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Original Research

Impact of second opinion pathology review in the diagnosis and management of atypical melanocytic lesions: A prospective study of the Italian Melanoma Intergroup (IMI) and EORTC Melanoma Group

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Abstract ***Background:*** The clinical value of an expert pathological review in patients with an atypical melanocytic lesion diagnosis remains unclear. Herein, we evaluate its impact in a prospective clinical study.
Methods: Patients with newly diagnosed or suspected atypical melanocytic proliferations and challenging skin tumours were reviewed prospectively by a specialised dermatopathologist through the nationwide 'Second Opinion Platform' of the Italian Melanoma Intergroup (IMI) network. The primary aim was the rate of major discrepancies that impacted patient management. Major discrepancies in diagnosis between referral and specialised review were blindly re-analysed by a panel of European Organisation for Research and Treatment (EORTC) Melanoma pathologists.

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Results: The samples submitted to central review included 254 lesions from 230 patients. The most frequent referral diagnoses were atypical melanocytic nevi of different subtypes (74/254, 29.2%), invasive melanomas (61/254, 24.0%), atypical melanocytic proliferations (37/254, 14.6%), AST (21/254, 8.3%) and in situ melanomas (17/254, 6.7%). There was disagreement between referral diagnosis and expert review in 90/254 cases (35.4%). Most importantly, 60/90 (66.7%) were major discordances with a change to the patient's clinical management. Among the 90 discordant cases, the most frequent new diagnosis occurred in World Health Organisation (WHO) Pathway I, followed by WHO Pathway IV (64/90 and 12/90, respectively). In total, 51/60 cases with major discrepancies were blindly re-evaluated by EORTC Melanoma pathologists with a final interobserver agreement in 90% of cases.

Conclusion: The study highlights that a second opinion for atypical melanocytic lesions affects clinical management in a minor, but still significant, proportion of cases. A central expert review supports pathologists and clinicians to limit the risk of both over- and under-treatment.

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1. Introduction

Second opinion review of pathology specimens is a common practice among pathologists within an institution or between different institutions (extradepartmental consultation), and many healthcare institutions require systematic in-house review of outside histopathology material prior to entering patients into routine therapeutic management or clinical studies, irrespective of the complexity of the case [1]. Consultations may also be requested by patients in case of diagnostic uncertainty when facing rare or unusual tumours, or when seeking confirmation because of the perceived severity of the first diagnosis, or when different therapeutic options are proposed.

Skin tumours are frequently the object of disagreements in pathology [2,3]. In particular, previous studies have reported high interobserver variability in atypical melanocytic lesions, which comprise a wide and heterogeneous group of tumours, usually showing high cytoarchitectural diversity, especially if the diagnosis falls in the middle diagnostic categories of the spectrum, for example, moderately dysplastic nevi to early-stage invasive melanoma [4–6] or Spitz tumours [7].

The recent World Health Organisation (WHO) multidimensional classification led to profound changes in taxonomy based on nine different pathways and the ensuing recognition of novel entities recognised on the basis of morphology coupled with molecular-genetic changes [8,9]. Accurate subclassification within WHO pathways is critical for treatment optimisation and may require ancillary analyses, including immunohistochemistry, fluorescence in situ hybridisation (FISH) and molecular analyses [10]. However, some healthcare facilities do not have timely access to novel antibodies, complex analyses and molecular platforms, thus causing inadequate or delayed diagnostic processes that may affect clinical management and patient outcome. Thus, a national Second Opinion consultation service from the Italian Melanoma Intergroup (IMI) was recently established in Italy with the aim of providing IMI centres with a systematic and timely pathologic review of newly diagnosed ambiguous melanocytic lesions and to further optimise patient care. Prospective studies are lacking in this field and, consequently, the impact of an expert pathological review on patients with an atypical melanocytic lesion diagnosis remains unclear.

The two co-primary end-points of the current study were the following: (i) to analyse the overall frequency of major discrepancies between referral and final diagnoses by the IMI Second Opinion consultation service; (ii) to assess the clinical and therapeutic impact of the centralised IMI pathology consultation in case of major discrepancies. Secondary end-points included (i) the overall frequency of minor discrepancies between referral and IMI diagnoses; (ii) consistency of the IMI specialised diagnosis compared with a panel of subspecialty experts from European Organisation for Research and Treatment (EORTC) Melanoma Group; (iii) use of ancillary tests instrumental to the improved diagnosis.

2. Materials and methods

2.1. IMI Second Opinion Platform review process

From August 2018 and June 2022, IMI members were invited, prospectively, to submit cases with an inconclusive diagnosis of atypical melanocytic lesion or diagnostically ambiguous/difficult melanocytic tumours through the IMI Second Opinion Platform (https://www.melanomaimi.it/). The purpose of the request was specified in each case. Each case was submitted with representative H&E slides, and/or paraffin block(s), clinical history and, if available, molecular testing, and clinical and dermoscopical images.

A board-certified dermatopathologist (D.M.), with academic medical centre affiliation and subspecialty

training of more than 24 years and expertise, reviewed the original histopathological slides and requested additional immunohistochemical, FISH or molecular analyses, when deemed necessary. These criteria were assessed as indicated by the current recommendations of the WHO Classification of melanocytic tumours [8,9]. The cases and therapy options were discussed with referral clinicians and with IMI dermatologists and IMI oncologists, when appropriate, and a final histopathological second opinion report was released within 10–14 d.

Data collected from the reports included age, sex, date of the report, hospital system/lab and IMI centre where the original report was generated, type of ancillary analyses (conventional histopathological examination, ancillary immunohistochemical, FISH and molecular analyses), anatomical site, original (provisional/uncertain or definite) referral and expert diagnosis. The use of terminology conveying various degrees of certainty and lack of unequivocal diagnosis ('consistent with, suggestive of, suspicious for, highly consistent with, highly suggestive of, some features of') from referral pathologists was recorded.

