68: 957–966.
16. Green A, Williams G, Neale R, Hart V, Leslie D, Parsons P, et al. Daily sunscreen application and betacarotene supplementation in prevention of basal-cell and squamous-cell carcinomas of the skin: a randomised controlled trial. Lancet 1999; 354:723–729.
17. Jambusaria-Pahlajani A, Kanetsky PA, Karia PS, Hwang WT, Gelfand JM, Whalen FM, et al. Evaluation of AJCC tumor staging for cutaneous squamous cell carcinoma and a proposed alternative tumor staging system. JAMA Dermatol 2013; 149: 402–410.
18. Canueto J, Burguillo J, Moyano-Bueno D, Vinolas-Cuadros A, Conde-Ferreiros A, Corchete-Sanchez LA, et al. Comparing the eighth and the seventh editions of the American Joint Committee on Cancer staging system and the Brigham and Women's Hospital alternative staging system for cutaneous squamous cell carcinoma: implications for clinical practice. J Am Acad Dermatol 2019; 80: 106–113 e2.
19. Brantsch KD, Meisner C, Schonfisch B, Trilling B, Wehner-Caroli J, Rocken M, et al. Analysis of risk factors determining prognosis of cutaneous squamous-cell carcinoma: a prospective study. Lancet Oncol 2008; 9: 713–720.
20. Jambusaria-Pahlajani A, Miller CJ, Quon H, Smith N, Klein RQ, Schmults CD. Surgical monotherapy versus surgery plus adjuvant radiotherapy in high-risk cutaneous squamous cell carcinoma: a systematic review of outcomes. Dermatol Surg 2009; 35: 574–585.
21. Fox M, Brown M, Golda N, Goldberg D, Miller C, Pugliano-Mauro M, et al. Nodal staging of high-risk cutaneous squamous cell carcinoma. J Am Acad Dermatol 2019; 81: 548–557.
22. de Bondt RB, Nelemans PJ, Hofman PA, Casselman JW, Kremer B, van Engelshoven JM, et al. Detection of lymph node metastases in head and neck cancer: a meta-analysis comparing US, USgFNAC, CT and MR imaging. Eur J Radiol 2007; 64: 266–272.
23. Land R, Herod J, Moskovic E, King M, Sohaib SA, Trott P, et al. Routine computerized tomography scanning, groin ultrasound with or without fine needle aspiration cytology in the surgical management of primary squamous cell carcinoma of the vulva. Int J Gynecol Cancer 2006; 16: 312–317.
24. Ruiz ES, Karia PS, Morgan FC, Schmults CD. The positive impact of radiologic imaging on high-stage cutaneous squamous cell carcinoma management. J Am Acad Dermatol 2017; 76: 217–225.
25. Stratigos AJ, Garbe C, Dessinioti C, Lebbe C, Bataille V, Bastholt L, et al. European interdisciplinary guideline on invasive squamous cell carcinoma of the skin: Part 2. Treatment. Eur J Cancer 2020 Feb 26. pii: S0959-8049(20)30019-8.
26. Chren MM, Linos E, Torres JS, Stuart SE, Parvataneni R, Boscardin WJ. Tumor recurrence 5 years after treatment of cutaneous basal cell carcinoma and squamous cell carcinoma. J Invest Dermatol 2013; 133: 1188–1196.
27. Schmitt AR, Brewer JD, Bordeaux JS, Baum CL. Staging for cutaneous squamous cell carcinoma as a predictor of sentinel lymph node biopsy results: meta-analysis of American Joint Committee on Cancer criteria and a proposed alternative system. JAMA Dermatol 2014; 150: 19–24.
28. Jansen P, Petri M, Merz SF, Brinker TJ, Schadendorf D, Stang A, et al. The prognostic value of sentinel lymph nodes on distant metastasis-free survival in patients with high-risk squamous cell carcinoma. Eur J Cancer 2019; 111: 107–115.
29. Cuperus E, Leguit R, Albregts M, Toonstra J. Post radiation skin tumors: basal cell carcinomas, squamous cell carcinomas and angiosarcomas. A review of this late effect of radiotherapy. Eur J Dermatol 2013; 23: 749–757.
30. Harris BN, Pipkorn P, Nguyen KNB, Jackson RS, Rao S, Moore MG, et al. Association of adjuvant radiation therapy with survival in patients with advanced cutaneous squamous cell carcinoma of the head and neck. JAMA Otolaryngol Head Neck Surg 2019; 145: 153–158.
31. Brewster AM, Lee JJ, Clayman GL, Clifford JL, Reyes MJ, Zhou X, et al. Randomized trial of adjuvant 13-cis-retinoic acid and interferon alfa for patients with aggressive skin squamous cell carcinoma. J Clin Oncol 2007; 25: 1974–1978.
