

University of Groningen

Clinical, histopathological and molecular features of dedifferentiated melanomas

EORTC Melanoma Group; Hench, Juergen; Mihic-Probst, Daniela; Agaimy, Abbas; Frank, Stephan; Hultschig, Claus; Simi, Sara; Alos, Lucia; Balamurugan, Thiagarajah; Blokx, Willeke

Published in:
European Journal of Cancer

DOI:
[10.1016/j.ejca.2023.03.032](https://doi.org/10.1016/j.ejca.2023.03.032)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2023

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

EORTC Melanoma Group, Hench, J., Mihic-Probst, D., Agaimy, A., Frank, S., Hultschig, C., Simi, S., Alos, L., Balamurugan, T., Blokx, W., Bosisio, F., Cappellesso, R., Griewank, K., Hadaschik, E., van Kempen, L. C., Kempf, W., Lentini, M., Mazzucchelli, L., Rinaldi, G., ... Massi, D. (2023). Clinical, histopathological and molecular features of dedifferentiated melanomas: An EORTC Melanoma Group Retrospective Analysis. *European Journal of Cancer*, 187, 7-14. Advance online publication. <https://doi.org/10.1016/j.ejca.2023.03.032>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



Original Research

Clinical, histopathological and molecular features of dedifferentiated melanomas: An EORTC Melanoma Group Retrospective Analysis



Juergen Hench ^{a,1}, Daniela Mihic-Probst ^{b,1}, Abbas Agaimy ^{c,1},
 Stephan Frank ^a, Peter Meyer ^a, Claus Hultschig ^a, Sara Simi ^d,
 Lucia Alos ^e, Thiagarajah Balamurugan ^f, Willeke Blokk ^g,
 Francesca Bosisio ^h, Rocco Cappellesso ⁱ, Klaus Griewank ^j,
 Eva Hadaschik ^j, Leon C. van Kempen ^{k,1}, Werner Kempf ^m,
 Maria Lentini ⁿ, Luca Mazzucchelli ^o, Gaetana Rinaldi ^p,
 Piotr Rutkowski ^q, Dirk Schadendorf ^j, Bastian Schilling ^r,
 Anna Szumera-Cieckiewicz ^s, Joost van den Oord ^h, Mario Mandalà ^{t,*,2},
 Daniela Massi ^{d,2}, on behalf of the EORTC Melanoma Group

^a Institute of Medical Genetics and Pathology, Division of Neuropathology, University Hospital Basel, Basel, Switzerland

^b Institute of Pathology and Molecular Pathology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

^c Institute of Pathology, Friedrich-Alexander-University Erlangen-Nürnberg, University Hospital, Erlangen, Germany

^d Section of Pathology, Department of Health Sciences, University of Florence, Florence, Italy

^e Department of Pathology, Hospital Clinic of Barcelona, Barcelona, Spain

^f Unit of Pathology, Royal Surrey County Hospital, Guildford, UK

^g Department of Pathology, Division of Laboratories, Pharmacy and Biomedical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands

^h Department of Imaging & Pathology, Laboratory of Translational Cell & Tissue Research and Department of Pathology, University Hospitals Leuven, KU Leuven, Leuven, Belgium

ⁱ Pathological Anatomy Unit, Padua University Hospital, 35121 Padua, Italy

^j Department of Dermatology, University Hospital of Essen, West German Cancer Center, University Duisburg-Essen and the German Cancer Consortium (DKTK), Essen, Germany

^k Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

^l Department of Pathology, University Hospital Antwerp, Antwerp, Belgium

^m Department of Dermatology, University Hospital Zurich, Zurich, Switzerland

ⁿ Department of Human Pathology, University of Messina, Messina, Italy

^o Laboratory of Molecular Pathology, Institute of Pathology, Ente Ospedaliero Cantonale, Locarno, Switzerland

^p Sezione di Oncologia, Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche (Di. Chir. On.S.), Università degli Studi di Palermo, Palermo, Italy

* Corresponding author: Unit of Medical Oncology, University of Perugia, Santa Maria della Misericordia Hospital, Perugia 06129, Italy.
 E-mail address: mario.mandalà@unipg.it (M. Mandalà).

¹ Equally contributed first authorship.

² Equally contributed senior authorship.

^a Maria Skłodowska-Curie National Research Institute of Oncology, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw, Poland

^r Department of Dermatology, Venereology and Allergology, University Hospital Würzburg, Würzburg, Germany

^s Maria Skłodowska-Curie National Research Institute of Oncology, Department of Soft Tissue/Bone Sarcoma and Melanoma, Warsaw, Poland

^t University of Perugia, Unit of Medical Oncology, Santa Maria della Misericordia Hospital, Perugia, Italy

Received 26 January 2023; Received in revised form 26 March 2023; Accepted 28 March 2023

Available online 2 April 2023

KEYWORDS

Dedifferentiated melanoma;
Epigenetics;
DNA methylation;
Copy number profiling

Abstract Purpose: Dedifferentiated melanoma (DedM) poses significant diagnostic challenges. We aimed to investigate the clinical, histopathological and molecular features of DedM. Methylation signature (MS) and copy number profiling (CNP) were carried out in a subgroup of cases.

