Research Article

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Biomarkers in Medicine

Translation of a circulating miRNA signature of melanoma into a solid tissue assay to improve diagnostic accuracy and precision

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Aim: Successful treatment of cutaneous melanoma depends on early and accurate diagnosis of clinically suspicious melanocytic skin lesions. Multiple international studies have described the challenge of providing accurate and reproducible histopathological assessments of melanocytic lesions, highlighting the need for new diagnostic tools including disease-specific biomarkers. Previously, a 38-miRNA signature (MEL38) was identified in melanoma patient plasma and validated as a novel biomarker. In this study, MEL38 expression in solid tissue biopsies representing the benign nevi to metastatic melanoma spectrum is examined. **Patients & methods:** Nanostring digital gene expression assessment of the MEL38 signature was performed on 308 formalin-fixed paraffin-embedded biopsies of nevi, melanoma *in situ* and invasive melanoma. Genomic data were interrogated using hierarchical clustering, univariate and multivariate statistical approaches. Classification scores computed from the MEL38 signature were analyzed for their association with demographic data and histopathology results, including MPATH-DX class, AJCC disease stage and tissue subtype. **Results:** The MEL38 score can stratify higher-risk melanomas (MPATH-Dx class V or more advanced) from lower-risk skin lesions (class I–IV) with an area under the curve of 0.97 ($p < 0.001$). The genomic score ranges from 0 to 10 and is positively correlated with melanoma progression, with an intraclass correlation coefficient of 0.85 with stage 0–IV disease. Using an optimized classification threshold of ≥2.7 accurately identifies higher-risk melanomas with 89% sensitivity and 94% specificity. Multivariate analysis showed the score to be a significant predictor of malignancy, independent of technical and clinical covariates. Application of the MEL38 signature to Spitz nevi reveals an intrasubtype profile, with elements in common to both nevi and melanoma. **Conclusion:** Melanoma-specific circulating miRNAs maintain their association with malignancy when measured in the hypothesized tissue of origin. The MEL38 signature is an accurate and reproducible metric of melanoma status, based on changes in miRNA expression that occur as the disease develops and spreads. Inclusion of the MEL38 score into routine practice would provide physicians with previously unavailable, personalized genomic information about their patient's skin lesions. Combining molecular biomarker data with conventional histopathology data may improve diagnostic accuracy, healthcare resource utilization and patient outcomes.

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Over the past two decades, the annual incidence of melanoma has increased by 4–6% in fair-skinned populations of Northern Europe, North America, New Zealand and Australia [1]. Australia leads the world in melanoma incidence, with two out of three people diagnosed with some form of skin cancer by the age of 70 years old [2]. Unfortunately, public awareness campaigns and advances in diagnosis and therapeutics have not translated to prolonged improvements in the age-standardized mortality rates for this cancer type in many countries [3–6].

Currently, melanocytic lesions suspected of being malignant are diagnosed almost exclusively using visual methods, including dermoscopy, followed by biopsy and histopathologic examination. Previous advances in screening

Table 1. Summary of melanoma histopathology studies reporting diagnostic accuracy.

Study of melanoma histopathology accuracy	Study (year)	Cases (n)	Rate of major/minor diagnostic discordance (%)	Estimated rate of change in management (%)	Ref.
Discordance in diagnosis of melanocytic lesions and its impact on clinical management: a melanoma referral center experience with 1521 cases	2021	1521	20/49	11	[12]
Pathologists' diagnosis of invasive melanoma and melanocytic proliferations: observer accuracy and reproducibility study	2017	240	17/48	17	[11]
Discordance of histopathologic parameters in cutaneous melanoma: clinical implications	2016	588	19/NA	18	[13]
Pathology review significantly affects diagnosis and treatment of melanoma patients: an analysis of 5011 patients treated at a melanoma treatment center	2014	5011	5/22	11	[22]
Histomorphologic assessment and interobserver diagnostic reproducibility of atypical spitzoid melanocytic neoplasms with long-term follow-up	2014	75	70/NA	N/A	[14]
Histologically challenging melanocytic tumors referred to a tertiary care pigmented lesion clinic	2012	478	35/NA	13	[20]
Discordance in the histopathologic diagnosis of melanoma at a melanoma referral center	2010	392	14/NA	14	[21]
Interobserver variability on the histopathologic diagnosis of cutaneous melanoma and other pigmented skin lesions	1996	140	39/NA	39	[18]
Mean		680	27/40	17.5	

