

# Molecular deconstruction of a biphasic malignant skin tumour

Joy Ursula Lauren Staniforth

James Yiu Hon Chan

Olivier Thierry Giger

## Abstract

We present the case of a diagnostically challenging biphasic cutaneous melanoma. We show how tumour microdissection and next generation sequencing revealed the molecular signature allowing confirmation of the diagnosis.

**Keywords** malignant melanoma; mutational profiling; next-generation sequencing

## Case report

A 72 year old male had a scalp lesion, clinically suspicious for squamous cell carcinoma and excised accordingly. Two years prior an atypical fibroxanthoma (AFX) was removed from the contralateral side of his scalp.

Histology showed a biphasic malignant dermal tumour extending into the subcutis (Figure 1). This was formed predominantly of vimentin-positive pleomorphic spindle cells (>99%), with small nests of an epithelioid component showing melanocytic marker positivity (Figure 2) in keeping with a malignant melanoma (MM). The BRAF V600E mutation gene product specific antibody VE1 was negative in both components. Immunohistochemistry results are summarized in Table 1. An actinic keratosis with low grade dysplasia was present in the overlying epidermis, but there was no atypical epidermal melanocytic lesion or background naevus. Even though the most likely diagnosis was dedifferentiated MM, the histogenesis of the pleomorphic spindle cell component remained unconfirmed. A tumour-to-tumour metastasis of MM to pleomorphic dermal sarcoma (PDS) or an MM-PDS collision tumour could not be excluded. The existence of the previous tumour additionally raised the possibility of metastasis from this. As these

**Joy Ursula Lauren Staniforth** MA(Cantab) MB BChir FRCPath  
Histopathology Speciality Registrar, North West Anglia NHS  
Foundation Trust, Peterborough, UK. Conflicts of interest: none  
declared.

**James Yiu Hon Chan** BM BSc(Hons) MRCP FRCPath Consultant  
Histopathologist, Cambridge University Hospitals NHS Foundation  
Trust, Cambridge, UK declared. Conflicts of interest: none declared.

**Olivier Thierry Giger** MD PhD University Lecturer and Honorary  
Consultant Histopathologist, University of Cambridge, Cambridge,  
UK. Conflicts of interest: none declared.

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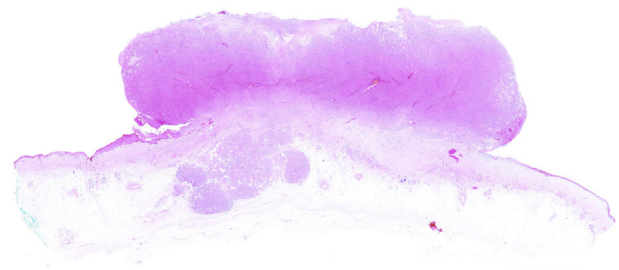
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possibilities could generate significantly different management strategies, molecular exploration of both components of the tumour was felt to be indicated.