32. O'Bryan K, Sherman W, Niedt GW, Taback B, Manolidis S, Wang A, et al. An evolving paradigm for the workup and management of high-risk cutaneous squamous cell carcinoma. J Am Acad Dermatol 2013; 69: 595–602 e1.
33. Lewis CM, Glisson BS, Feng L, Wan F, Tang X, Wistuba, II, et al. A phase II study of gefitinib for aggressive cutaneous squamous cell carcinoma of the head and neck. Clin Cancer Res 2012; 18: 1435–1446.
34. Jenni D, Karpova MB, Muhleisen B, Mangana J, Dreier J, Hafner J, et al. A prospective clinical trial to assess lapatinib effects on cutaneous squamous cell carcinoma and actinic keratosis. ESMO Open 2016; 1: e000003.
35. Gross N, Ferrarotto R, Nagarajan P, Bell D, El-Naggar A, Johnson JM, et al. Phase II study of neoadjuvant cemiplimab prior to surgery in patients with stage III/IV (M0) cutaneous squamous cell carcinoma of the head and neck (cSCC-HN). Presented at the European Society for Medical Oncology meeting, Barcelona, September 28–30, 2019.
36. Veness MJ, Morgan GJ, Palme CE, Gebski V. Surgery and adjuvant radiotherapy in patients with cutaneous head and neck squamous cell carcinoma metastatic to lymph nodes: combined treatment should be considered best practice. Laryngoscope 2005; 115: 870–875.
37. Ch'ng S, Maitra A, Allison RS, Chaplin JM, Gregor RT, Lea R, et al. Parotid and cervical nodal status predict prognosis for patients with head and neck metastatic cutaneous squamous cell carcinoma. J Surg Oncol 2008; 98: 101–105.
38. Hillen U, Leiter U, Haase S, Kaufmann R, Becker J, Gutzmer R, et al. Advanced cutaneous squamous cell carcinoma: a retrospective analysis of patient profiles and treatment patterns – results of a non-interventional study of the DeCOG. Eur J Cancer 2018; 96: 34–43.
39. Moloney FJ, Comber H, O'Lorcain P, O'Kelly P, Conlon PJ, Murphy GM. A population-based study of skin cancer incidence and prevalence in renal transplant recipients. Br J Dermatol 2006; 154: 498–504.
40. Kambayashi Y, Fujimura T, Aiba S. Comparison of immunosuppressive and immunomodulatory cells in keratoacanthoma and cutaneous squamous cell carcinoma. Acta Derm Venereol 2013; 93: 663–668.
41. Lippman SM, Parkinson DR, Itri LM, Weber RS, Schantz SP, Ota DM, et al. 13-cis-retinoic acid and interferon alpha-2a: effective combination therapy for advanced squamous cell carcinoma of the skin. J Natl Cancer Inst 1992; 84: 235–241.
42. Slater NA, Googe PB. PD-L1 expression in cutaneous squamous cell carcinoma correlates with risk of metastasis. J Cutan Pathol 2016; 43: 663–670.
43. Pickering CR, Zhou JH, Lee JJ, Drummond JA, Peng SA, Saade RE, et al. Mutational landscape of aggressive cutaneous squamous cell carcinoma. Clin Cancer Res 2014; 20: 6582–6592.
44. Migden MR, Rischin D, Schmults CD, Guminski A, Hauschild A, Lewis KD, et al. PD-1 blockade with cemiplimab in advanced cutaneous squamous-cell carcinoma. N Engl J Med 2018; 379: 341–351.
45. Grob JJ, Gonzales Mendoza R, Basset Seguin N, Vornicova O, Schachter J, Joshi A, et al. Pembrolizumab for recurrent/metastatic cutaneous squamous cell carcinoma (cSCC):

efficacy and safety results from the Phase 2 KEYNOTE-629 study. Presented at the European Society for Medical Oncology meeting, Barcelona, September 28–30, 2019.
46. Maubec E, Boubaya M, Petrow P, Basset-Seguin N, Grob JJ, Dreno B, et al. Pembrolizumab as first-line therapy in patients with unresectable cutaneous squamous cell carcinoma: Phase 2 results from CARSKIN. Presented at the American Society of Clinical Onclology meeting, Chicago, May 31 – June 4, 2019.
47. Sadek H, Azli N, Wendling JL, Cvitkovic E, Rahal M, Mamelle G, et al. Treatment of advanced squamous cell carcinoma of the skin with cisplatin, 5-fluorouracil, and bleomycin. Cancer 1990; 66: 1692–1696.
48. Guthrie TH, Jr, Porubsky ES, Luxenberg MN, Shah KJ, Wurtz KL, Watson PR. Cisplatin-based chemotherapy in advanced basal and squamous cell carcinomas of the skin: results in 28 patients including 13 patients receiving multimodality therapy. J Clin Oncol 1990; 8: 342–346.
49. Khansur T, Kennedy A. Cisplatin and 5-fluorouracil for advanced locoregional and metastatic squamous cell carcinoma of the skin. Cancer 1991; 67: 2030–2032.