NA: Not applicable.

and diagnosis have increased the incidence of early-stage melanoma diagnoses worldwide but have not reduced overall mortality rates [3]. Over the past decade, new treatments including targeted therapies and immunotherapy have shown promise in reducing mortality. However, these treatments are approved for late-stage melanoma patients only, can cost more than US$100,000 per patient per year and their long-term benefit is still being studied [7,8].

The MPATH-Dx structure for describing melanocytic lesions is increasingly adopted by pathologists as a method to improve diagnostic consistency and communication between healthcare providers [9,10]. Despite this and other improvements in related areas such as dermoscopy, multiple studies into the accuracy and reproducibility of melanoma diagnosis by histopathology alone show that it is not adequately accurate or reproducible (see Table 1) [11–18]. Elmore *et al.* showed that 28% of MPATH-Dx class V melanomas, in other words, those requiring the widest excision margins and consideration of lymph node screening and adjuvant therapy, were significantly underdiagnosed [11]. Reproducibility of class V diagnoses was also lacking, with 17% of class V lesions examined receiving a lower classification when the same diagnostic slides were re-analyzed by the same pathologist at a different time point. There is consensus among experts that the introduction of standardized pathology terminology and new molecular tests are needed to improve diagnostic accuracy, patient outcomes and resource use efficiency [4,12,13,19–21].

Genomic profiling of miRNAs is a precise molecular technique that can be performed on many tissue types including blood, urine, tears and solid tissue (Figure 1). miRNAs are post-transcriptional regulators of gene expression which have tissue- and disease-specific patterns of expression [23]. Their role in melanoma oncogenesis and progression is well documented, including their crucial role in formation of the dermal tumor niche and activation of cancer-associated fibroblasts through exosomal secretion. Studies have shown that miRNAs can have either pro- or anti-oncogenic actions, resulting in increasing or decreasing levels of their expression as dermal cells develop malignant properties, interact with their microenvironment and invade nearby or distant tissues [24].

Previously, a signature of 38 circulating miRNAs ('MEL38') was identified by genomic profiling of plasma from individuals with stage I–IV melanoma and comparison with data from nonmelanoma controls. The biological functions of the MEL38 miRNAs include regulators of angiogenesis and inflammation (n = 2), invasion and metastasis (n = 14), immune response and treatment resistance (n = 11), and tumor suppression and oncogene activation (n = 8) [25,26]. The protein-coding mRNAs identified as those regulated by MEL38 overlap significantly with the MAPK signaling pathway ($p = 1.3 \times 10^{-11}$) [27]. Modulation of this pathway is responsible for therapeutically targetable BRAF and NRAS mutations in melanoma cells [27,28].

To express the information represented by the MEL38 signature in an easily interpretable and patient-personalized form, a gene-weighting algorithm is applied to the Nanostring-quantified miRNA counts to generate a classification score (i.e., MEL38 score). MEL38 scores range from 0 to 10 and are positively associated with increasing levels of