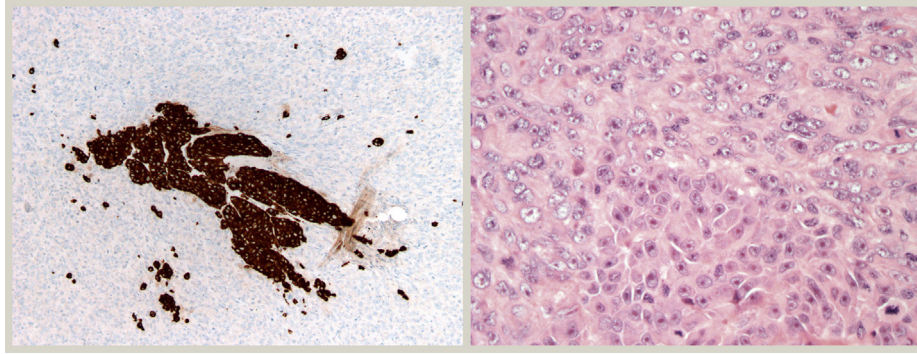
Cancer mutation analysis by next generation sequencing (NGS; Ion Torrent 50 cancer gene panel) was performed on both tumour components. Both shared identical pathogenic variants in *ATM*, *CDKN2A*, *FBXW7*, *FGFR2* and *KIT* (Table 2). The epithelioid component showed an additional pathogenic *NRAS* variant, as observed in 15–20% of cutaneous MMs.<sup>1</sup> A *BRAF* non-V600E mutation was not detected. Mutational analysis of the previously excised AFX using the same NGS panel revealed no overlapping mutations but a pathogenic *TP53* variant. This confirmed that both components in the current lesion represented two separate subclones of the same tumour, i.e. biphasic epithelioid and dedifferentiated malignant melanoma. The molecular findings further clearly separated the present tumour from the previously diagnosed AFX, which contained a characteristic *TP53* mutation attributed to ultraviolet light exposure<sup>2</sup> (though this in itself is not diagnostic of AFX, as *TP53* mutations are also common in malignant melanoma for the same reason). Fluorescent in situ hybridization using a 1p32 probe (Vysis LSI CDKN2C SpectrumGreen) showed a significant amplification of the short arm of chromosome 1p in both tumour components (image not shown).

## Discussion and conclusion

Dedifferentiated malignant melanoma (DMM) can be diagnostically challenging. It is likely under-recognized and may be mistaken for other malignancies such as undifferentiated sarcoma, especially in the setting of metastases.<sup>3</sup> In cases of primary cutaneous DMM, examining the skin lesion's junctional edge or the base of an epidermal ulcer (if present) may help to identify conventional MM components.<sup>4</sup> Examination for a precursor



**Figure 1** H&E, low power whole slide view (scale bar 4 mm).



**Figure 2** Melan-A immunohistochemistry (left): strong and diffuse positivity in a conventional melanoma focus; these comprised <1% of the lesion. H&E (right): a conventional melanoma focus (bottom left) with adjacent pleomorphic component, demonstrating the cytomorphological differences between the two components.

### Immunohistochemistry results

Component	Vimentin	CD68P	CD10	CK5/6, p63	CD45	CD34	Desmin, MSA	SOX-10, S100	Melan A, HMB-45	BRAF V600E
Pleomorphic	+	+ (focal)	–	–	–	–	–	–	–	–
Epithelioid	+	–	–	–	–	–	–	+	+	–
Conclusion	Non-specific	Non-contributory	Not supportive of AFX (but non-specific)	Not supportive of squamous cell carcinoma	No evidence of haematogenous differentiation	No evidence of vascular differentiation	No evidence of smooth muscle differentiation	Supportive of malignant melanoma, lacking a BRAF V600E mutation, in the epithelioid component.		

Details of primary antibodies: anti-vimentin antibody [Vim 3B4], Dako, Cat # M7020; anti-CD68P antibody [PG-M1], Dako, Cat # M0876; anti-CD10 antibody [56C6], Leica, Cat # PA0131; anti-CK5/6 antibody [D5/16 B4], Dako, Cat # M7237; anti-p63 antibody [7JUL], Dako, Cat # PA0103; anti-CD45 antibody [2B11 + PD7/26], Dako, Cat # M0701; anti-CD34 antibody [QEnd-10], Dako, Cat # M7165; anti-desmin antibody [D33], Dako, Cat # M0760; anti-MSA antibody [HHF35], Leica, Cat # PA0258; anti-SOX-10 antibody [SP267], Roche, Cat # 760–4968; anti-S100 antibody, Dako, Cat # Z0311; anti-Melan A antibody [A103], Leica, Cat # PA044; anti-HMB-45 antibody [HMB-45], Leica, Cat # PA0027; anti-BRAF antibody [VE1], Roche, Cat # 709–4855.

