Melanoma subtypes: genomic profiles, prognostic molecular markers and therapeutic possibilities

Roy Rabbie1,2, Peter Ferguson3,4, Christian Molina-Aguilar5, David J Adams1* and Carla D Robles-Espinoza1,5

1 Experimental Cancer Genetics, The Wellcome Sanger Institute, Hinxton, UK
2 Cambridge Cancer Centre, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK
3 Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Sydney, Australia
4 Melanoma Institute Australia, The University of Sydney, Sydney, Australia
5 Laboratorio Internacional de Investigación sobre el Genoma Humano, Universidad Nacional Autónoma de México, Santiago de Querétaro, Mexico

*Correspondence to: David Adams, Experimental Cancer Genetics, Wellcome Sanger Institute, Hinxton, Cambridge CB10 1HH, UK. E-mail: da1@sanger.ac.uk

Abstract
Melanoma is characterised by its ability to metastasise at early stages of tumour development. Current clinico-pathologic staging based on the American Joint Committee on Cancer criteria is used to guide surveillance and management in early-stage disease, but its ability to predict clinical outcome has limitations. Herein we review the genomics of melanoma subtypes including cutaneous, acral, uveal and mucosal, with a focus on the prognostic and predictive significance of key molecular aberrations.

Keywords: melanoma; cutaneous; desmoplastic; acral; uveal; mucosal; mutations; driver genes; biomarkers; prognostic; predictive; 31-gene expression profile

Introduction
Historically, melanoma has been classified into subtypes based on the tissue from which the primary tumour arises. The major such subtypes are cutaneous melanoma (CM), which arises in non-glaborous skin; acral melanoma (AM), a distinct form that originates in glaborous skin of the palms, soles and nail beds; mucosal melanoma (MM), the rarest subtype, which arises from melanocytes in the mucosal lining of internal tissues; and uveal melanoma (UM) which develops from melanocytes in the uveal tract of the eye (Figure 1). These subtypes have well recognised epidemiological, clinical and histopathological characteristics, and recent studies have described the molecular alterations that underpin some of these attributes. Site of origin seems to correlate best with tumoural somatic profile, with melanomas arising from chronically sun damaged (CSD) sites having a higher mutational burden than tumours arising from non-CSD sites [1] – a direct consequence of the UV-induced C>T transitions at dipyrimidines that dominate the majority of CM genomes [2–4].

Based solely on the occurrence of driver mutations, melanomas have further been classified into four genomic subtypes: BRAF-mutant, NRAS-mutant, NFI-loss and triple wild-type (TWT) [3,4]. These subtypes do not have distinguishing histopathological features or sites of origin, although there are notable trends; for example, nearly all UMs and the majority of AMs and MMs fall into the TWT category [3]. The BRAF, NRAS and NFI driver alterations all activate the mitogen-activated protein kinase (MAPK) pathway and generally occur at the earlier stages of tumour evolution [5]. In CM, it has been proposed that subsequent mutations occur in the TERT promoter and in regulators of the cell cycle such as CDKN2A, which precede mutations in chromatin remodelers such as members of the SWI/SNF complex and TP53, the latter being associated with more advanced stages of primary tumour progression [5]. Whether some cells are inherently able to metastasise or whether further genomic alterations are necessary to gain this ability, remains under active investigation [6,7].

Herein, we describe how melanoma subtypes are shaped by their genomic profiles, and outline our current understanding of prognostic and predictive molecular markers.
Melanoma subtypes

CM: dominated by ultraviolet-induced mutations

The genomic landscape of CM

CM generally affects people of European descent and is the commonest reported melanoma subtype. Consequently, the majority of genomic and transcriptomic studies have been performed on CM cases. CM has the highest burden of somatic mutations across the major cancer subtypes, with a mutational landscape that is dominated by the UV mutational signature, primarily C>T transitions as described earlier [2–4]. About 45–50% of CM are \textit{BRAF}-mutant (principally...
Recent studies have found great heterogeneity in melanoma gene expression profiles, with some studies suggesting that BRAF-mutant tumours confer a poorer prognosis relative to BRAF wild-type melanoma. In particular, BRAF-mutated melanoma has been linked to a shorter overall survival in patients with stage IV disease when compared to those with BRAF WT disease [9,23]. While the majority of studies investigating the relationship between BRAF mutations and clinical outcomes are focused on patients with metastatic disease, recent studies have demonstrated that BRAF-mutant melanomas are associated with a shorter disease-free survival and melanoma-specific survival in patients with early-stage disease [24,25]. Historically, NRAS-mutant disease has been associated with thicker primary lesions and higher mitotic activity [12]. However, there have been conflicting reports on its prognostic significance. In particular, no impact on survival was seen when NRAS mutations were measured in primary disease [26,27], however when measured from metastases, NRAS mutations were associated with improved survival compared to tumours with BRAF mutations or TWT tumours [28,29]. Despite the undisputed prognostic relevance of American Joint Committee on Cancer (AJCC) classification and certain driver mutations, our ability to predict those early-stage patients at highest metastatic risk remains conspicuously limited.

Gene expression profiles and their prognostic implication

A gene expression profile (31-GEP) test has been proposed that evaluates the expression of 31 gene targets in the primary tumour, providing a binary classification of ‘low risk’ (Class 1) or ‘high risk’ (Class 2) of metastases within 5 years of diagnosis [30]. The test assesses the expression of three control genes, four genes with proven prognostic utility for UMs [31] and 24 genes previously reported to be differentially expressed in metastatic compared to primary tumours [32–38]. The performance of this test has been evaluated in several retrospective [30,39,40] as well as prospective validation studies [41,42] and has been shown to enhance current prognostic accuracy in particular through identifying clinically and pathologically sentinel lymph node (SLN)-negative patients with high-risk of metastases. However, although there is great promise in reproducibility and clinical validity, the clinical utility for the 31-GEP test on clinical decision-making is still incompletely defined, and will require evidence from further large-scale prospective multi-institutional registry studies before it can be considered for inclusion in any national or professional association guideline recommendations.