50. Huis In 't Veld EA, Grunhagen DJ, Deroose JP, Nijsten TEC, Wouters M, Verhoef C, et al. Isolated limb perfusion for unresectable extremity cutaneous squamous cell carcinoma; an effective limb saving strategy. Br J Cancer 2018; 119: 429–434.
51. Maubec E, Petrow P, Scheer-Senyarich I, Duvillard P, Lacroix L, Gelly J, et al. Phase II study of cetuximab as first-line single-drug therapy in patients with unresectable squamous cell carcinoma of the skin. J Clin Oncol 2011; 29: 3419–3426.
52. Dereure O, Missan H, Girard C, Costes V, Guillot B. Efficacy and tolerance of cetuximab alone or combined with chemotherapy in locally advanced or metastatic cutaneous squamous cell carcinoma: an open study of 14 patients. Dermatology 2016; 232: 721–730.
53. Necchi A, Giannatempo P, Lo Vullo S, Raggi D, Nicolai N, Colecchia M, et al. Panitumumab treatment for advanced penile squamous cell carcinoma when surgery and chemotherapy have failed. Clin Genitourin Cancer 2016; 14: 231–236.
54. Foote MC, McGrath M, Guminski A, Hughes BG, Meakin J, Thomson D, et al. Phase II study of single-agent panitumumab in patients with incurable cutaneous squamous cell carcinoma. Ann Oncol 2014; 25: 2047–2052.
55. William WN, Jr, Feng L, Ferrarotto R, Ginsberg L, Kies M, Lippman S, et al. Gefitinib for patients with incurable cutaneous squamous cell carcinoma: a single-arm phase II clinical trial. J Am Acad Dermatol 2017; 77: 1110–1113 e2.
56. Gold KA, Kies MS, William WN, Jr, Johnson FM, Lee JJ, Glisson BS. Erlotinib in the treatment of recurrent or metastatic cutaneous squamous cell carcinoma: a single-arm phase 2 clinical trial. Cancer 2018; 124: 2169–2173.
57. Necchi A, Lo Vullo S, Perrone F, Raggi D, Giannatempo P, Calareso G, et al. First-line therapy with dacomitinib, an orally available pan-HER tyrosine kinase inhibitor, for locally advanced or metastatic penile squamous cell carcinoma: results of an open-label, single-arm, single-centre, phase 2 study. BJU Int 2018; 121: 348–356.
58. Cavalieri S, Perrone F, Miceli R, Ascierto PA, Locati LD, Bergamini C, et al. Efficacy and safety of single-agent pan-human epidermal growth factor receptor (HER) inhibitor dacomitinib in locally advanced unresectable or metastatic skin squamous cell cancer. Eur J Cancer 2018; 97: 7–15.
59. Lu SM, Lien WW. Concurrent radiotherapy with cetuximab or platinum-based chemotherapy for locally advanced cutaneous squamous cell carcinoma of the head and neck. Am J Clin Oncol 2018; 41: 95–99.
60. Jansen MHE, Kessels J, Nelemans PJ, Kouloubis N, Arits A, van Pelt HPA, et al. Randomized trial of four treatment approaches for actinic keratosis. N Engl J Med 2019; 380: 935–946.
61. Chen AC, Martin AJ, Dalziell RA, McKenzie CA, Lowe PM, Eris JM, et al. A phase II randomized controlled trial of nicotinamide for skin cancer chemoprevention in renal transplant recipients. Br J Dermatol 2016; 175: 1073–1075.
62. Lindelöf B, Jarnvik J, Ternesten-Bratel A, Granath F, Hedblad MA. Mortality and clinicopathological features of cutaneous squamous cell carcinoma in organ transplant recipients: a study of the Swedish cohort. Acta Derm Venereol 2006; 86: 219–222.
63. Genders RE, Osinga JAJ, Tromp EE, O'Rourke P, Bouwes Bavinck JN, Plasmeijer EI. Metastasis risk of cutaneous squamous cell carcinoma in organ transplant recipients and immunocompetent patients. Acta Derm Venereol 2018; 98: 551–555.
64. Dantal J, Morelon E, Rostaing L, Goffin E, Brocard A, Tromme I, et al. Sirolimus for secondary prevention of skin cancer in kidney transplant recipients: 5-year results. J Clin Oncol 2018; 36: 2612–2620.
65. Aguirre LE, Guzman ME, Lopes G, Hurley J. Immune checkpoint inhibitors and the risk of allograft rejection: a comprehensive analysis on an emerging issue. Oncologist 2019; 24: 394–401.
66. Leard LE, Cho BK, Jones KD, Hays SR, Tope WD, Golden JA, et al. Fatal diffuse alveolar damage in two lung transplant patients treated with cetuximab. J Heart Lung Transplant 2007; 26: 1340–1344.