Patients and methods: A retrospective series of 78 DedM tissue samples from 61 patients retrieved from EORTC (European Organisation for Research and Treatment of Cancer) Melanoma Group centres were centrally reviewed. Clinical and histopathological features were retrieved. In a subgroup of patients, genotyping through Infinium Methylation microarray and CNP analysis was carried out.

Results: Most patients (60/61) had a metastatic DedM showing most frequently an unclassified pleomorphic, spindle cell, or small round cell morphology akin to undifferentiated soft tissue sarcoma, rarely associated with heterologous elements. Overall, among 20 successfully analysed tissue samples from 16 patients, we found retained melanoma-like MS in only 7 tissue samples while a non-melanoma-like MS was observed in 13 tissue samples. In two patients from whom multiple specimens were analysed, some of the samples had a preserved cutaneous melanoma MS while other specimens exhibited an epigenetic shift towards a mesenchymal/sarcoma-like profile, matching the histological features. In these two patients, CNP was largely identical across all analysed specimens, in line with their common clonal origin, despite significant modification of their epigenome.

Conclusions: Our study further highlights that DedM represents a real diagnostic challenge. While MS and genomic CNP may help pathologists to diagnose DedM, we provide proof-of-concept that dedifferentiation in melanoma is frequently associated with epigenetic modifications.

© 2023 Elsevier Ltd. All rights reserved.

1. Introduction

Melanoma is the most aggressive type of skin cancer that arises from melanocytes, neuroectoderm-derived pigment-producing cells with highly polarised dendritic morphology [1]. The diagnosis of primary and metastatic melanoma is based on well-established morpho-immunophenotypic features and genotyping. Whereas melanocytic differentiation in primary melanoma is often immediately apparent making their diagnostic recognition straightforward, metastatic melanomas may adopt potentially misleading cyto-architectural characteristics, can show loss of expression of immunohistochemical markers or present with aberrant morpho-immunophenotypes, all of which may give rise to differential diagnostic confusion in the differentiation of respective lesions against diverse non-melanocytic neoplasms of epithelial, mesenchymal, neurogenic or even hematolymphoid and histiocytic origin [2–8].

Genotyping (specifically, *BRAF*, *NRAS* and *NFI*) has been suggested and used as a surrogate marker in classifying such difficult-to diagnose dedifferentiated metastatic melanoma cases [3–5,9]. Dedifferentiation reflects a high degree of plasticity with the ability of differentiated melanoma cells to dedifferentiate into a more primitive neural crest-like phenotype, either *de novo* or upon systemic therapy [10–15].

To minimise the risk of misdiagnosis, clinical, histopathological and genotypic criteria have been defined recently in order to capture diagnosis of dedifferentiated melanoma (DedM) in clinically unclear situations. Major clues to the diagnosis include (1) melanoma history, (2) presence of a minimally differentiated clone, (3) emergence of non-specific histology such as pleomorphic undifferentiated, pleomorphic rhabdomyoblastic or osteochondroblastic, (4) detection of a melanoma-associated mutation, and (5) absence of an alternative primary [5]. More recently, another terminology has been proposed,

also reflecting the partially unexplored biology and molecular presentation of these tumours [7].

Dedifferentiation and melanoma plasticity are both important consequences of the biological heterogeneity governing clinical progression, metastasis as well as resistance to targeted approaches and immunotherapy in melanoma [8,12].

Although the exact molecular mechanisms leading to dedifferentiation are largely unknown, it is conceivable that intratumoral stem cells may be essential for the expansion of new lines of differentiation [2,16]. In these cells, activation of lineage-specific transcription factors may change lineage commitment causing aberrant gene expression patterns and phenotypes. In addition, epigenetic alterations caused by malfunction of genes involved in maintaining chromatin marks and/or DNA methylation patterns, may be involved [17].

Large consecutive series with centralised pathologic review and in depth comprehensive genetic analyses are lacking so far, which has hampered specific taxonomic tumour classification. Here, we investigated the clinical, histopathological and molecular features of DedM. Methylation signature (MS) and the copy number profile (CNP) were obtained for a subgroup of cases with sufficient biopsy tissue.

2. Materials and methods

2.1. Patients and archival biopsy tissue

Tissue samples from metastatic DedM and, when available, matched primary cutaneous melanomas were retrospectively collected at EORTC Melanoma Group centres. Samples from systemic treatment-naïve patients as well as from patients on treatment (BRAFi, BRAFi + MEKi, immunotherapy) were collected along with clinical data on age, gender, primary melanoma diagnosis, locoregional therapy, and systemic therapy (Table 1S). Representative H&E-stained slides were scanned at $\times 400$ magnification using the Aperio AT2 platform (Leica Biosystems, Wetzlar, Germany). Each digitised slide was imported into the HALO Link® (Indica Labs, Albuquerque, NM) image management system and reviewed by three pathologists with recognised expertise in melanoma and soft tissue pathology (D.M., D.M.P., A.A.) to reach a consensus diagnosis based on previously proposed morphological, immunohistochemical, and genetic criteria [5].

The diagnosis of a 'genuine DedM' was rendered as defined above if the tumour fulfilled the above criteria and in addition showed lack of morphological and immunophenotypic features of melanoma, that it is negative for all routinely used melanoma markers (pS100, SOX10, MelanA, HMB45 and melanoma cocktail). DedM is the main pattern observed in primary tumours showing transition from conventional melanoma to a sarcoma-like component associated with loss of melanoma markers.