By undertaking unsupervised hierarchical clustering of gene expression profiles, Jönnsson and collaborators were able to categorise melanomas into four biologically relevant subgroups; MITF-low/proliferative, high-immune response, MITF-high/pigmentation and normal-like [19]. Importantly, the MITF-low/proliferative subtype, characterised by an absence of the expression of immune-response genes, had only BRAF/NRAS-mutated samples and more
Table 1. Overview of genomic profile of melanoma subtypes

| Biological pathways | Genes | CM | DM-subtype | AM | UM | MM |
|---------------------|-------|----|------------|----|----|----|
| MAPK genomic subtypes | ~Total %mut* | ~90-95% | ~73% | ~50-60% | ~100% | ~50-60% |
| | **BRAF** | ~45-50% | ~0-5% [13,43] | ~10-35% | rarely seen | ~0-21% |
| | | [3,4] | [4,4-47] | [48,49] | [3,4,45,51] | |
| | **RAS (mainly NRAS)** | ~30% [3,4] | ~0-6% [13,43] | ~8-22% | rarely seen | ~5-25% |
| | | [3,4,47] | [48,49] | [3,45,51] | |
| | **NFI** | ~10-15% [3,4] | ~52-93% [13,43] | ~11-23% | rarely seen | ~0-18% [3,51] |
| | | [3,4,47] | [48,49] | | |
| | **TWT** | ~5-10% [3,4] | ~7-48% [13,43] | ~45-58% | ~100% [48,49] | ~65-75% |
| | | [3,4,47] | [48,49] | | |
| | **KIT (mut or gain)** | ~1.5-2.1% [3,55] | rarely seen | ~3-36% [44,46,47,52] | ~11% [53] | ~7-25% |
| | | [3,4,47] | [48,49,65,7] | | |
| | **GNAQ** | rarely seen | rarely seen | rarely seen | ~4-49% [48,49] [56] | ~1% [51] |
| | | [3,4,47] | [48,49] | | |
| | **MAP2K1 & 2** | ~4% [3] | ~7% [13] | ~8% [3] | ~9% [48] | ~0-11% [3,50] |
| Cell Cycle | ~Total %mut* | ~57% [3] | ~70-75% | ~90% | ~85% | ~36-75% |
| | **CDKN2A (mut)** | ~13-40% [3,4] | ~20-29% [13,58] | ~0-3% [3,44] | rarely seen, methylated in | rarely seen |
| | | [3,4,47] | [48,59] | | | [3,50,51,54] |
| | **CDKN2A (loss)** | ~45% [3] | ~18% [13] | ~35% [44] | rarely seen | ~12% [48] | ~10-38% |
| | | [3,4,47] | [48,59] | | | [3,50] |
| | **CDK4 (mut or gain)** | ~5-6% [3,4] | ~5% [13] | ~9% [3,44] | rarely seen | ~3% [48] | ~5-25% [3,50] |
| | | [3,4,47] | [48,59] | | | |
| | **RBT** | ~4-15% [3,4] | ~15-18% [3,4] | ~9-17% [3,44] | ~3% [48] | ~0-21% [3,50] |
| | | [13,43,58] | [48,59] | | | |
| | **TP53** | rarely seen | rarely seen | rarely seen | ~70-83% (but the great majority of metastatic UM) [48,49] | ~25% [3] | rarely seen |
| | | [3,4,47] | [48,59] | | | [3,50,51,54] |
| | **CCND1** rarely seen | ~2% [13] | rarely seen | ~6-54% [3,44] | ~6% [48] | ~25% [3,50,51,54] |
| | | [3,4,47] | [48,59] | | | |
| | **BAP1 (mut or loss)** | ~13-13% [3,4] | rarely seen [3] | ~7-43% [3,44] | ~6-54% [3,44] | ~6-54% [3,44] | ~6-54% [3,44] |
| | | [3,4,47] | [48,59] | | | |
| PI3K/AKT | **PTEN (mut or loss)** | ~8.5-40% [3,4] | rarely seen [13] | ~26-28% [3,44] | ~6-11% up to 76% with LOH [48,60] | 4-25% [3,50,51,54] |
| Number of mutations | **** | **** | ** | | | ** |
| Chromosomal aberrations | - | - | - | | | - |
| Transcription factors | **NFkBIE** promoter | ~5% [3] | ~15-33% [3,13] | not seen [3] | NA | rarely seen [3] |
| | | [3,18] | [3,4,47] | | | |
| | **MITF** | ~10-20% [3,18] | rarely seen | ~15% [3] | ~63% samples are reported to include deletions or amplifications in MITF [48] | ~5-25% [3,51] |
| | | [3,4,47] | [48,59] | | | |
| Telomerase pathway | **TERT (mut or gain)** | ~85% [3] | ~85% [13] | ~9-45% [3,4,47] | ~2-9% [48,61] | ~5-13% [3,50,51] |

*Estimates based on the literature, and on the genes listed on the table including mutations and copy number aberrations.

*Represents the mutational load.

© 2018 The Authors. The Journal of Pathology published by John Wiley & Sons Ltd on behalf of Pathological Society of Great Britain and Ireland. www.pathsoc.org

J Pathol 2019; 247: 539–551
www.thejournalofpathology.com
tumours with CDKN2A deletions, and was significantly associated with a poorer prognosis. Classification of primary melanomas by gene expression also resulted in these four classes, which could be collapsed into two classes associated with clinical outcome [62]. The multi-institutional TCGA (The Cancer Genome Atlas) initiative subsequently identified three transcriptomic subclasses, an immune group, a keratin group, and a MITF-low group [4,63]. A subsequent analysis showed that these classifications comprised very similar biological entities (TCGA immune ~ Lund high-immune, TCGA keratin~Lund normal-like and MITF-high/pigmentation, TCGA MITF-low ~ Lund MITF-low/proliferative) [63].