The study was approved by the Ethical Committee of Azienda USL Toscana Centro Regione Toscana (#11988 CEAVC) on 29th March 2018, and by the scientific IMI board committee. The IMI second opinion was included in the IMI website (www.melanomaimi.it) and the service was provided neither at cost to the clinicians nor to the patients. Dermatopathologists, dermatologists and medical oncologists belonging to IMI had free access to the centralised diagnostic platform. For each case, written informed consent was obtained from the patient, and a referral clinician, to whom the final diagnosis was posted by email, was identified.

2.2. Immunohistochemical, FISH and molecular tests in adjunct to morphological diagnosis

Incoming ancillary analyses (e.g. IHC, FISH and molecular tests) and additional tests performed at a central site were recorded. Additional IHC stains were performed on Benchmark Ultra Immunostainer (Ventana Medical Systems, Tucson, AZ, USA) upon central review. Melanoma and 9p21 FISH probes were performed on VP2000 Vysis (Abbott Molecular Inc, Des Planes, IL).

For melanoma, multicolour FISH DNA kit (Vysis/Abbott Molecular, Des Plaines, IL) was used, composed of LSI *RREB1* (6p25) SpectrumRed/LSI *MYB* (6q23) SpectrumGold/LSI *CCND1* (11q13) SpectrumGreen/CEP6 (6p11.1-q11 Alpha Satellite DNA) SpectrumAqua. For 9p21, Vysis LSI *CDKN2A* SpectrumOrange/CEP 9 Spectrum Green probe kit (Abbott Molecular Inc, Des Plaines, IL) was used.

NGS was performed on Myriapod NGS Cancer panel DNA. Normalised libraries were mixed (library pool) and sequenced in parallel on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). The data generated by the sequencer were analysed locally with dedicated Myriapod NGS Data Analysis Software (Diatech Pharmacogenetics, Jesi, Italy); other molecular tests were performed on MALDI-TOF mass spectrometry associated with Single Base Extension technology with CE-IVD marked system Myriapod® COLON status validated on MassARRAY platform (Diatech Pharmacogenetics, Jesi, Italy).

2.3. Evaluation of diagnostic discrepancy between submitted diagnoses and IMI second review

The concordance rate was assessed as the number of cases of each subtype with the same diagnosis from both the referral and expert pathologists. Discordances between the pathology reports from referring institutions and the centralised IMI report were classified as major or minor. Discordance was categorised as major if therapeutic management changes occurred, while changes that did not affect stage and/or clinical care were classified as minor discordance; finally, if reports were substantially overlapping, cases were classified as no discordance.

2.4. Interobserver reproducibility within a panel of expert EORTC Melanoma Group pathologists

Among cases that had a major discrepancy with the referral diagnosis, samples with sufficient quality of representative slides were additionally digitalised by Aperio AT2 platform (Leica Biosystems, Wetzlar, Germany) and, after anonymisation, brought to the consensus assessment within a panel of three dermatopathologists subspecialty experts from the EORTC Melanoma Pathology Group (A.S.C., L.A., M.G.C.) on a HALO Link platform (Indica Labs, Albuquerque, NM, USA).

2.5. Potential impact of a change in diagnosis on clinical management

Major discordance with management change was defined as a discordance that led to modification in surgical procedures and/or systemic therapy. For cases with major discordance with therapeutic strategy, change in management was categorised as (i) requiring surgery or a significant change in planned surgery; (ii) surgery cancelled (no longer deemed necessary); (iii) requiring systemic therapy with or without surgery.

3. Results

3.1. Distribution frequency of diagnostic categories from the IMI Second Opinion review

Fig. 1 shows the flow chart of the study. Overall, from January 2018 to June 2022, 254 consecutive second opinion

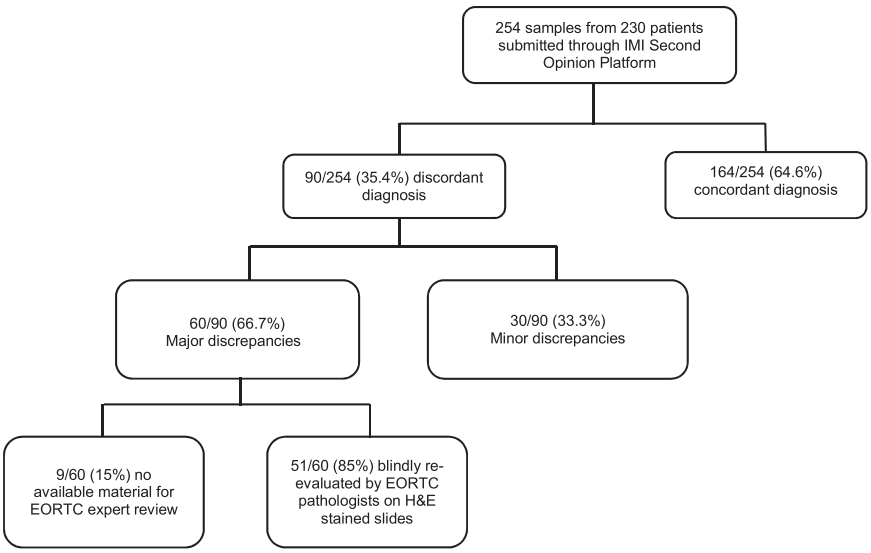

Fig. 1. Flow chart of the study.

cases for 230 patients from 43 Italian institutions were included. Requests were submitted from Northern (n = 61), Central (n = 25) and Southern Italy (n = 168) through the website (www.melanomaimi.it). Consultations were requested from pathologists (n = 61) or treating physicians (n = 193). Ninety-six patients were males (41.7%). Median age was 40 years (range 4–90 years). Paediatric cases (≤18 years) constituted 10% of all submitted cases. Excised lesions were taken from skin (n = 231), sentinel lymph nodes (SLN, n = 8), lymph nodes (n = 2), mucosal sites (vulva, vagina and conjunctiva n = 3), soft tissue (n = 5), nail matrix (n = 1), parotid gland (n = 1) and visceral sites (brain, stomach and peritoneum, n = 3). For 16 cases, histopathological material (glass slides and/or IHC stains) was submitted with an undetermined/provisional diagnosis, while in 238 cases a definitive and conclusive diagnosis was delivered. Among the cases submitted for expert review, the most frequent diagnoses were atypical melanocytic nevi of different subtypes (74/254, 29.2%), atypical melanocytic proliferations (37/254, 14.6%), invasive melanomas (61/254, 24.0%), AST (21/254, 8.3%) and in situ melanomas (17/254, 6.7%). A detailed description of the submitted referral diagnoses is reported in Table 1 and examples of submitted cases are shown in Figs. 2–4.