While primary DedM as a composite tumour is usually straightforward diagnosis, metastatic undifferentiated melanoma should fulfil the diagnostic criteria proposed recently by Agaimy et al. [5] and outlined above. In case of melanoma of unknown primary (MUP), the metastasis should show a melanoma-typical mutation such as NRAS, BRAF or NF1 and the metastasis should not correspond to a specific histological pattern, but displays non-specific morphologies known to occur in diverse undifferentiated malignancies such as undifferentiated pleomorphic sarcoma (UPS)-like, unspecified spindle cell sarcoma-like, undifferentiated round cell sarcoma-like, undifferentiated epithelioid/rhabdoid morphology, pleomorphic rhabdomyoblastic, osteoblastic or chondroblastic patterns [3].

Similar criteria were applied to diagnose tumours as 'compatible with DedM' when there was no previous history of melanoma or where the genotype of the primary melanoma was unknown [5]. In those uncommon cases with metastatic MUP, only cases in which at least one metastasis showed unequivocal melanocytic differentiation were considered. Moreover, in patients with previous melanoma history, specific sarcoma types such as leiomyosarcoma, Ewing's sarcoma, and synovial sarcoma were considered independent (secondary) tumours, and therefore excluded.

The study was approved by the institutional Research Ethical Board (ID 132/19 ASST Papa Giovanni XXIII Bergamo, Italy).

2.2. DNA extraction

In all specimens meeting inclusion criteria and having sufficient archival tissue, DNA extraction was performed with an automated commercial isolation system (Maxwell FFPE RSC DNA kit, Promega). DNA was spectrometrically quantified (NanoDrop, Thermo Fisher).

2.3. DNA methylation analysis and copy number profiling

DNA was analysed on the Illumina Infinium Human Methylation bead chip microarray platform according to manufacturer's instructions (service provided by Life & Brain AG, Bonn). Data were normalised (SWAN (Subset quantile Within-Array Normalization)) and beta values obtained with minfi [18] as described [19–21]. Genome-wide CNPs were computed from microarray data with conumee [22] as described [19,23].

2.4. Comparative analysis of methylation data

DNA methylation profiles were integrated in a pan-cancer dimension reduction plot using the EpiDiP (www.epidip.org) platform that calculates Uniform Manifold Approximation Projection [24] plots based on

top differentially methylated probes as described [19–21]. In short, reference data contain large fractions of previously published methylation data from The Cancer Genome Atlas (TCGA legacy portal), Gene Expression Omnibus (GEO). Furthermore, at the time of this writing, the EpiDiP data lake contains more than 6000 currently non-annotated datasets from anonymous external sources. This dataset also contains large numbers of *bona fide* melanoma samples, in particular from the TCGA, but also the GEO repositories GSE90496 [23] and GSE140686 [25]. Of note, EpiDiP provides a comprehensive set of artefact-laden datasets which typically resulted from degraded or quantitatively insufficient DNA. In such cases, the microarray data show a discrete methylation pattern which reproducibly clusters, allowing exclusion of data from inappropriate samples. The nearest 15 annotated neighbour cases were scored concerning methylation-based classification. Hence, a case surrounded by 15 neighbours annotated as melanoma would receive a 100% melanoma score, while cases falling at the edge of a cluster would consecutively score lower. The maximum score was considered diagnostic for each sample.

3. Results

Demographics, clinical presentation, morphological features, immunohistochemical and molecular-genetic findings for the 61 patients (78 formalin-fixed and paraffin-embedded [FFPE] tissue samples) meeting our inclusion criteria of DedM are summarised in Table 1S. The median age of our cohort was 57 years (range 24–89 years), 30 (49%) patients were males. Overall, 60/61 were metastatic melanoma patients, while 1/61 presented with undifferentiated primary melanoma. Of the 60 metastatic DedM cases, 53 patients had a prior history of histologically confirmed primary melanoma, while 7 patients were diagnosed as metastatic MUP (11.6%).

Specifically, the histological pattern of the undifferentiated melanoma was assessed based on defined morphological features in analogy to the classification of soft tissue and bone sarcomas as well as other rare patterns. An unclassified pleomorphic and spindle cell morphology akin to UPS-like was the most frequently encountered pattern ($n = 21$), followed in decreasing order of frequency by small round cell sarcoma-like, admixed with medium-sized epithelioid cells ($n = 20$), large rhabdoid/epithelioid cell morphology ($n = 12$), spindle cell sarcoma-like, occasionally mimicking low-grade fibromyxoid sarcoma ($n = 11$), small round cells admixed with spindled cells ($n = 9$), myxoid/myxofibrosarcoma-like ($n = 3$), chondroblastic/osteogenic sarcoma ($n = 1$), and adenocarcinoma-like ($n = 1$). A focal pleomorphic rhabdomyosarcoma-like component was noted in 2 cases with otherwise UPS-like morphology. One tumour with spindle cell sarcoma-like features showed focal osteogenic features.

Genetic findings are reported in Table 1S. Anatomical sites of metastatic DedM were skin/soft tissue ($n = 25$), lymph nodes ($n = 19$), lung ($n = 10$), and viscera ($n = 23$). In one case, disseminated disease was diagnosed. DedM metastasis occurred synchronously to the primary tumour in 3 patients (cases 32, 37, and 47).