Molecular markers of response/resistance to targeted therapy and immune-checkpoint inhibition

The development of approved targeted therapies for patients with metastatic and early-stage melanomas has been remarkable and driven by significant discoveries around the molecular mechanisms of melanoma-genesis. Combined treatment with BRAF and MEK inhibitors achieves radiological responses in ~70% of patients with BRAFV600 mutations [68]. A proportion of patients are intrinsically resistant to BRAF inhibitors, and most patients who initially respond will eventually exhibit resistance. The need to maximise the long-term clinical benefit of this strategy remains a key challenge and molecular profiling may play a particularly important role in deciphering the mechanisms of response and resistance to targeted therapy.

One of the most frequently reported mutations leading to intrinsic BRAF resistance is loss of the phosphatase and tensin homolog (PTEN) gene. Decreased responses to BRAF inhibition in patients with PTEN loss is thought to be attributed to constitutive activation of the PI3K/AKT pathway which leads to cell proliferation and survival [69]. MITF has also been shown to be an important regulator of response, and high MITF levels allow melanoma cells to evade cell death triggered by BRAF and MEK inhibitors [70,71]. Intriguingly, it has been shown that very low levels of MITF when co-existing with high levels of receptor tyrosine kinase AXL (MITFlow/AXLhigh phenotype) represent a de-differentiated cellular state that displays innate resistance to BRAF inhibitors and increased invasiveness [20]. A number of other mechanisms of intrinsic resistance have been suggested, reviewed in [72]. The most common mechanism of acquired resistance is via reactivation of the MAPK/ERK pathway [72]. Recently, studies have reported non-mutational mechanisms

Figure 2. Molecular representation of the mutations associated with the RAS/RAF/MEK/ERK pathways in melanoma, including the MITF signalling cascade. GPCR, G-protein coupled receptor; RTK, receptor tyrosine kinase. *KIT amplifications are seen in ~10% of CMs, ~9.5% of AMs, ~15% MMs [64]. †Cyclin D1 is also amplified in ~18% of CMs [65]. ‡MDM2 is also amplified in ~6% of CMs [66]. Adapted from [67].
for the acquisition of resistance through phenotype switching [21,73,74].

Immune checkpoint inhibitor (ICI) therapy has revolutionised melanoma therapy and resulted in unprecedented rates of long-term disease control and survival in patients with metastatic disease. It is hypothesised that the mutational status of a cancer influences anti-tumour immune and ICI responses, presumably by virtue of enhanced neoantigen formation due to increased number of non-synonymous single-nucleotide variants [75,76]. This phenomenon probably reflects an increased likelihood of forming neoantigens that will elicit T-cell reactivity. Consistent with this notion, tumours with microsatellite instability resulting from acquired deficiency of DNA mismatch repair are also associated with enhanced response to PD-1 blockade [77,78]. This has formed the basis for the first site-agnostic drug approval made by the FDA, for anti-PD1 therapy [79,80]. Studies ex vivo strongly support the dominance of mutational neoantigens as the targets for lymphocyte recognition of a tumour, and neoantigen expression and HLA binding characteristics have been shown to be surrogates for treatment response [75,76,81,82]. In keeping with this, mutational and neoantigen load have also recently been linked with clinical benefit from adoptive T cell immunotherapy [83]. Further evidence suggests that clonal neoantigens may be particularly relevant [84]. While genomic instability may feasibly provide sufficient genomic variation to promote an effective immune response, the mechanism relating DNA damage and genomic instability to ICI response is not fully understood and mutational load does not sufficiently explain all cases [85]. Significant genomic heterogeneity between tumours can contribute to heterogenous clinical responses and this may account for some of the conflicting results seen in separate cohorts [86,87].

Immune activation gene-expression signatures have been shown to define distinct CM subtypes [19] and the prevalence of pre-existing tumour infiltrating T cells has been shown to correlate with clinical response to anti-PD1 immunotherapy [88]. Although previous reports have suggested that the expression of cytolytic markers might correlate with response to anti-CTLA4 [76], these are based on small retrospective analyses and there has yet to be any specific gene expression signature that has been independently validated in this context. It is increasingly appreciated that the relationship of the tumour’s mutational profile to immune dynamics is moderated by additional factors that affect expression, processing and immunogenicity of putative neoantigens. Accordingly, predictive approaches are now being paired with additional filters as well as expression data to evaluate somatic mutations which are adequately expressed and processed.

Desmoplastic melanoma (DM): a CM subtype with an elevated mutational load

DM is a variant of CM, consisting of intradermal proliferations of spindled melanocytes, commonly associated with lymphoid aggregates, and typically found on chronically sun-damaged skin of older individuals (Figure 1). The term DM initially referred to the association of invasive tumour cells with abundant stromal collagen, and therefore DM can be classified as pure and mixed, based on the degree of desmoplasia [89]. Pure DMs have less frequent lymph node involvement and tend to display a less aggressive clinical course than mixed DM. DMs rank among the most heavily mutated types of cancer, with a mutation rate on average four-fold higher than CMs, of which the great majority are attributed to UV mutagenesis [13]. DMs also tend to have lower DNA copy number alterations than other melanoma subtypes; the few focal deletions that have been observed target CDKN2A and NF1, whereas amplifications affect EGFR, CDK4, MDM2, TERT, MAP3K1, MET, YAP1 and NFkBIE [13]. The promoter of NFkBIE has been identified as a recurrently mutated locus in 15–33% of samples [3,13]. This gene, coding for IkBε, inhibits downstream nuclear factor kappa B (NFκB) signalling by sequestering NFκB transcription factors in the cytoplasm (Figure 2) [13]. Although also mutated in CM, promoter mutations are enriched in DM [3]. No melanoma hotspot mutations in BRAF or NRAS have been identified in studies focusing on DM [13,90,91]; the MAPK pathway seems instead to be activated by other mutations [13] (Table 1). Indeed, possible oncogenic MAPK mutations in this subtype of melanoma include alterations detected in NF1, CBL, ERBB2, MAP2K1 and MAP3K1, as well as mutations that are hotspot in other types of cancers such as BRAF G469E, G466E and D594N and NRAS Q61H [13].