Table 1
Original diagnoses submitted to consultation to the IMI Second Opinion Platform (n = 254).

Submitted diagnosis	N	%
Benign nevus (Spitz nevus, acral nevus, congenital nevus, blue nevus, Reed nevus)	45	17.8
Intermediate (BIN, DPN, atypical nevus, low/high grade dysplastic nevus)	29	11.4
Atypical melanocytic lesion/neoplasm/hyperplasia/proliferation	37	14.6
AST	21	8.3
PEM	5	1.9
MelTUMP	5	1.9
SAMPUS	2	0.8
MIS	17	6.7
Invasive melanoma	61	24.0
Melanoma metastasis	16	6.3
Undetermined/provisional[a]	16	6.3

BIN: BAP-1 inactivated nevus; DPN: deep penetrating nevus; AST: atypical spitz tumour; PEM: pigmented epithelioid melanocytoma; MelTUMP: melanocytic tumour of uncertain malignant potential; SAMPUS: superficial atypical melanocytic proliferation of uncertain significance; MIS: in situ melanoma.
[a] Provisional report without a final diagnosis.

3.2. Immunohistochemical, FISH and molecular tests in adjunct to morphological diagnosis

Incoming ancillary analyses (e.g. IHC, FISH and molecular tests) and additional tests performed at the central site were recorded. Specifically, additional IHC stains were performed in 176/254 cases (69.3%), FISH analyses (*CDKN2A; CCND1; RREB1; MYB; CCND1;* CEP6) in 31/254 cases (12.2%), PCR-real time in 5/254 cases (1.9%), MALDI-TOF mass spectrometry in 2/254 cases (0.8%) and NGS in 5/254 cases (1.9%).

Ancillary immunohistochemical tests most commonly performed included a panel of antibodies: HMB-45; SOX10; tyrosinase, MART-1; p16; Ki-67/MIB1; VE1 (BRAFV600E); ALK1; ROS1; Pan-TRK; BAP-1; beta-catenin; PRAME; PRKAR1A. FISH analyses included *CDKN2A; CCND1; RREB1; MYB; CCND1;* CEP6 in 31/254 cases (12.2%). Molecular analyses included: PCR-real time in 5/254 cases (1.9%), MALDI-TOF mass spectrometry in 2/254 cases (0.8%) and NGS in 5/254 cases (1.9%).

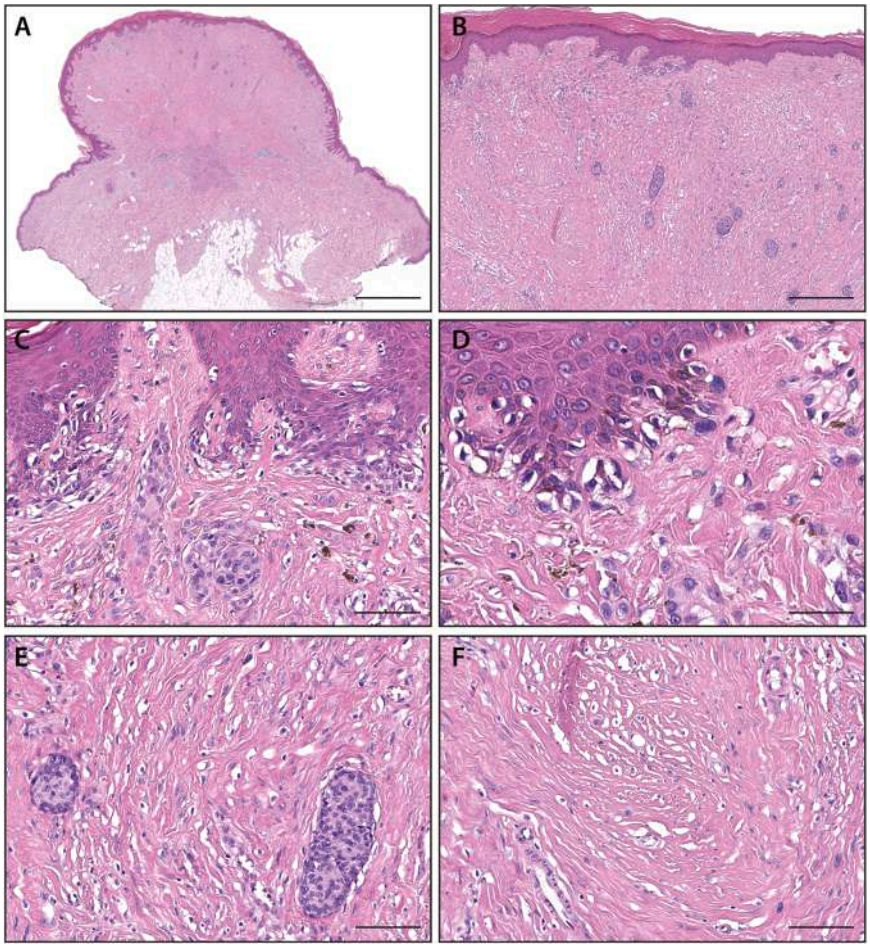

Fig. 2. Female, 52 years (case 058, Table 1). Amelanotic nodular lesion of the left elbow clinically diagnosed as amelanotic melanoma. Referral histopathological diagnosis: Lentiginous in situ melanoma on soft fibroma with associated intradermal melanocytic nevus. IMI Second Opinion diagnosis: Desmoplastic congenital nevus with atypia. A: Scanning magnification shows a nodular compound (mostly dermal) paucicellular melanocytic proliferation (H&E, 1×, scale bar 1 mm). B: Higher magnification shows aggregates of melanocytes surrounded by a dense fibrotic stroma (H&E, 4×, scale bar 250 μm). C: Disordered junctional architecture (H&E; 10×, scale bar 100 μm). D: The junctional component comprises of basilar single cells and disordered junctional nesting with no significant pagetoid spread (H&E, 20×, scale bar 50 μm). E: Dermal aggregates of melanocytes with uniform nuclei (H&E, 20×, scale bar 50 μm). F: Prominent desmoplastic stroma composed of thick, eosinophilic collagen bundles (H&E, 40×, scale bar 25 μm).