Among 61 patients with DedM, 33 received locoregional and/or systemic therapy (Table 1S), 29 patients received first-line systemic therapy. Median age of treated patients was 51 years. Fourteen patients received first-line immunotherapy, 9 BRAF plus MEK inhibitors, 4 locoregional therapy (3 radiotherapy and 1 electrochemotherapy), 1 patient concomitant RT and immunotherapy. Five patients received chemotherapy because of an initial diagnosis of an undifferentiated tumour. An undifferentiated metastasis occurred after, during, or prior to systemic therapy in 9, 7, and 4 patients, respectively. In 7 patients, dedifferentiation occurred during and after therapy (2 patients), before and during treatment (2 patients) and before and after therapy (3 patients). Partial or complete response to systemic therapy was observed in 12 patients (41%), whereas 16 patients experienced progressive disease (55%); in 4 cases (14%) response was unknown. Among patients, who received systemic therapy, with stages M1b, M1c, and M1d ($n = 21$), 11 (52%) achieved response. Among patients receiving first-line immunotherapy 7/14 achieved an objective response, among patients BRAF mutated who received first-line BRAFi plus MEKi 5/9 responded to the treatment. Nine of the 33 treated patients were still alive, at a median follow-up of 48 months. Median overall survival from starting systemic therapy was 12 months, and a long-term response (> 6 months) was observed in 9 patients (27%).

4. Chromosomal copy number variation and epigenetic features

Combined methylation and CNP was performed on specimens from 20/61 patients with confirmed DedM and yielded interpretable data for 16/61 patients. Overall, in 20 tissue samples from 16 patients, we found non-melanoma MS in 13 tissue samples and melanoma MS in 7 tissue samples.

Two patients (patient 23 and 64) had three samples taken from different sites, two patients (patient 32 and 48) from two different sites and one patient (patient 22) from four different sites investigated. Interestingly, the tumour in patient 22 exhibited a melanoma MS in a lymph node metastasis while the MS in the separately analysed tumour fraction growing into the extranodal adipose tissue had a MS more similar to osteosarcoma (OS). Another metastasis occurring 1 year later under targeted therapy again showed an OS-like MS. Patient 23 who was initially diagnosed as cancer of unknown primary demonstrated a melanoma-typical MS in a