Following the recognition that somatic non-synonymous mutational load might be associated with improved immune checkpoint responses, Eroglu and colleagues hypothesised that patients with DM may respond well to ICI therapies [92]. In a retrospective analysis of pathology reports from 1058 patients with advanced melanoma treated with anti–PD-1 or anti–PD-L1 antibodies, Eroglu et al identified 60 patients with advanced DM, who overall had a high response rate to PD-1 blockade. Whole-exome sequencing data from 17 patients revealed driver NF1 mutations in 14/17 samples (82.4%) and enrichment of loss-of-function mutations in TP53 and ARID2. However, these mutations were not associated with response to PD-1 blockade. These findings suggest that, despite the dense fibrous stroma that had been expected to limit immune infiltration, PD-1/PD-L1 blockade may be effective in patients with DM, supporting further clinical investigation of immune checkpoint blockade in these patients.

Additional rarer categories of CMs that have distinctive histopathological and molecular features include spitzoid melanoma [93], melanoma arising from giant
congenital naevus [94] and melanoma in childhood [95], not reviewed herein.

AM: numerous copy number changes and low point mutation burden

AM is a rarer histological variant arising on the palms, soles and nail beds and accounts for a greater proportion of melanomas in patients of African, Asian and Latin American descent [96–99]. When compared to CM tumours, AMs have a much lower single nucleotide mutational burden yet display a higher number of somatic structural aberrations [3,44]. The few AM samples sequenced to date demonstrate a lower contribution of the UV signature [3,44]. A handful of cases from subungual sites, however, do demonstrate a significant proportion of UV-associated mutations, which might suggest that skin in these locations might not be completely protected from sun-induced UV-radiation [100].

A large proportion of AMs fall into the TWT subtype, with only 42–55% of tumours having mutations in BRAF, NRAS or NF1 [3,44] (Table 1). KIT mutation and amplifications are also AM drivers, with between 3 and 36% of tumours bearing these alterations [44,52]. A fraction of AMs also carry activating mutations in the promoter of TERT (between 9 and 41% of patients depending on the study [44,46]) and TERT gene amplifications are currently the only recognised adverse molecular prognostic indicators [101]. TERT inhibition has been shown to be cytotoxic for AM cells in vitro [44] and, following both in vivo and in vitro evidence, TERT inhibitors are currently being proposed for clinical use [102]. Interestingly, although TERT deregulation in UV-exposed melanomas is caused by point mutations, about 45% of AMs have TERT copy number gains [3]. TERT copy number may predict the outcome of high-dose (HD)-IFNα-2b treatment in AM [103].

Genes frequently targeted by amplifications are KIT, TERT, PAK1, CDK4 and CCND1, and genes recurrently deleted include CDKN2A, PTEN and NF1 [44,104] (Table 1). A study of 514 primary AM samples showed that the overall frequency of at least one aberration in CDK4, CCND1 or P16INK4a was 82.7%. In this study, AM cell lines and patient-derived xenografts containing cyclin dependent kinase 4 (CDK4) pathway aberrations were sensitive to CDK4/6 inhibitors [105] and clinical studies are anticipated (NCT034534919). There are other, infrequently altered genes identified by AM sequencing studies; for example, mutations of MAP2K2 and loss of ARID2 [44]. Another subset of AMs show, like CMs, MITF amplifications [3]. Interestingly, very few point mutations have been described in TP53, PTEN, RAC1 or RB1, with these genes instead being targeted via amplifications or deletions.

Although AMs harbouring BRAF or KIT mutations may respond to the appropriate inhibitors, the majority of patients do not currently have any genotype-specific treatment options. In light of the lower somatic mutation burden, it might be thought that the efficacy of ICIs may be lower in this subtype [106]. However, small retrospective series have so far demonstrated that response rates are comparable to those in CM [107,108]. It remains to be seen whether the association of mutational and neoantigen load to ICI response is also observed in this subtype.

UM: a sparsely mutated melanoma subtype with poor responses to modern systemic therapies

The genomic landscape of UM

UM has one of the lowest observed mutational densities across all tumours, estimated to be about 1.1 somatic mutations per Mb [4]. Indeed, the burden of coding somatic mutations is comparable to that of paediatric cancers such as medulloblastoma and neuroblastoma [48]. Nonetheless, a small number of recurrent somatic mutations have been observed. Activating mutations in the guanine-nucleotide proteins GNAQ and GNA11 occur in the great majority of tumours (a combined frequency of ~85–92.5%), and in CYSLT2 (4%) and PLCB4 (2.5%), all in a mutually exclusive manner [4,56], as these may all activate the MAPK pathway [56,109] (Figure 2, Table 1). Other significantly mutated genes in UM are BAP1, EIF1AX and SF3B1, which also form a second mutually-exclusive subgroup [56] (Table 2). In addition to providing an insight into key molecular signalling and progression pathways, UM driver genes also associate with molecular subclasses and bear important prognostic implications (Table 2). Different studies have found a number of mutational signatures in these tumours, most notably one associated with ageing and explained by spontaneous deamination of 5-methylcytosine, and others related to defects in nucleotide excision and in DNA mismatch repair [4,48]. There are currently no known drugs that target GNAQ/GNA11 and alternative pathway inhibitors have so far not shown clinical benefit in early phase trials, reviewed in [110]. A number of trials are ongoing, targeting a range of UM signalling cascades [111]. The clinical responses to ICIs have similarly been disappointing [112].