3.3. Discrepancy between submitted diagnoses and IMI second review

There was disagreement between submitted and IMI diagnoses in 90/254 cases (35.4%). Among the discordant cases, 60/90 (66.7%) represented a major discordance with change in management (Fig. 1, Table 2). In case of major discrepancy, the diagnosis was downgraded from malignant to intermediate or benign in 24/60 (40%) cases and, vice versa, from benign or intermediate to malignant in 34/60 (57%) cases. Despite correct melanoma diagnosis, pathological T misclassifications (from pT3a to pT3b, and from pT1b to pT1a) were identified in 2/60 (3%) original reports and were included among major discrepancies. Another case was re-classified from pT2a to pT1b and was included among minor discrepancies.

3.4. Interobserver reproducibility within a panel of expert EORTC Melanoma Group pathologists

To validate the IMI Second Opinion diagnosis, 51/60 (85%) cases with available material and major discrepancies in diagnosis were re-evaluated blindly by two EORTC Melanoma pathologists (L.A., A.S.C.). The two EORTC Melanoma pathologists were in agreement with IMI Second Opinion diagnosis in 41/51 cases (80%) (Table 1S). In the remaining 10 cases, the central panel included a third EORTC Melanoma pathologist (M.G.C.) resulting, at the end of the external review, in an interobserver agreement with the second opinion diagnosis in 9/10 cases (90%).