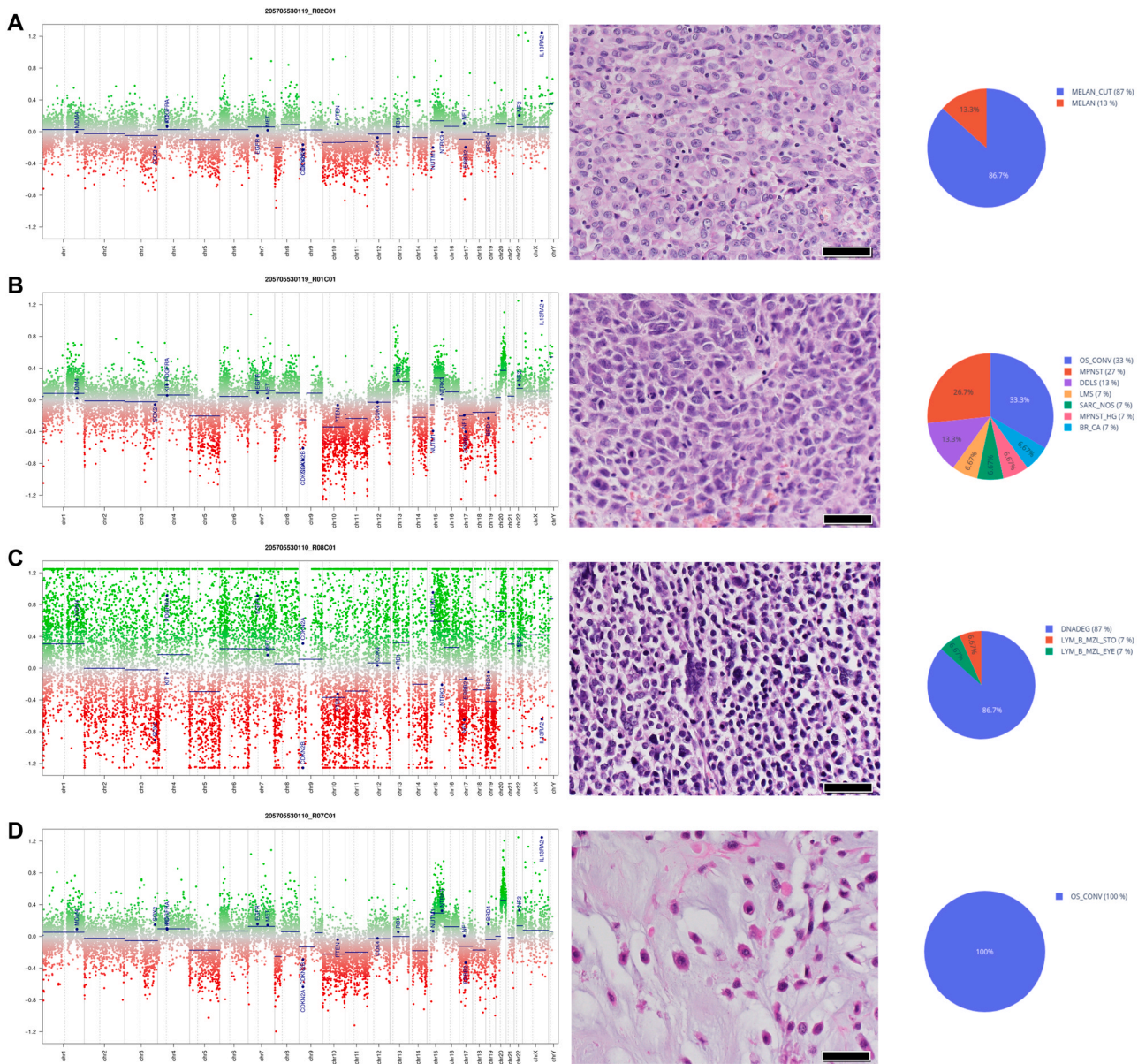


Fig. 1. Patient 22. Axillary lymph node metastasis (A) with extranodal fat involvement (B) before therapy and metastasis during therapy (C, D). All copy number profiles from DNA methylation microarrays exhibit similar copy number changes, indicating clonal relationship of all biopsy specimens. Conventional H&E histology reflects increasing degrees of dedifferentiation; scale bars: 50 μ m. Pie charts show Uniform Manifold Approximation Projection methylation scores, indicating epigenetic similarity to melanoma (A), conventional osteosarcoma (B), degraded DNA (C), and conventional osteosarcoma (D); scores generated with www.epidip.org.

lymph node metastasis whereas concurrent soft tissue metastases had a MS most similar to epigenetic reference data of malignant peripheral nerve sheath tumours (MPNST). A visceral metastasis to the jejunum occurring during immunotherapy 2 years later exhibited a melanoma-typical MS again (Figs. 1 and 2).

In addition to the above-mentioned aberrant MS in DedM, tumours of further 10 DedM patients with clinico-histologically confirmed melanoma diagnosis also showed deviant MSs: Again, epigenetic resemblance to malignant soft tissue tumours, mostly OS and

MPNST, were predominant (Table S2). CNP derived from methylation microarray data independently of DNA methylation profiles exhibited changes typical of cutaneous melanoma (loss of 1p, 6q, 9p and gains of 1q, 6p or 8q) in 11/16 patients. As such, these profiles suggest the presence of melanoma despite atypical MSs.

Comparison of CNVs (Copy number variations) from different metastases of the same patients (patients 22–23) confirmed clonal relationship, even if the methylation profile was replaced with OS-like, MPNST-like or UPS-like MS (Figs. 1 and 2).