Chromosomal copy number gains and losses (copy number alterations [CNAs]) are more common in UM, and the largest genomic studies have focused on these aberrations. Unsupervised hierarchical clustering of UM genomes based on CNAs reveals two main chromosomal subsets [4,48,113]. Aberrations in chromosome 3 are the main distinguishing feature between the two main subsets and TCGA refers to these subsets as Disomy 3 (D3) and Monosomy 3 (M3) [49]. It has long been recognised that M3 is associated with poor prognosis and high metastatic risk, while tumours with D3 correlate with good prognosis and rarely lead to disseminated disease [114]. The metastatic rate for tumours with M3 ranges from 0 to 48% [115], and the M3 genotype has been shown to be superior to clinicopathologic factors as a prognostic indicator [114]. Gain of the long arm of chromosome 8q is also associated with poor prognosis [116]. The M3 cluster is characterised by aberrations in BAP1,
as well as 8q gain, but the extent and type of this chromosomal gain varies between the two sub-clusters. The gain of 8q, where MYC is located, does not contain this oncogene and MYC transcript levels do not correlate with 8q status [4,48], so perhaps this gain is targeting another genomic region. The D3 cluster further subdivides into two subsets, one characterised by a high level of chromosome 6p (short-arm) and somatic mutations in **EIF1AX**, and the second with gains of chromosomes 6p and 8q (long-arm) and somatic mutations in **SF3B1**. The prevalence of observed alterations, it has been proposed that mutations in **GNAQ, GNA11, CYSLTR2**, or **PLCB4** represent an early event, followed by loss of chromosome 3 and mutation of **BAP1** in the case of M3, and by mutation of **EIF1AX** or **SF3B1** in the case of D3 [48]. The TCGA study also clustered samples using transcriptional and methylation profiles, which largely aligned with the original CNA clusters [4].

**Gene expression profiling in UM**

UMs can also be stratified according to the GEP classification described earlier, and into the same prognostically relevant molecular classes [33] and this has become the standard of care for molecular testing in a number of oncology centres [124]. Recently, Class 1 tumours have been subdivided into two subgroups, **Class 1A** (2% of patients 5-year metastatic risk) and **Class 1B** (21% of patients 5-year metastatic risk) [125], based on the differential expression of **CDH1** and **RAB31**. Class 1A tumours are also associated with D3 and **EIF1AX** mutations. Class 2 UM tumours exhibit a dedifferentiated stem-cell-like and epithelioid phenotype that is associated with M3 and **BAP1** mutations and confers a high metastatic risk [118,125]. They can be subclustered into Class 2A and 2B, where Class 2B cases harbour a loss of chromosome 8p that makes them even more aggressive with an earlier onset of metastases relative to Class 2A [126]. Unlike CM, multiple groups have shown that the prognostic accuracy of GEP outperforms clinicopathologic features and chromosomal gains and losses in predicting metastases [127–129].

**MM: a rare and aggressive subtype**

MMs are rare and have a particularly aggressive clinical course [130,131]. Similar to AM, MM is characterised by a higher number of chromosomal structural aberrations and a lower mutational burden than CM [3,54]. Mutations in **BRAF, NRAS** or **NF1** in MM are less prevalent than in CM, with loss of **PTEN** (4–25% of samples [3,54]) mutation or amplification of **KIT** (7–25% of MM samples [3,54,132]) and **CCND1** or **CDK4** (104) being more common (Table 1). In fact, Hayward and colleagues identified a previously unappreciated set of driver genes shared between UM and MM, with two-thirds of TWT MM showing activating mutations in **GNAQ** and **SF3B1**. Additionally, some studies [52] suggest that losses of **CDKN2A** are more common in AM and MM than in CM, though estimates vary [3,44,50].

Targeted therapies against mutation of **KIT** have failed to show convincing therapeutic efficacy in MMs [133]. The immune checkpoint blocking antibodies have shown variable efficacy in phase II and retrospective studies [134,135].

**Conclusion**

Despite significant progress in the understanding of CM biology, our ability to assess the likelihood of recurrence
and death for any individual patient remains conspicuously limited. Assessment of an individual CM patient’s risk is currently based on the AJCC recommendations, which consider traditional staging factors such as Breslow thickness and ulceration [136]. However, over two-thirds of CM-related deaths occur in patients diagnosed with stage I or II disease [137], and, as the incidence of melanoma continues to increase, the absolute number of such ‘low risk’ patients who ultimately relapse and die is rising [138]. National guidelines do not currently recommend intensive surveillance and adjuvant therapy for stage I-IIA disease [139]. Additional strategies for prognostication in this early-stage CM cohort, particularly those with biological propensity to metastasise and who might benefit from modern survival-prolonging adjuvant therapies [140–142], would clearly be beneficial. However it seems clear that while there is tremendous enthusiasm to integrate molecular biomarkers into clinical practice, no such markers or signatures fulfill the necessary criteria for inclusion into the AJCC melanoma staging or as a component of any validated clinical tool [136]. More studies are also needed to determine which type of specimen and approach yields the highest success rate. The creation of large, prospective, multi-institution registry studies that harness the power of electronic data sharing should improve on some of the shortcomings of current prognostic tools including; relatively small study populations, short follow-up and lack of internal and external validation. These studies will be needed to address this unmet clinical need for patient stratification in CM.

Distinct melanoma subtypes harbour somatic aberrations on the same key pathways, but the affected genes may be different (Table 1). Accordingly, it is evident that the current genomic classification of melanomas devised by TCGA may work well with CM and with DM to some extent, but a similar classification that informs therapeutic options is needed for AM and MM, where more than 50% of tumours may fall into the TWT subtype.

Why melanomas from non-CSD regions have such different genomic landscapes to those from CSD tissues remains an open question. Apart from the fact that cells from these different regions have varying exposure to UV-induced mutagenesis, another possible explanation may lie in the different lineages from which these melanocytes originate, and the different microenvironments they inhabit. Therefore, the question remains, if the CM driver mutations arose in melanocytes from glabrous skin, and vice versa, would melanocytes transform and form tumours? Or, are the melanocyte lineages sufficiently different that different mutations are needed to progress to malignancy? Would the microenvironment play a significant role in melanocyte transformation? In vitro experiments with cell lines from different melanocytic lineages and in vivo experiments in model organisms such as mice and zebrafish should help address this fundamental question. Clearly, although good progress has been achieved for patients with CM, therapeutic options and response remain poor in patients with other melanoma subtypes. Recent studies exploring prognostic markers and potential therapeutic targets are helping bridge this gap, and as more genomes from rarer melanoma subtypes are sequenced, our understanding of targeted therapy and response should improve. Most of these tumoural genomes originate from patients of European descent, and a further important question is whether findings in these populations can be translated to patients from other ethnicities – particularly in AM and MM which constitute a higher proportion of melanoma cases in non-European descent populations.