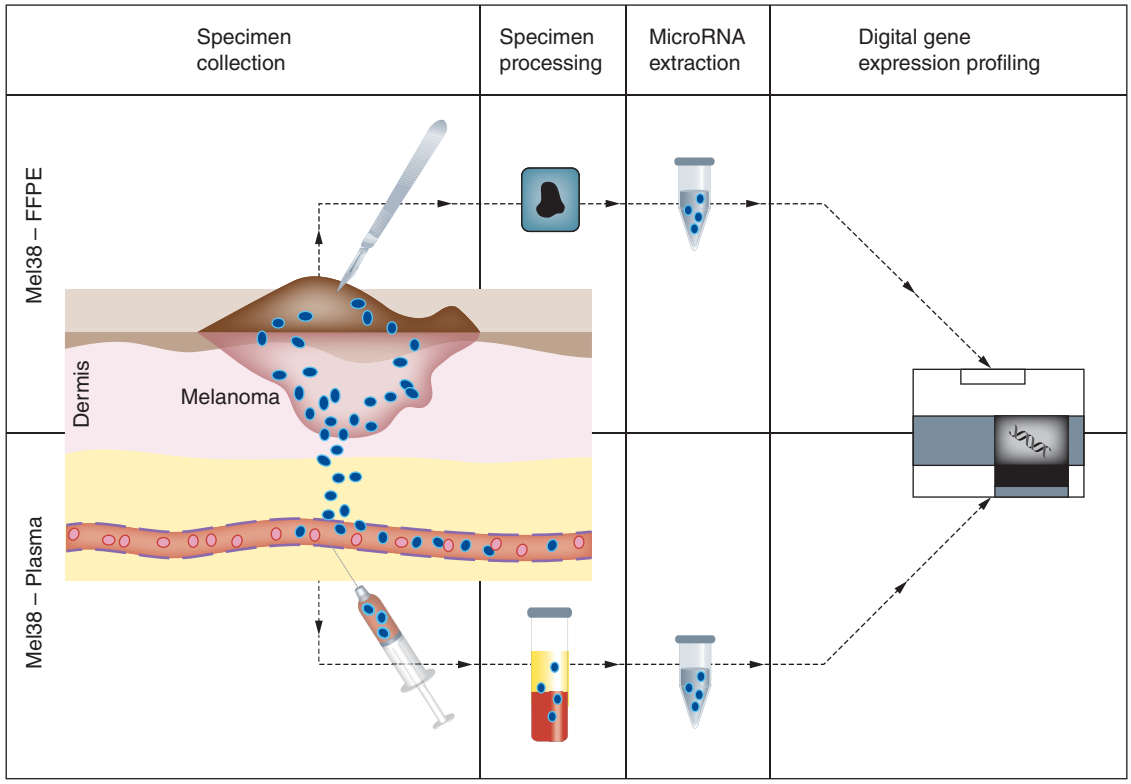

Figure 1. Schematic diagram of the development of MEL38, a miRNA signature of melanoma originally identified in plasma (lower section), and its adaptation to FFPE melanocytic skin lesions (upper section) using digital gene expression-profiling methods and classification algorithms previously described.
Data taken from [25–27].

melanoma malignancy, from benign nevi to metastatic disease. In multiple validation cohorts comprised of varied specimen types, the MEL38 score has shown robust and statistically significant associations with melanoma status, treatment response and prognosis [25–27].

In this study we perform MEL38 miRNA profiling of 308 clinically annotated, formalin fixed paraffin embedded melanocytic skin lesions, representing the most common specimen and histological subtypes analysed in skin pathology practices. The clinical and technical accuracy of the MEL38 signature is evaluated and contrasted with equivalent metrics for conventional diagnostic approaches to demonstrate the potential benefit of including genomic profiling in the diagnostic workup of melanocytic lesions.

Patients & methods

Sample population

This retrospective study was performed using melanocytic lesions that were submitted for diagnostic assessment (Australian Clinical Labs) as part of normal healthcare over the previous 5 years. Specimens were selected to represent the progression from benign nevi through to advanced invasive adult melanoma (18 years and older), including multiple common histological subtypes and disease stages (AJCC 8th Edition), as summarised in Table 2 [29]. An additional 14 samples of Spitz nevi from patients younger than 18 years old at the time of diagnosis were also included.