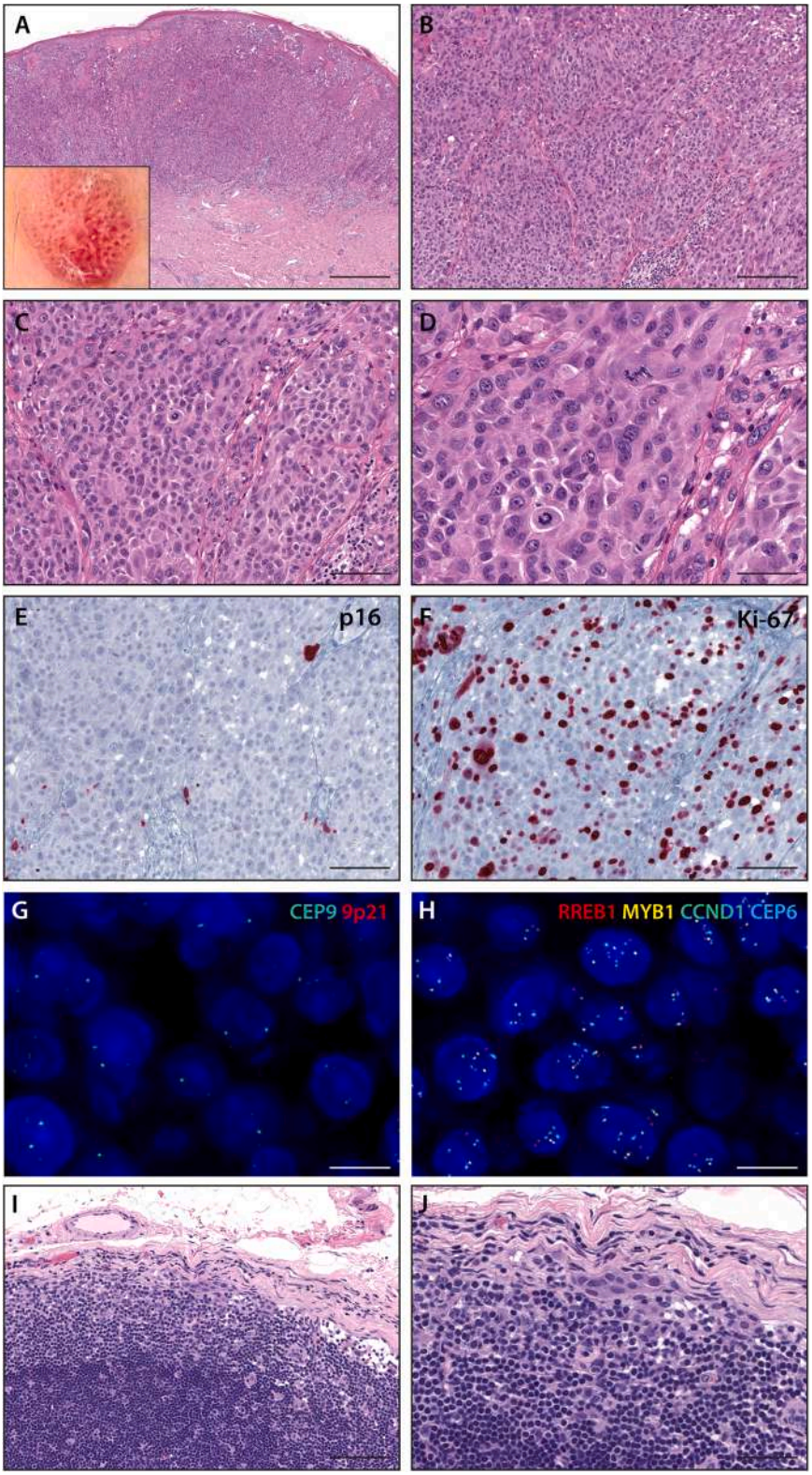

Fig. 3. Female, 16 years (case 060, Table 1). Cutaneous lesion of the right leg clinically diagnosed as hemangioma. Referral histopathological diagnosis: AST versus Spitzoid Melanoma. IMI Second Opinion diagnosis: Spitz Melanoma pT2a N1a(sn) with 9p21 homozygous deletion (Breslow 1.8 mm, absence of ulceration); *BRAF*wt. A: Bulky compound melanocytic tumour composed of epithelioid melanocytes extending to the deep dermis (H&E, 4×, scale bar 250 μm); inset: dermoscopic image showing an atypical vascular pattern. B: Confluent, expansile nests and sheet-like growth pattern of epithelioid melanocytes (H&E, 10×, scale bar 100 μm). C: At higher magnification, melanocytes show high grade nuclear atypia, thickened and irregular nuclear membranes, hyperchromatism and enlarged eosinophilic nucleoli (H&E, 20×, scale bar 50 μm). D: Mitoses including atypical and deep forms are seen (13 mitoses/mm^2; H&E, 20×,

scale bar 50 μm). E: By immunohistochemistry, melanocytes show diffuse lack of expression of p16 (20×, scale bar 50 μm). F: Ki67 proliferation index is > 20% (20×, scale bar 50 μm). G: FISH (*CDKN2A*/CEP9) shows 9p21 homozygous deletion (100×, scale bar 5 μm). H: Melanoma multicolour FISH probes show alterations in *CCND1* and *RREB1* (100×, scale bar 5 μm). I: Sentinel lymph node with subcapsular deposits (H&E, 20×, scale bar 50 μm). J: Higher magnification illustrates non-pigmented melanocytes with epithelioid cytology (H&E, 40×, scale bar 25 μm).

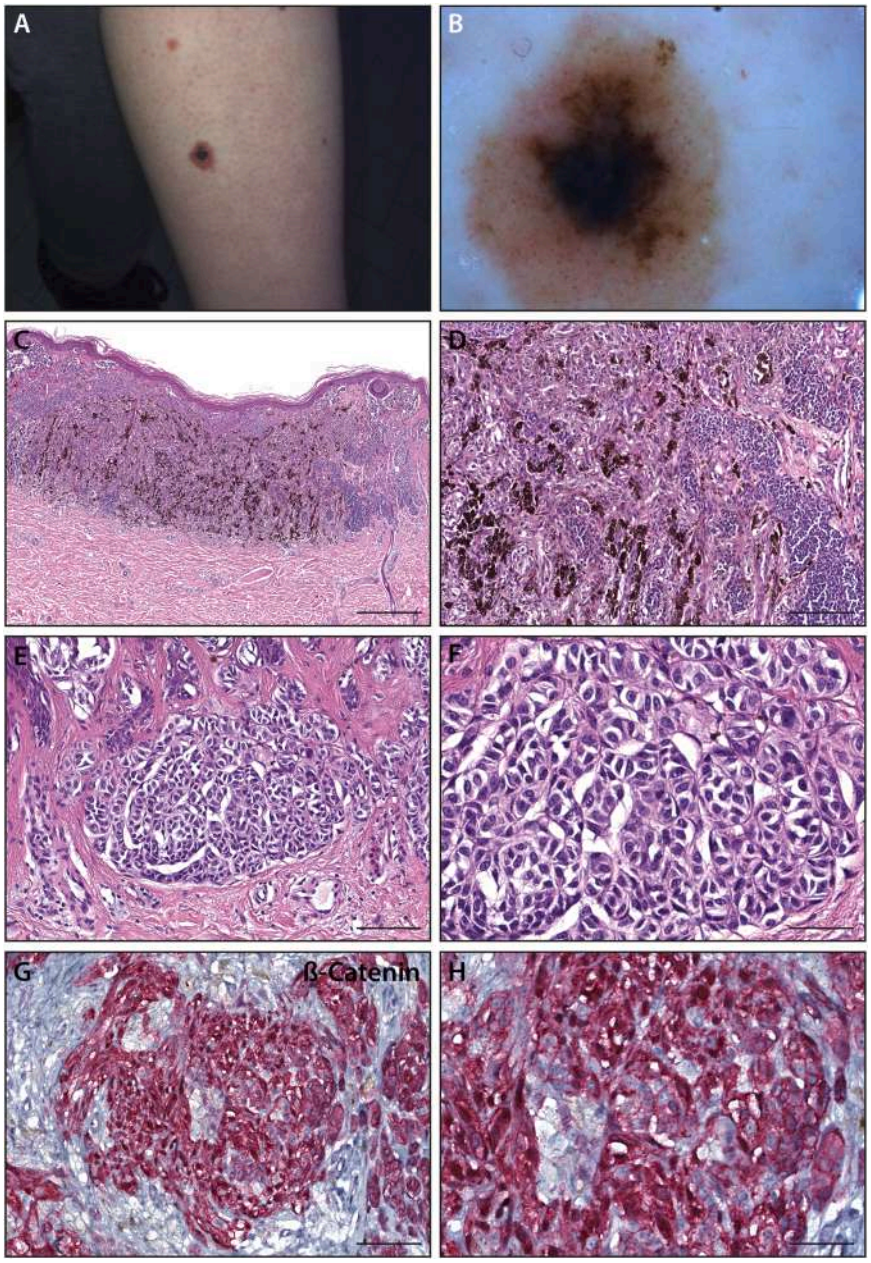

Fig. 4. Female (albino patient), 12 years (case 023, Table 2S). Irregularly pigmented lesion 11 × 9 mm in size, on the right leg, present for 2 years, in slow growth. Clinical diagnosis: Nevus? Melanoma? Referral histopathological diagnosis: Spitz tumour with uncertain potential. IMI Second Opinion diagnosis: Combined nevus with deep penetrating nevus component. A: Clinical image showing an irregularly pigmented lesion with shades of brown and black. B: Unusual polychromatic appearance by dermoscopy. C: Scanning magnification shows a dermal-based melanocytic lesion consisting of pigmented melanocytes intermingled with abundant melanophages (H&E, 4×, scale bar 250 μm). D: Enlarged epithelioid to spindle-shaped melanocytes, melanophages and a conventional nevus component are seen (H&E, 10×, scale bar 100 μm). E: Epithelioid melanocytes are arranged in fascicles and bundles of variable size surrounded by thickened collagen bundles (H&E, 20×, scale bar 50 μm). F: Melanocytes are relatively uniform and show enlarged nuclei (H&E, 40×, scale bar 25 μm). G: Melanocytes show ß Catenin positivity by immunohistochemistry (20×, scale bar 50 μm). H: Higher magnification more clearly illustrates nuclear ß Catenin positivity (40×, scale bar 25 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Cases with major discrepancies between original referral diagnosis and IMI Second Opinion diagnosis and therapeutic implications ($n = 60$).