Acknowledgements

We would like to thank Nick Hayward, Patricia Possik and Sarah Welsh for very helpful discussions, as well as Phillip Ball and Richard Poulson for their support in figure formatting. This work was supported by Cancer Research UK and The Wellcome Trust. CDR-E is supported by a Wellcome Trust Seed Award in Science (204562/Z/16/Z), by a UNAM PAPIIT Award (IA200318), a Stimulus for Medical Research from the Miguel Aleman Trust, and by a UC-MEXUS Collaborative Award (CN-18-121). CM-A is supported by a postdoctoral salary from the Wellcome Trust (204562/Z/16/Z).

Author contributions statement

RR described the prognostic and therapeutic implications of molecular aberrations across the melanoma subtypes, and drafted sections of the introduction and conclusion. CDRE and CMA described the genomic landscape of melanoma subtypes, as well as sections of the introduction and conclusion. PF provided the H&E images and caption, and commented on the histopathologic aspects of the manuscript. DIA and CDRE provided overall supervision and leadership, as well as comments and suggestions across the entire manuscript. All authors approved the final version.

References

1. Shain AH, Bastian BC. From melanocytes to melanomas. Nat Rev Cancer 2016; 16: 345–358.
2. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature 2013; 500: 415–421.
3. Hayward NK, Wilmott JS, Waddell N, et al. Whole-genome landscapes of major melanoma subtypes. Nature 2017; 545: 175–180.
4. Cancer Genome Atlas Network. Genomic and transcriptomic characterization of cutaneous melanoma. Cell 2015; 161: 1681–1696.
5. Shain AH, Joseph NM, Yu R, et al. Genomic and transcriptional analysis reveals incremental disruption of key signalling pathways during melanoma evolution. Cancer Cell 2018; 34: 45–55.e44.
6. Celia-Terrassa T, Kang Y. Distinctive properties of metastasis-initiating cells. Genes Dev 2016; 30: 892–908.
et al.

27. Ellerhorst JA, Eghazy S, Ringborg U, et al. Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. Mol Oncol 2008; 1: 395–405.

28. Omholt K, Platza, Kantor L, et al. NRAS and BRAF mutations arise early during melanoma pathogenesis and are preserved throughout tumor progression. Clin Cancer Res 2003; 9: 6483–6488.

29. Platza A, Eghazy S, Ringborg U, et al. Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. Mol Oncol 2008; 1: 395–405.

30. Gerami P, Cook RW, Russell MC, et al. Gene expression profiling for molecular staging of cutaneous melanoma in patients undergoing sentinel lymph node biopsy. J Am Acad Dermatol 2015; 72: 780–785.e783.

31. Omken MD, Worley LA, Tuscan MD, et al. An accurate, clinically feasible multi-gene expression assay for predicting metastasis in uveal melanoma. J Mol Diagn 2010; 12: 461–468.

32. Bittner M, Meltzer P, Chen Y, et al. Molecular classification of cutaneous malignant melanoma by gene expression profiling. Nature 2000; 406: 536–540.

33. Omken MD, Worley LA, Ehlers JP, et al. Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death. Cancer Res 2004; 64: 7205–7209.

34. Haqq C, Nosrati M, Sudilovsky D, et al. The gene expression signatures of melanoma progression. Proc Natl Acad Sci U S A 2005; 102: 6092–6097.

35. Smith AP, Hoeck K, Becker D. Whole-genome expression profiling of the melanoma progression pathway reveals marked molecular differences between nevi/melanoma in situ and advanced-stage melanomas. Cancer Biol Ther 2005; 4: 1018–1029.

36. Jaeger J, Koczlan D, Thiesen HJ, et al. Gene expression signatures for tumor progression, tumor subtype, and tumor thickness in laser-microdissected melanoma tissues. Clin Cancer Res 2007; 13: 806–815.

37. Scatolini M, Grand MM, Grosso E, et al. Altered molecular pathways in melanocytic lesions. Int J Cancer 2010; 126: 1869–1881.

38. Maurer A, Roesch A, Hafner C, et al. Identification of new genes associated with melanoma. Exp Dermatol 2011; 20: 502–507.

39. Zager JS, Gastman BR, Leachman S, et al. Performance of a prognostic 31-gene expression profile in an independent cohort of 523 cutaneous melanoma patients. BMC Cancer 2018; 18: 130.

40. Ferris LK, Farber AS, Middlebrook B, et al. Identification of high-risk cutaneous melanoma tumors is improved when combining the online American joint committee on cancer individualized melanoma patient outcome prediction tool with a 31-gene expression profile-based classification. J Am Acad Dermatol 2017; 76: 818–825.e813.

41. Hsueh EC, DeBloom JR, Lee J, et al. Interim analysis of survival in a prospective, multi-center registry cohort of cutaneous melanoma tested with a prognostic 31-gene expression profile test. J Hematol Oncol 2017; 10: 152.

42. Gastman BR, Gerami P, Kurlay SJ, et al. Identification of patients at risk for metastasis using a prognostic 31-gene expression profile in subpopulations of melanoma patients with favorable outcomes by standard criteria. J Am Acad Dermatol 2018; 80: 149–157. https://doi.org/10.1016/j.jaad.2018.07.028.

43. Wiesner T, Kiuru M, Scott SN, et al. NF1 mutations are common in desmoplastic melanoma. Am J Surg Pathol 2015; 39: 1357–1362.

44. Liang WS, Hendricks W, Kiefer J, et al. Integrated genomic analyses reveal frequent TERT aberrations in acral melanoma. Genome Res 2017; 27: 524–532.

45. Curtin JA, Friddy J, Kangishita T, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med 2005; 353: 2315–2324.

46. Vazquez V de L, Vicente AL, Carloni A, et al. Molecular profiling, including TERT promoter mutations, of acral lentiginous melanomas. Melanoma Res 2016; 26: 93–99.
Melanoma subtypes

47. Moon KR, Choi YD, Kim JM, et al. Genetic alterations in primary acral melanoma and acral melanocytic nevus in Korea: common mutated genes show distinct cytomorphological features. *J Invest Dermatol* 2018; **138**: 933–945.