Each specimen was reviewed by two or more experienced dermatopathologists, with the majority diagnosis recorded as the result. The study was approved by Australian Clinical Labs internal medical advisory board and satisfies criteria for use of human tissue by diagnostic pathology companies as outlined by the Australian Government's National Health and Medical Research Council [30]. A study sample size of $\geq$300 specimens was set based on the methods described by Hajian-Tilak, assuming an area under the curve (AUC) of 0.78 or higher with 80% power and 95% confidence [31].

Table 2. Patient and specimen demographics.

Descriptor	n	%
Age:		
– Mean	60	
– <30	19	7
– 31–40	37	13
– 41–50	34	12
– 51–60	41	14
– >60	157	54
Gender		
– Males	154	53
– Females	134	47
Nevi		
– Intradermal	16	17
– Compound	37	40
– Junctional	16	17
– Spitz	20	22
– Other	4	4
Melanoma *in situ*		
– Lentigo maligna	33	69
– Superficial spreading	6	12
– Other	9	19
Invasive melanoma		
– Superficial spreading	84	50
– Nodular	29	17
– Lentigo maligna	8	5
– Other	46	28
AJCC clinical stage		
– IA	58	35
– IB	19	11
– IIA	17	10
– IIB	21	13
– IIC	13	8
– III/IV	39	23
MPATH-Dx class		
– I	33	11
– II	44	15
– III	50	17
– IV	54	18
– V	113	38

RNA extraction & genomic analysis

A minimum of two unstained slides containing a total of 20 µm of tissue with 20% or greater cellularity was obtained from each specimen. The reviewing pathologist indicated the area of interest for each specimen using a hematoxylin- and eosin-stained slide, which then guided the macrodissection process on the unstained slides. Total RNA was extracted from deparaffinated tissue using the miRNeasy FFPE kit (QIAGEN Cat no./ID: 217504, Hilden, Germany) according to manufacturer guidelines and quantified using a Qubit 2.0 fluorometer (Invitrogen, MA, USA). miRNA profiling was performed using the Nanostring nCounter Human v3 miRNA Panel with the nCounter SPRINT or nCounter MAX platform (Nanostring, Inc., WA, USA), according to the manufacturer's guidelines.

Data processing & statistical analysis

Log_2 scaled raw data were adjusted for technical variation by positive control scaling and 'top 100' normalization using the NanoStringNorm R library [32]. Normalized data were then examined for quality by comparing ten quality metrics with predetermined internal standards, including assessments of positive and negative controls, ligation efficiency controls, global mean, standard deviation and the degree of normalization applied. A classification score ('MEL38 score') was calculated for each specimen that passed the data quality control analysis, using the support vector machine (SVM) gene-weighting algorithm, described previously [26]. The SVM miRNA expression weights used to compute a MEL38 score were updated for differences between plasma and FFPE derived data by performing a partial retraining using representative subset of nevi (n = 12) and invasive melanoma samples (n = 12).

All data normalization, quality control and MEL38 score calculations were performed using customized R scripts and the results stored in a relational database. Statistical analysis and visualization were performed using