Case ID	Age	Gender	Primary site	First referral diagnosis	IMI Second Opinion diagnosis	Surgery/medical therapy change		
001	8	M	Left limb	Unusual melanocytic proliferation, with conflicting morphological features and unpredictable biological behaviour	Spitz melanoma (pT3b)	Re-excision	SLNB	Adjuvant therapy
002	29	F	Left elbow	MelTUMP	Spitz melanoma (pT3a)	Yes, 20 mm	Yes, performed positive	Yes
003	42	M	Trunk	MelTUMP	Melanoma arising on congenital nevus (pT3a)	Yes, 20 mm	Yes, performed negative	No
004	44	F	Left limb	Spitz nevus versus MIS	SSM (pT1a)	Yes, 20 mm	Yes, performed negative	No
005[a]	53	F	Left arm	Compound melanocytic nevus with Spitzoid features	SSM (pT1a)	Yes,10 mm	No	No
006[a]			Left arm	Atypical Spitz nevus	MIS	Yes, 10 mm	No	No
007	20	F	Trunk	Atypical Spitz nevus	SSM (pT1a)	Yes, 5 mm	No	No
008	28	M	Trunk	AST	SSM (with nevus remnants) (pT1a)	Yes, 10 mm	No	No
009	39	M	Nail matrix	Atypical melanocytic hyperplasia	Acral MIS	Yes, 10 mm	No	No
010	29	F	3° finger	Melanoma in VGP with epithelioid cells, naevoid and Spitzoid features arising on melanocytic congenital nevus (pT3a)	Combined congenital nevus with an atypical (epithelioid cell) Spitzoid component	Yes, 5 mm	No	No
011	24	F	Back	Dysplastic compound nevus with MIS component	Dysplastic nevus (high grade)	No	No	No
012[a]	30	F	Right arm	SSM (pT3a)	Blue nevus	No	No	No
013[a]			1° axillary sentinel lymph node	Rare epithelioid Melan-A+ cells	Nodal nevus	No	No	No
014[a]			2° axillary sentinel lymph node	Rare epithelioid Melan-A+ cells	Nodal nevus	No	No	No
015	55	F	Neck	Dysplastic nevus with areas of transition in atypical melanocytic intraepithelial neoplasm	MIS arising on nevus	No	No	No
016	24	F	Abdomen	AST versus Naevoid melanoma (pT1b)	Combined melanocytic nevus with BAP-1 loss	Yes, 5 mm	No	No
017	48	M	Left ear lobe	Dermal localisation of melanoma	Atypical melanocytic tumour with BAP-1 loss combined with dermal nevus	No	No	No
018	21	M	Left leg	SSM with Spitzoid features (pT1b)	AST	No	No	No
019[a]	18	F	Left flank	SSM (pT1a)	Compound nevus	Yes, 5 mm	No	No
020[a]			Back	MIS	Compound nevus	No	No	No
021	18	F	Axillary sentinel lymph node	Epithelioid cells (MART-1+, HMB-45+, SOX10+) suggestive, but not conclusive, for metastatic deposit (0.5 mm)	Capsular Blue nevus	No	No	No
022	13	F	Scalp	NM (pT2a)	Atypical melanocytic tumour with BAP-1 loss/melanocytoma combined with congenital dermal nevus	No	No	No
023	52	F	Right flank	Compound melanocytic lesion with junctional component with moderate/severe atypia	MIS arising on nevus	No	No	No
024	25	F	Back	NM (pT3a)	NM (pT3b) (misclassification in pT)	Yes, 5 mm	No	No
025[a]	62	F	Back	Dysplastic junctional nevus (low grade)	MIS	No	No	No
026[a]			Back	Dysplastic junctional nevus (low grade)	MIS	Yes, 5 mm	No	No
027	70	F	Right subscapular region	MelTUMP	SSM with Spitzoid features (pT2a)	Yes, 5 mm	No	No

(*continued on next page*)

Table 2 (*continued*)