48. Royer-Bertrand B, Torsello M, Rimoldi D, et al. Comprehensive genetic landscape of uveal melanoma by whole-genome sequencing. *Am J Hum Genet* 2016; **99**: 1190–1198.

49. Robertson AG, Shih J, Yau C, et al. Integrative analysis identifies four molecular and clinical subsets in uveal melanoma. *Cancer Cell* 2017; **32**: 204–220.e215.

50. Hintzsche JD, Gorden NT, Amato CM, et al. Whole-exome sequencing identifies recurrent SF3B1 R625 mutation and conmutation of NF1 and KIT in mucosal melanoma. *Melanoma Res* 2017; **27**: 189–199.

51. Cosgarea I, Ugurel S, Sucker A, et al. Targeted next generation sequencing of mucosal melanomas identifies frequent NF1 and RAS mutations. Oncotarget 2017; **8**: 40683–40692.

52. Curtin JA, Busam K, Pinkel D, et al. Somatic activation of KIT in distinct subtypes of melanoma. *J Clin Oncol* 2006; **24**: 4340–4346.

53. Wallander ML, Layfield LJ, Emerson LL, et al. Mutation of PTEN in cutaneous melanoma. *Oncogene* 2002; **21**: 4581–4587.

54. Furney SJ, Turajlic S, Stamp G, et al. Somatic mutations of GNAQ in uveal melanoma and blue naevi. *Genes Chromosomes Cancer* 2014; **53**: 5303–5306.

55. Moore AR, Ceraudo E, Sher JJ, et al. Recurrent activating mutations of G-protein-coupled receptor CYSLTR2 in uveal melanoma. *Mod Pathol* 2016; **29**: 675–680.

56. Dono M, Angelini G, Cecconi M, et al. Amplification of CDK4 and MDM2 in malignant melanoma. *Genes Chromosomes Cancer* 2006; **45**: 447–454.

57. Van Raamsdonk CD, Bezrookove V, Green G, et al. Mutational dichotomy in uveal melanoma by whole-exome sequencing of mucosal melanomas identifies frequent NF1 and RAS mutations. *Oncotarget* 2017; **8**: 70847–70855.

58. Jahn SW, Kashofer K, Halbwedl I, et al. Promoter hypermethylation: a common cause of reduced p16(INK4a) expression in uveal melanoma. *J Invest Ophthalmol* 2014; **1031–1035.

59. Catalanotti F, Cheng DT, Shoushtari AN, et al. PTEN loss-of-function alterations are associated with intrinsic resistance to BRAF inhibitors in metastatic melanoma. *JCO Precis Oncol* 2017; **1**: 1–15; doi: 10.1200/po.16.00054.

60. Abdel-Rahman MH, Yang Y, Zhou XP, et al. Somatic activation of KIT in distinct subtypes of melanoma. *Melanoma Res* 2017; **27**: 205–213.

61. Sauter ER, Yeo UC, von Stemm A, et al. Integrative analysis identifies recurrent SF3B1 R625 mutation and conmutation of NF1 and KIT in mucosal melanoma. *Melanoma Res* 2017; **27**: 189–199.

62. Harbst K, Staaf J, Lauss M, et al. The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. *Cancer Discov* 2014; **4**: 94–109.

63. Salgia R, Kulkarni P. The genetic/non-genetic duality of drug ‘resistance’ in cancer. *Trends Cancer* 2018; **4**: 110–118.

64. Tong X, Kuilman T, Shahrabi A, et al. Cancer drug addiction is relayed by an ERK2-dependent phenotype Switch. *Nature* 2017; **550**: 270–274.

65. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* 2014; **371**: 2189–2199.

66. Van Allen EM, Miao D, Schilling B, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* 2015; **350**: 207–211.

67. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* 2014; **371**: 2189–2199.

68. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012; **366**: 2455–2465.

69. van Rooij N, van Buuren MM, Philips D, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in a plasmablastic lymphoma. *J Clin Oncol* 2013; **31**: e439–e442.

70. Gabin MM, Zhang X, Schuster H, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* 2014; **515**: 577–581.

71. Lauss M, Donia M, Harbst K, et al. Mutational and putative neoantigen load predict clinical benefit of adoptive T cell therapy in melanoma. *Nat Commun* 2017; **8**: 1738.

72. van Rooij N, van Buuren MM, Philips D, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in a plasmablastic lymphoma. *J Clin Oncol* 2013; **31**: e439–e442.

73. Hugo W, Zaretsky JM, Sun L, et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell* 2016; **165**: 35–44.

74. Riaz N, Havel JJ, Makarov V, et al. Tumor and microenvironment evolution during immunotherapy with Nivolumab. *Cell* 2017; **171**: 934–949.e915.

75. Rob W, Chen PL, Reuben A, et al. Integrated molecular analysis of tumor biopsies on sequential CTLA-4 and PD-1 blockade...
reveals markers of response and resistance. Sci Transl Med 2017; 9: eaah3560.

88. Daud AI, Loo K, Pauli ML, et al. Tumor immune profiling predicts response to anti-PD-1 therapy in human melanoma. J Clin Invest 2016; 126: 3447–3452.

89. Busam KJ, Mujumdar U, Hummer AJ, et al. Cutaneous desmoplastic melanoma: reappraisal of morphologic heterogeneity and prognostic factors. Am J Surg Pathol 2004; 28: 1518–1525.

90. Kim J, Lazaar AJ, Davies MA, et al. BRAF, NRAS and KIT sequencing analysis of spindle cell melanoma. J Cutan Pathol 2012; 39: 821–825.

91. Davison JM, Rosenbaum E, Barrett TL, et al. Absence of V599E BRAF mutations in desmoplastic melanomas. Cancer 2005; 103: 788–792.

92. Eroglu Z, Zaretsky JM, Halaban R, et al. CDK4/6 inhibitors in targeted therapy. J Clin Oncol 2017; 35: 1758834018757175.