**Table 1**

### NGS results

Gene	Exon	Coding	Protein	Allele frequency in %	
				Dedifferentiated	Epithelioid
<i>NRAS</i>	2	c.35G>A	p.Gly12Asp	0	62.3
<i>KIT</i>	13	c.1946A>G	p.Asn649Ser	38.43	44.27
<i>FBXW7</i>	10	c.1435C>T	p.Arg479Ter	45	48.02
<i>CDKN2A</i>	2	c.341_342delCCinsTT	p.Pro114Leu	80.83	87.72
<i>FGFR2</i>	9	c.1112C>T	p.Ala371Val	43.73	40.64
<i>ATM</i>	26	c.3887C>T	p.Pro1296Leu	43.54	46.57

**Table 2**

lesion in the epidermis (e.g. superficial spreading malignant melanoma or lentigo maligna) and for a background naevus is also important. Finally, molecular profiling can be helpful as in this case.

In the case presented, a “conventional” epithelioid MM component was identified in the tumour bulk and at its edges. Whereas the diagnosis of the DMM component remained unconfirmed after histomorphological and immunohistochemical

### Multiple-choice questions

1. Which gene is commonly mutated in AFXs and MMs due to UV light exposure?

- ATM
- FBXW7
- FGFR2
- KIT
- TP53

2. Which area of a DMM may be useful to examine for a conventional component?

- At the dermal/subcuticular peripheries of the lesion
- At the epidermal junctional edge of the lesion
- Foci flanking an ulcerated area of the lesion
- Within the background epidermis
- All of the above

3. Why should caution be exercised when using NGS to ascertain DMM aetiology?

- Analysis often fails due to poor DNA quality
- Analysis uses up all the tissue in the block
- Microdissection cannot separate out different tumour components
- There may be significant tumour mutational heterogeneity
- UV light-induced mutations completely preclude its use in skin tumours

Correct answers: 1e, 2e, 3d

assessment, multipanel mutational analysis clearly demonstrated that this was the same tumour. The difference within the mutation spectrum (NRAS) was reflected in the histological appearance.

The application of NGS has been illustrated in gallbladder, lung and brain composite neoplasms as a possible aid to distinguishing between possibilities of clonal tumours with divergent phenotypes, collision tumours, and tumour-to-tumour metastases.<sup>1</sup> Microdissection of the different tumour components and separate mutational profiling is technically viable in experienced hands. In our case, the epithelioid nests were scanty (<1%) but nevertheless a tumour component purity of >60% was achieved by microdissection. This purity figure was ascertained through the lack of an NRAS mutation in the spindle cell component and a >60% allele frequency in the epithelioid component, assuming a single allelic variant to be present. NRAS lies on the short arm of chromosome 1 (1p13.2). A significant 1p amplification often described in MMs<sup>5</sup> was also observed in our

### Practice points

- It is imperative to search for conventional or well-differentiated heterologous components in a dedifferentiated skin tumour. Extensive sampling and molecular analysis may be helpful.
- Molecular profiling of more than one tumour component may be required.
- Primary dedifferentiated cutaneous tumours may show characteristic mutations which relate to their aetiopathogenesis (e.g. TP53 mutations in AFX and MM, and NRAS mutations and chromosome 1p amplifications in MM).

case, however we were not able to demonstrate a copy number variation between the two components by FISH.

Taken together, the identification of a minor tumour component as a MM in an otherwise poorly differentiated tumour, the shared mutation profile and the 1p32 amplification status between the two components confirmed this lesion to be a biphasic epithelioid and dedifferentiated malignant melanoma. This case further shows that care in interpretation must always be applied in the diagnostic use of mutational sequencing. Genomic heterogeneity and a high mutational burden are common in primary cutaneous MMs<sup>6</sup> and molecular profiling of different tumour components may be required. As illustrated by this case, skilled correlation between morphological and molecular features is essential for accurate, unified diagnosis. ◆

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