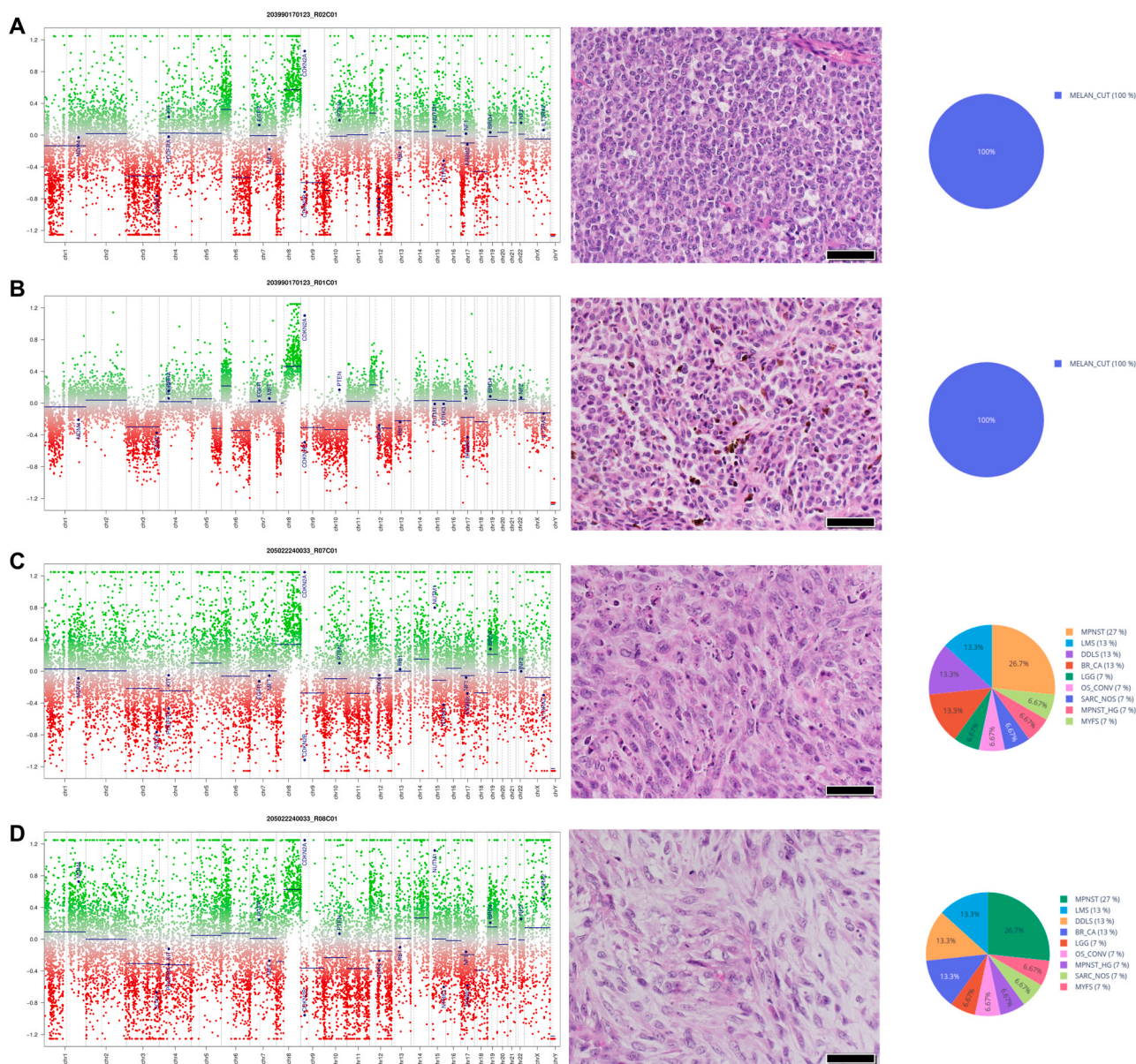


Fig. 2. Patient 23. Copy number profiles of three different metastatic sites before (A, C, D) and after treatment 3 years later (B) featuring four distinct morphological patterns indicating clonal relationship. Initial lymph node metastasis (A) and peritoneal metastasis under therapy with retained melanoma methylation signature in Uniform Manifold Approximation Projection plotting (pie charts). Histology is shown on H&E-stained slides; scale bar 50 μ m. Note that in specimen B melanin pigment can be identified. Initial soft tissue metastasis (C, D) with divergent histological patterns; DNA was extracted separately from two sites within the same tumour specimen. The epigenome aligns with malignant peripheral nerve sheath tumours (MPNST) and other high-grade mesenchymal neoplasms.

5. Discussion

Our study conveys four potential relevant results: i) loss of MS occurs in DedM to an extent that it is no longer possible to recognise these tumours epigenetically as melanomas; ii) the epigenetic features may also confer to a misdiagnosis of specific types of soft tissue sarcomas in a large proportion of patients such as OS and MPNST; iii) in most cases of DedM, the CNP is preserved despite loss of the melanoma MS; iv) clinical features, mutation profile, CNP and MS should be integrated to achieve a correct diagnosis of DedM. Finally, a significant

proportion of patients with DedM achieve a clinical response to systemic therapy. These findings are clinically and translationally novel, timely and relevant both for pathologists and clinicians.

In our study, a significant proportion of melanomas lost their melanoma-like MS. In the case of low epigenetic concordance with a wide range of sarcomatous tumours, chromosomal CNPs should be checked against the chromosomal aberrations typically present in cutaneous melanoma [23] (reference cases on www.epidip.org from the TCGA). In case primary tumour specimens from the same patient are available, these

should be subjected to methylation and CNP in parallel to the current metastatic disease in order to detect potential clonal relationships. In cases where dedifferentiation has altered the neoplastic epigenome to the extent that it is no longer matching cutaneous melanoma, alternative approaches such as targeted parallel sequencing should be performed. This is particularly helpful if a given tumour exhibits MS of MPNST or OS, while its CNP is not reminiscent of such entities.

From a practical point of view, the current study adds on available criteria for DedM [5] and for challenging cases suggests a step-wise approach which includes the following: the medical history of prior melanoma and in case of unknown primary, at least one differentiated melanoma metastasis with melanoma-typical mutation such as NRAS, BRAF or NF1. Moreover, analysis of more than one sample in patients with probable metastatic MUP is more promising to find at least one metastasis with melanoma-compatible MS or CNP, especially in cases with BRAF/NRAS/NF1-triple negative genotypes. Clinicians and pathologists should be aware of the challenging significance of MS alone which can lead to a misinterpretation of pathologic findings and misdiagnoses in > 50% of cases, if used in isolation. For this reason, an integration of MS and CNP is suggested by our study. In general, combined methylation and copy number analysis seems to be a helpful technology complementing histological workup, capable of potentially eliminating the need for costly large parallel sequencing approaches.

Our study results illustrate that a diagnosis of DedM should not be made on the basis of a MS alone. For example, patient 23 would have been diagnosed with MPNST. This patient with unknown primary and DedM metastases on the thigh received a correct diagnosis thanks to clinics with clear-cut inguinal melanoma metastasis, NRAS mutation, ‘melanoma specific’ CNP despite MS of an MPNST. None of the divergent epigenomes in our study showed epigenetic similarity to epithelial or hematolymphoid neoplasms.

Our study extends and validates in a clinical context some preclinical findings suggesting that epigenetic mechanisms are crucial in the regulation of the melanoma cell differentiation state [17,26]. The shift in MS likely represents a process of retrograde differentiation of melanocytes in which melanoma cells re-gain a stem cell feature, and, in turn, could shift to a neuroectodermal or mesodermal lineage. Interestingly, with regards to our series, the majority of patients presented with an OS-like MS, suggesting indeed a mesodermal lineage. It has been reported that neural crest dedifferentiation is an adverse phenotype and that dedifferentiation could be a state of cellular resistance to ICIs (Immune checkpoint inhibitors) and targeted therapies since cancer cells revert to a more primitive cellular phenotype. In our series 12/29 (41%) patients responded to systemic therapy, and 26% showed a long-term

response. Notably, among patients with M1b, M1c, M1d disease, 50% of patients responded to systemic therapy. Our results are in partial disagreement with previous reports [27,28] but in agreement with Kim et al. [12], who demonstrated an IFN- γ -induced dedifferentiation signature as a biological explanation of response in patients with DedM.