93. Lazova R, Pornputtapong N, Halaban R, et al. Prognostic implications of monosomy 3 in uveal melanoma. Lancet 1996; 347: 1222–1225.

94. Abdel-Rahman MH, Christopher BN, Faramawi MF, et al. Frequency, molecular pathology and potential clinical significance of partial chromosome 3 aberrations in uveal melanoma. Mod Pathol 2011; 24: 954–962.

95. Dogrusoz M, Baggar M, van Duinen SG, et al. The prognostic value of AJCC staging in uveal melanoma is enhanced by adding chromosome 3 and 8q status. Invest Ophthalmol Vis Sci 2017; 58: 833–842.

96. Grie wank KG, van de Nes J, Schilling B, et al. Genetic and clinico-pathologic analysis of metastatic uveal melanoma. Mod Pathol 2014; 27: 175–183.

97. Harbour JW, Onken MD, Roberson ED, et al. Frequent mutation of BAP1 in metastasizing uveal melanomas. Science 2010; 330: 1410–1413.

98. Kalirai H, Dodson A, Faqui S, et al. Lack of BAP1 protein expression in uveal melanoma is associated with increased metastatic risk and has utility in routine prognostic testing. Br J Cancer 2014; 111: 1373–1380.

99. Ewens KG, Kanetsky PA, Richards-Yutz I, et al. Chromosome 3 status combined with BAP1 and EIF4AX mutation profiles are associated with metastasis in uveal melanoma. Invest Ophthalmol Vis Sci 2014; 55: 5160–5167.

100. Yavuzi yigitoglu S, Koopmans AE, Verdijk RM, et al. Uveal melanomas with SF3B1 mutations: a distinct subclass associated with late-onset metastases. Ophthalmology 2016; 123: 1118–1128.

101. Harbour JW, Roberson ED, Anbunathan H, et al. Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma. Nat Genet 2013; 45: 133–135.

102. Furney SJ, Pedersen M, Gentien D, et al. SF3B1 mutations are associated with alternative splicing in uveal melanoma. Cancer Discov 2013; 3: 1122–1129.

103. Aaberg T Jr, Cook RW, Oelschlager K, et al. Current clinical practice: differential management of uveal melanoma in the era of molecular tumor analyses. Clin Ophthalmol 2014; 8: 2449–2460.

104. Field MG, Harbour JW. Recent developments in prognostic and predictive testing in uveal melanoma. Curr Opin Ophthalmol 2014; 25: 234–239.

105. Onken MD, Worley LA, Harbour JW. A metastasis modifier locus on human chromosome 8p in uveal melanoma identified by integrative genomic analysis. Clin Cancer Res 2008; 14: 3737–3745.

106. Singh AD, Sisley K, Xu Y, et al. Reduced expression of autotaxin predicts survival in uveal melanoma. Br J Ophthalmol 2007; 91: 1385–1392.
128. Worley LA, Onken MD, Person E, et al. Transcriptomic versus chromosomal prognostic markers and clinical outcome in uveal melanoma. Clin Cancer Res 2007; 13: 1466–1471.
129. Petrausch U, Martus P, Tonnie H, et al. Significance of gene expression analysis in uveal melanoma in comparison to standard risk factors for risk assessment of subsequent metastases. Eye (Lond) 2008; 22: 997–1007.
130. Mihajlovic M, Vlajkovic S, Jovanovic P, et al. Primary mucosal melanomas: a comprehensive review. Int J Clin Exp Pathol 2012; 5: 739–753.
131. Postow MA, Hamid O, Carvajal RD. Mucosal melanoma: pathogenesis, clinical behavior, and management. Curr Oncol Rep 2012; 14: 441–448.
132. Yun J, Lee J, Jang J, et al. KIT amplification and gene mutations in acral/mucosal melanoma in Korea. APMS 2011; 119: 330–335.
133. Kim KB, Airwas A. Treatment of KIT-mutated metastatic mucosal melanoma. Chin Clin Oncol 2014; 3: 35.
134. D’Angelo SP, Larkin J, Sosman JA, et al. Efficacy and safety of nivolumab alone or in combination with ipilimumab in patients with mucosal melanoma: a pooled analysis. J Clin Oncol 2017; 35: 226–235.
135. Studentova H, Kalabova H, Koranda P, et al. Immunotherapy in mucosal melanoma: a case report and review of the literature. Oncotarget 2018; 9: 17971–17977.
136. Gershenwald JE, Scolyer RA, Hess KR, et al. Melanoma staging: evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin 2017; 67: 472–492.
137. Morton DL, Thompson JF, Cochran AJ, et al. Final trial report of sentinel-node biopsy versus nodal observation in melanoma. N Engl J Med 2014; 370: 599–609.
138. Whiteman DC, Baade PD, Olsen CM. More people die from thin melanomas (1 mm) than from thick melanomas (>4 mm) in Queensland, Australia. J Invest Dermatol 2015; 135: 1190–1193.
139. Coti DG, Thompson JA, Algazi A, et al. Melanoma, version 2.2016, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2016; 14: 450–473.
140. Maio M, Lewis K, Demidov L, 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.
141. Long GV, Hauschild A, Santinami M, et al. Adjuvant dabrafenib plus trametinib in stage III BRAF-mutated melanoma. N Engl J Med 2017; 377: 1813–1823.
142. Weber J, Mandala M, Del Vecchio M, et al. Adjuvant nivolumab versus ipilimumab in resected stage III or IV melanoma. N Engl J Med 2017; 377: 1824–1835.

50 Years Ago in The Journal of Pathology...

Histochemical types of acidic glycoprotein produced by mucous cells of the tracheobronchial glands in man

D. Lamb and Lynne Reid

Hodgkin’s disease: A follow-up study of patients surviving more than twenty years after the original diagnosis

H. F. Smetana

Teratomata in infancy and childhood: A review of 91 cases

C. L. Berry, Jean Keeling and Clare Hilton

To view these articles, and more, please visit: www.thejournalofpathology.com

Click ‘BROWSE’ and select ‘All issues’, to read articles going right back to Volume 1, Issue 1 published in 1892.

The Journal of Pathology

Understanding Disease