Case ID	Age	Gender	Primary site	First referral diagnosis	IMI Second Opinion diagnosis	Surgery/medical therapy change		
028	45	F	Prevesical peritoneum	Osteogenic melanoma arising on ovarian teratoma versus undifferentiated carcinoma	Poorly differentiated carcinoma	Yes, 10 mm	Yes	No
029	62	F	Sternal region	Lentiginous melanocytic proliferation	LM	No	No	No
030	43	M	Right limb	NM (pT1a)	Combined nevus (common acquired nevus and Spitz nevus)	Yes, 5 mm	No	No
031	39	M	Right flank	Junctional nevus	MIS	Yes, 10 mm	No	No
032	30	F	Left arm	Atypical melanocytic neoplasm	SSM with Spitzoid features (pT3a)	Yes, 5 mm	No	No
033	52	F	Interscapular region	Invasive melanoma (pT1a)	Dysplastic nevus (low grade)	Yes, 10 mm	Yes, performed negative	No
034	33	F	Inguinal sentinel lymph node	Micrometastasis of melanoma	Nodal nevus	No	No	No
035	18	F	Left limb	SSM (pT1a)	AST	No	No	No
036	61	M	Right leg	MIS	SSM (pT1a)	No	No	No
037	50	F	N.A.	SSM (pT1b)	SSM (pT1a) (misclassification in pT)	Yes, 10 mm	No	No
038	23	M	Right limb	Atypical Spitzoid melanocytic proliferation/AST	Spitz melanoma (pT3a)	Yes, 10 mm	No	No
039	36	M	Glans	The differential diagnosis is melanocytic tumour combined with aspects of compound genital nevus and PEM/borderline/MelTUMP and a malignant melanoma simulating blue nevus on nevus in the genital mucosa	Invasive mucosal melanoma arising on atypical genital nevus (pT2a)	Yes, 20 mm	Yes, performed negative	No
040	18	F	Abdomen	Junctional Spitz nevus associated with intradermal nevus	MIS arising on nevus	Yes, 10 mm	No	No
041	16	F	Right leg	Compound melanocytic proliferation with Spitzoid features and cytoarchitectural atypia	SSM with Spitzoid features (pT1a)	Yes, 5 mm	No	No
042	63	M	Left hand	Naevoid NM (pT3a)	Atypical cellular Blue nevus	Yes, 10 mm	No	No
043	52	M	Back	Compound melanocytic proliferation with severe junctional component, dysplasia and areas of MIS	Compound melanocytic nevus of superficial congenital type	No	No, not performed	No
044	79	M	Back	Poorly symmetrical and poorly demarcated compound melanocytic lesion. The junctional component shows a prevalent lentiginous growth pattern, with cytoarchitectural atypia consistent with the diagnosis of melanoma (pT1a)	SSM (pT1a)	No	No	No
045[a]	13	M	Right shoulder	Naevoid melanoma (pT1a)	Compound nevus, mitotically active	Yes, 10 mm	No	No
046[a]			Left flank	Naevoid melanoma (pT1a)	Dysplastic nevus (low grade)	No	No	No
047	36	M	Left shoulder	MIS with regressive phenomena	SSM (pT1a)	No	No	No
048	12	F	Left popliteal fossa	AST	Melanoma Spitz (pTx, shave biopsy)	Yes, 10 mm	No	No
049	39	F	Back	MIS	Dysplastic nevus (low grade)	Yes, 10 mm	Yes, performed negative	No
050	73	M	Right limb	MIS	SSM (pT1a)	No	No	No
051	38	F	Back	Reed nevus	MIS	Yes, 10 mm	No	No
052	62	M	Right shoulder	Junctional melanocytic proliferation, with severe borderline atypia with melanoma in situ, on pigmented compound superficial congenital nevus, desmoplastic type	MIS arising on nevus	Yes, 5 mm	No	No
053	55	F	Left arm	Composite melanocytic nevus with dysplastic features (Clark nevus)	SSM arising on nevus (pT1a)	Yes, 5 mm	No	No

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Table 2 (*continued*)

Case ID	Age	Gender	Primary site	First referral diagnosis	IMI Second Opinion diagnosis	Surgery/medical therapy change		
054	70	F	Left paravertebral region	Dysplastic compound nevus (high grade)	MIS arising on nevus	Yes, 10 mm	Yes, performed negative	No
055	32	M	Right arm	Intradermal melanocytic lesion with cytological atypia	Naevoid melanoma, papillomatous variant (pT2a)	Yes, 5 mm	No	No
056	30	M	Deltoid region	AST with high atypia	MIS arising on nevus	Yes, 10 mm	Yes	No
057	66	M	Right shoulder	Junctional and atypical intradermal melanocytic proliferation	Melanoma arising on congenital melanocytic nevus (nevus spilus) (pT1b)	Yes, 5 mm	No	No
058	52	F	Left elbow	Lentiginous MIS on soft fibroma and associated intradermal melanocytic nevus	Desmoplastic congenital compound nevus with junctional atypia	Yes, 10 mm	Yes	No
059	21	F	Back	SSM arising on congenital nevus (pT1a)	Congenital compound nevus	No	No	No
060	16	F	Right leg	AST versus Spitzoid melanoma	Spitz melanoma (pT2a)	No	No	No
						Yes, 10 mm	Yes, performed positive	Yes

AST: atypical spitz tumour; MIS: in situ melanoma; MelTUMP: melanocytic tumour of uncertain malignant potential; SSM: superficial spreading melanoma; ALM: acral lentiginous melanoma; NM: nodular melanoma; LM: Lentigo Maligna, PEM: pigmented epithelioid melanocytoma.

[a] Patient with more than one sample included in the current study.

3.5. Categorisation of diagnostic changes and their impact on clinical management

Table 1 illustrates therapeutic changes and management (clinical impact) in case of major discrepancies between referral and expert diagnoses for patients sent with a formal diagnosis. In 34/60 (57%) cases, a diagnostic change from benign or intermediate to malignant was observed. For SLN (8 cases), in four cases, there was a downgrading of metastatic melanoma to nodal nevus. Thus, considering the inclusion criteria of phase III adjuvant trials, at least four patients would have been falsely qualified for adjuvant therapy.

3.6. Minor discrepancies/misclassifications between referral diagnoses and IMI second review

Minor discrepancies or misclassifications resulting in no impact on clinical care were observed in 30 cases (33.3%) and are summarised in Table 2S.

4. Discussion

The present prospective clinical study supports the importance of a real-time expert pathologic review for the diagnostic definition of challenging atypical melanocytic lesions, as we report 33.8% diagnostic changes, including 22.5% major discrepancies with predicted clinical impact on patient management. Importantly, IMI-EORTC interobserver concordance validated the IMI Second Opinion diagnosis in 90% of cases.

Major findings of the study are the following: (i) we provide evidence, in the context of a prospective study, that expert review for atypical melanocytic lesions is clinically relevant; (ii) therapeutic management is impacted in a significant proportion of patients; (iii) a central expert review should be considered routinely in the clinical management of challenging atypical melanocytic lesions.