Our study has several strengths including the following: i) the relatively large cohort of DedM, which has been centrally reviewed by pathologists with expertise in dermatopathology and soft tissue pathology; ii) CNP was informative even in melanoma samples that lost the melanoma-like MS; iii) the translational relevant finding that MS mechanisms may drive phenotypic presentation of melanoma to an extent that it is no longer possible to recognise these tumours epigenetically as melanoma. However, our study has some weaknesses including the following: i) the retrospective nature, ii) the lack of preclinical mechanistic studies and iii) the relatively limited cohort to reliably draw conclusions on dedifferentiated state as a poor prognostic feature in melanoma.

In summary, our study by reporting a relevant diagnostic tool for DedM diagnosis and identifying a translational mechanism of melanoma plasticity contributes to a better understanding of a challenging and rare phenomenon in melanoma biology. Moreover, our study represents a novel addition to the diagnostic features of dedifferentiation in malignant neoplasms as we show that not only cumulative molecular-genetic aberrations but also epigenetic reprogramming are involved in the cellular plasticity and dedifferentiation of cancer. Future studies will evaluate possible impact of epigenetic drugs and the most suitable systemic therapy scheme (melanoma versus sarcoma-tailored) as a potential therapeutic strategy to be implemented in DedM with non-melanoma-like MS.

Funding

This work was funded by an EORTC Melanoma Grant to M.M. and D.M. and supported by Fondazione AIRC ‘Programma di ricerca 5 per Mille’ (ID# 21073 2018 to D.M. and EPICA investigators).

CRedit authorship contribution statement

Conceptualisation: Daniela Massi and Mario Mandalà. **Data curation:** Juergen Hench, Daniela Mihic-Probst, Abbas Agaimy, Mario Mandalà and Daniela Massi. **Formal analysis:** Juergen Hench, Daniela Mihic-Probst, Abbas Agaimy, Mario Mandalà and Daniela Massi. **Funding acquisition:** Daniela Massi and Mario Mandalà. **Investigation:** Juergen Hench, Daniela Mihic-Probst, Abbas Agaimy, Mario Mandalà and Daniela Massi. **Methodology:** Juergen Hench, Daniela Mihic-Probst, Abbas Agaimy, Mario Mandalà and Daniela

Massi. **Project administration:** Juergen Hench^{S1}, Daniela Mihic-Probst, Abbas Agaimy, Mario Mandalà and Daniela Massi. **Resources:** all authors. **Software:** Juergen Hench, Stephan Frank, Peter Meyer, Claus Hultschig. **Supervision:** Juergen Hench, Daniela Mihic-Probst, Abbas Agaimy, Mario Mandalà and Daniela Massi. **Validation:** Juergen Hench, Daniela Mihic-Probst^{S2}, Abbas Agaimy, Mario Mandalà and Daniela Massi. **Visualisation:** Juergen Hench, Daniela Mihic-Probst, Abbas Agaimy, Mario Mandalà and Daniela Massi. **Roles/Writing – original draft:** Juergen Hench, Daniela Mihic-Probst, Abbas Agaimy, Mario Mandalà and Daniela Massi. **Writing – review & editing:** all authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ejca.2023.03.032](https://doi.org/10.1016/j.ejca.2023.03.032).

References

- [1] Miller AJ, Mihm Jr. MC. Melanoma. *N Engl J Med* 2006;355:51–65.
- [2] Gattenlöhner S, Brocker EB, Muller-Hermelink HK. Malignant melanoma with metastatic rhabdomyosarcomatoid transdifferentiation. *N Engl J Med* 2008;358:649–50.
- [3] Agaimy A, Specht K, Stoehr R, Lorey T, Märkl B, Niedobitek G, et al. Metastatic malignant melanoma with complete loss of differentiation markers (undifferentiated/dedifferentiated melanoma): analysis of 14 patients emphasizing phenotypic plasticity and the value of molecular testing as surrogate diagnostic marker. *Am J Surg Pathol* 2016;40:181–91.
- [4] Rupp NJ, Rechsteiner M, Freiberger SN, Lenggenhager D, Urosevic M, Burger IA, et al. New observations in tumor cell plasticity: mutational profiling in a case of metastatic melanoma with biphasic sarcomatoid transdifferentiation. *Virchows Arch* 2018;473:517–21.
- [5] Agaimy A, Stoehr R, Hornung A, Popp J, Erdmann M, Heinzerling L, et al. Dedifferentiated and undifferentiated melanomas: report of 35 new cases with literature review and proposal of diagnostic criteria. *Am J Surg Pathol* 2021;45:240–4.
- [6] Ferreira I, Droop A, Edwards O, Wong K, Harle V, Habeeb O, et al. The clinicopathologic spectrum and genomic landscape of de/trans-differentiated melanoma. *Mod Pathol* 2021;34:2009–19.
- [7] Ferreira I, Arends MJ, van der Weyden L, Adams DJ, Brenn T. Primary de-differentiated, trans-differentiated and undifferentiated melanomas: overview of the clinicopathological, immunohistochemical and molecular spectrum. *Histopathology* 2022;80:135–49.
- [8] Massi D, Mihic-Probst D, Schadendorf D, Dummer R, Mandalà M. Dedifferentiated melanomas: morpho-phenotypic profile, genetic reprogramming and clinical implications. *Cancer Treat Rev* 2020;88:102060.
- [9] Cipriani NA, Letovanec I, Hornicek FJ, Mullen JT, Duan Z, Borger DR, et al. BRAF mutation in 'sarcomas': a possible method to detect de-differentiated melanomas. *Histopathology* 2014;64:639–46.
- [10] Powell MR, Sheehan DJ, Kleven DT. Altered morphology and immunohistochemical characteristics in metastatic malignant melanoma after therapy with vemurafenib. *Am J Dermatopathol* 2016;38:e137–9.
- [11] Curry JL, Falchook GS, Hwu WJ, Torres-Cabala CA, Duvic M, Tetzlaff MT, et al. Changes in tumor morphology and cyclin-dependent kinase inhibitor expression in metastatic melanoma treated with selective second-generation BRAF inhibitor. *Am J Dermatopathol* 2013;35:125–8.
- [12] Kim YJ, Sheu KM, Tsoi J, Abril-Rodriguez G, Medina E, Grasso CS, et al. Melanoma dedifferentiation induced by IFN- γ epigenetic remodeling in response to anti-PD-1 therapy. *J Clin Invest* 2021;131:e145859.
- [13] Hossain SM, Gimenez G, Stockwell PA, Tsai P, Print CG, Rys J, et al. Innate immune checkpoint inhibitor resistance is associated with melanoma sub-types exhibiting invasive and de-differentiated gene expression signatures. *Front Immunol* 2022;13:955063.
- [14] Benboubker V, Boivin F, Dalle S, Caramel J. Cancer cell phenotype plasticity as a driver of immune escape in melanoma. *Front Immunol* 2022;13:873116.
- [15] Zhu EY, Riordan JD, Vanneste M, Henry MD, Stipp CS, Dupuy AJ. SRC-RAC1 signaling drives drug resistance to BRAF inhibition in de-differentiated cutaneous melanomas. *NPJ Precis Oncol* 2022;6:74.
- [16] Civenni G, Walter A, Kobert N, Mihic-Probst D, Zipser M, Belloni B, et al. Human CD271-positive melanoma stem cells associated with metastasis establish tumor heterogeneity and long-term growth. *Cancer Res* 2011;71:3098–109.
- [17] Sanna A, Phung B, Mitra S, Lauss M, Choi J, Zhang T, et al. DNA promoter hypermethylation of melanocyte lineage genes determines melanoma phenotype. *JCI Insight* 2022;7:e156577.
- [18] Fortin J-P, Triche TJ, Hansen KD. Preprocessing, normalization and integration of the Illumina HumanMethylationEPIC array with minfi. *Bioinformatics* 2017;33:558–60.
- [19] Hench J, Vlajnic T, Soysal SD, Obermann EC, Frank S, Muenst S. An integrated epigenomic and genomic view on phyllodes and phyllodes-like breast tumors. *Cancers* 2022;14:667.
- [20] Haefliger S, Tzankov A, Frank S, Bihl M, Vallejo A, Stebler J, et al. NUT midline carcinomas and their differentials by a single molecular profiling method: a new promising diagnostic strategy illustrated by a case report. *Virchows Arch* 2021;478:1007–12.
- [21] Saleh C, Jaszczuk P, Hund-Georgiadis M, Frank S, Cordier D, Hench IB, et al. Differentiation of rare brain tumors through unsupervised machine learning: clinical significance of in-depth methylation and copy number profiling illustrated through an unusual case of IDH wildtype glioblastoma. *Clin Neuropathol* 2021;40:17–24.
- [22] Hovestadt V, Zapatka M. *onumee*: enhanced copy-number variation analysis using Illumina DNA methylation arrays. R package version 1.9.0 Available from: (<http://bioconductor.org/packages/conumee>).
- [23] Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D, et al. DNA methylation-based classification of central nervous system tumours. *Nature* 2018;555:469–74.
- [24] McInnes L, Healy J, Melville J. UMAP: uniform manifold approximation and projection for dimension reduction. *ArXiv180203426 Cs Stat*. 2018.
- [25] Koelsche C, Schrimpf D, Stichel D, Sill M, Sahn F, Reuss DE, et al. Sarcoma classification by DNA methylation profiling. *Nat Commun* 2021;12:498.
- [26] van den Bogaard EH, Podolsky MA, Smits JP, Cui X, John C, Gowda K, et al. Genetic and pharmacological analysis identifies a physiological role for the AHR in epidermal differentiation. *J Invest Dermatol* 2015;135:1320–8.
- [27] Holzel M, Tuting T. Inflammation-induced plasticity in melanoma therapy and metastasis. *Trends Immunol* 2016;37:364–74.
- [28] Balletta P, Sanchez-Del-Campo L, Chauhan J, Effern M, Kenyon A, Kershaw CJ, et al. Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma. *Genes Dev* 2017;31:18–33.