Our results validate, prospectively, findings from previous retrospective studies. Change in diagnosis for referred melanocytic lesions has been reported in 14–35% of cases [11–13]. A recent survey suggested that most pathologists request second opinions for melanocytic tumours of uncertain malignant potential (85%) or atypical Spitzoid lesions (88%) [14]. By retrospective retrieval of 358 dermatopathology cases, a second-opinion diagnosis was found to be discordant in 37/358 cases (10.3%). In 32 of 358 cases (8.9%), second-opinion review impacted treatment management, with surgery cancellation in 28/32 (87.5%) cases [15]. Moreover, in a simulated model considering a population of 10,000 individuals undergoing excision of a melanocytic lesion, diagnostic disagreement was more likely to cause 'over-calling' than 'under-calling' of melanoma [16]. In a retrospective study, major and minor discordances were reported in 20.2% and 48.8% of cases, respectively [17].

However, retrospective studies can harbour some intrinsic biases, including patient and diagnosis selection. In our study, we received consecutive cases that have been considered worthy of a second opinion, either by pathologists or clinicians. Overall, our prospective longitudinal study extends and strengthens findings of the above-reported studies [11–13,15,17], suggesting that the disagreement between referral and expert diagnosis is observed in about one-third of cases, with change in management in a majority of them.

In our study, 130/254 (51.2%) submitted cases were 'intermediate lesions' according to WHO classification; [8,9] among them, only 69/130 (53%) cases were confirmed as such. The recent WHO classification has underlined the importance of acknowledging intermediate-grade melanocytic proliferations [8,9,18]. By WHO definition, an intermediate lesion is 'a junctional and sometimes also superficial dermal lesion considered to be benign or equivocal that is characterised by cytological and architectural atypia, intermediate between wholly benign and fully malignant lesions, characterised genomically by mutations of two or more genes, but less than in fully evolved malignancy' [8]. An expert group opinion suggested a pragmatic diagnostic approach for each pathway described by WHO classification, either in the setting of general pathology labs or expert centres [10]. Intrinsically, the WHO's new classification, by introducing a higher complexity associated with the histopathologic diagnosis integrated by molecular analysis, has led to an increased request for second opinion diagnoses from specialised centres. In our experience, focusing on main grey areas according to WHO Pathways, like Spitz tumours, FISH/NGS added critical information to the diagnosis in almost 16% of cases where specific WHO pathways were implicated.

In terms of therapeutic impact, in 34/60 (57%) cases, a diagnostic change from benign/intermediate to malignant was observed. Specifically, in 4/34 (11.7%) cases a re-excision of 20 mm, in 17/34 (50%) cases a re-excision of 10 mm and in 13/34 (38.3%) cases a re-excision of 5 mm was required. In 10/34 cases (29.4%) patients underwent SLN biopsy according to current guidelines [19]. Most importantly, with regard to SLN, 4/8 (50%) cases were downgraded from metastatic melanoma to nodal nevus. Such results agree with recent findings showing high interobserver discrepancy in SLN assessment [20]. Thus, considering the inclusion criteria of phase III adjuvant trials, at least four patients would have been falsely qualified for adjuvant therapy. Notably, two cases were diagnosed with SLN metastasis, potential candidates for adjuvant immunotherapy.

In our experience, narrative reports incomplete for pathological staging refining features and absence of synoptic reports more frequently prompt second opinion requests. In addition, pathologists may use different expressions, for example, 'consistent with,' 'suggestive of,' 'compatible with…,' 'features indicative of' to convey their level of uncertainty in a diagnosis and/or to minimise their own personal legal risk in relation to possible misdiagnosis. However, such phraseology is not used consistently and treating physicians do not entirely understand their intentional significance [21]. To improve quality communications between pathologists and clinicians, recently, the newly revised MPATH-Dx V2.0 schema was introduced as an adjunct for standardised diagnostic reporting of melanocytic lesions and decision-making recommendations [22].

Without proper clinical monitoring, a proportion of second opinions may critically delay treatment, thus resulting in patient uncertainty and disappointment. Second opinions might be perceived as signals of patient distrust, harming the doctor–patient relationship [23]. Thus, the IMI future agenda includes the following: IMI National database with clinical monitoring and long-term follow-up data and the development of programs supported by patient advocates to help patients in seeking a second opinion within the healthcare system. The program includes suggesting specialists for the specific patient's problem and providing tools to reconcile between discrepant opinions for the search of a consensus diagnosis.

The financial implications of a second opinion have previously been addressed [24]. It has been recognised that second opinions may lower healthcare costs while reducing both over- and under-treatment [25]. In Italy, the National Health Service covers the first pathological diagnosis accessible to all citizens, without discrimination based on income or age, while the pathologic second opinion is not reimbursed and currently not included in the core benefits package (LEA). The present findings might prompt the Ministry of Health to include the procurement of a second opinion into the charter of patient rights and prominently display these rights in outpatient facilities.

The strength of our study is its prospective design reinforced by the blinded external panel review of challenging cases with major discrepancies. The main limitation is the lack of long-term follow-up. In conclusion, our results highlight the importance of a second specialised review for atypical melanocytic tumours. Since clinical management was impacted in a significant proportion of patients, our data strongly support routine second opinion to be included and reimbursed for the effective management of atypical melanocytic tumours.

CRediT authorship contribution statement

Conceptualisation: **D.M.**, **M.M.**; Data curation: **D.M.**, **S.S.**, **F.U.**; Formal analysis: **D.M.**, **A.S.C.**, **L.A.**, **M.M.**; Funding acquisition: **D.M.**; Investigation: **D.M.**, **A.S.C.**, **L.A.**, **M.G.C.**; Methodology: **S.S.**, **F.U.**; Project administration: **D.M.**; Resources: **D.M.**; Supervision: **D.M.**, **M.M.**; Validation: **D.M.**, **A.S.C.**, **L.A.**, **M.G.C.**; Visualisation: **D.M.**, **A.S.C.**, **L.A.**, **M.G.C.**; Writing

original draft: **D.M.**, **M.M.**; Writing review & editing: **D.M.**, **A.S.C.**, **L.A.**, **G.P.**, **I.S.**, **M.G.C.**, **M.M**.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2023.05.009.

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