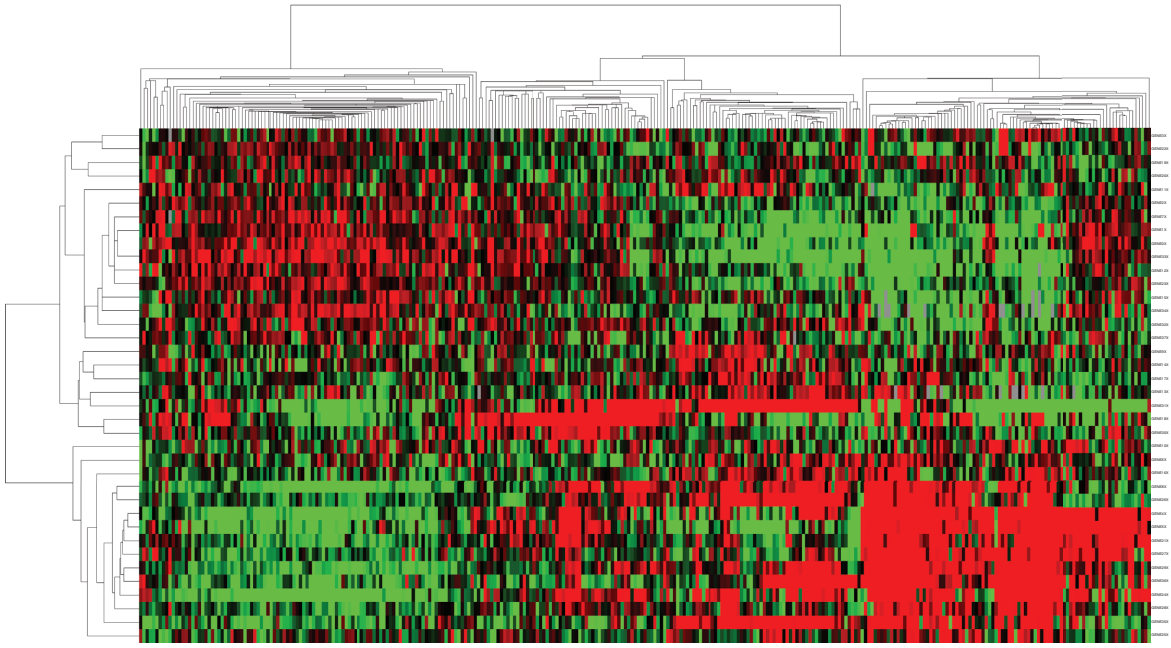

Figure 2. Supervised hierarchical clustering of the MEL38 signature data from 308 FFPE specimens of benign nevi, melanoma *in situ* and invasive melanoma. The clustering pattern revealed shows blocks of up and downregulation, reflecting the raw data used by the MEL38 classifier to compute each patient's individual MEL38 score.

Microsoft Excel and MedCalc [33,34]. All p-values calculated are two sided and when <0.05 were deemed to be statistically significant. Receiver operator curve (ROC) analysis was used to assess the sensitivity and specificity of the MEL38 score and to determine the optimal score threshold to classify specimens as either high or low risk of disease progression and a potentially poorer outcome [35]. Binary logistic regression was used to visualize the score as a continuous predictor of malignancy.

Results

Genomic profiling of melanocytic lesions representing the spectrum of benign melanocytic lesion to metastatic melanoma

The MEL38 signature was examined using total RNA from 308 FFPE tissue specimens with a melanocytic cellular content of 20% or greater. Patient and specimen details are summarized in Table 2. To visualize the relationship between the 38 miRNAs and specimen details, 2D hierarchical clustering of the complete cohort was performed (Figure 2). This resulted in an ordering of samples corresponding to an increasing degree of cancer progression (left to right). The gradual transition in levels of relative up and down miRNA expression reflects the continuum of nevi to invasive melanoma progression, as seen in clinical practice.

Notably, two miRNAs (hsa-mir-205 and hsa-mir-497) appear to have similarly low expression in the benign nevi and invasive/metastatic melanomas, but high expression in the early-stage invasive melanomas. These miRNAs may therefore have a specific role in aiding a melanoma cell to develop early invasive characteristics and may warrant further investigation.

The MEL38 score is positively correlated with increasing melanoma stage & is statistically significant independent to other variables

MEL38 scores for each specimen were calculated using SVM derived gene weights, as previously described. The scores range from 0 to 10 and are positivity correlated with the degree of malignancy, as shown by box plots of MEL38 versus AJCC stage (all samples) and versus MPATH-Dx classes (primary lesions only), in Figure 3A & B, respectively. The intraclass correlation of the score versus specimen status (nevi to metastatic melanoma) was 0.85.

To verify that the MEL38 score is a continuous predictor of malignancy, independent to other clinicopathological variables, general linear models were computed using patient age, gender, histological subtype and AJCC stage

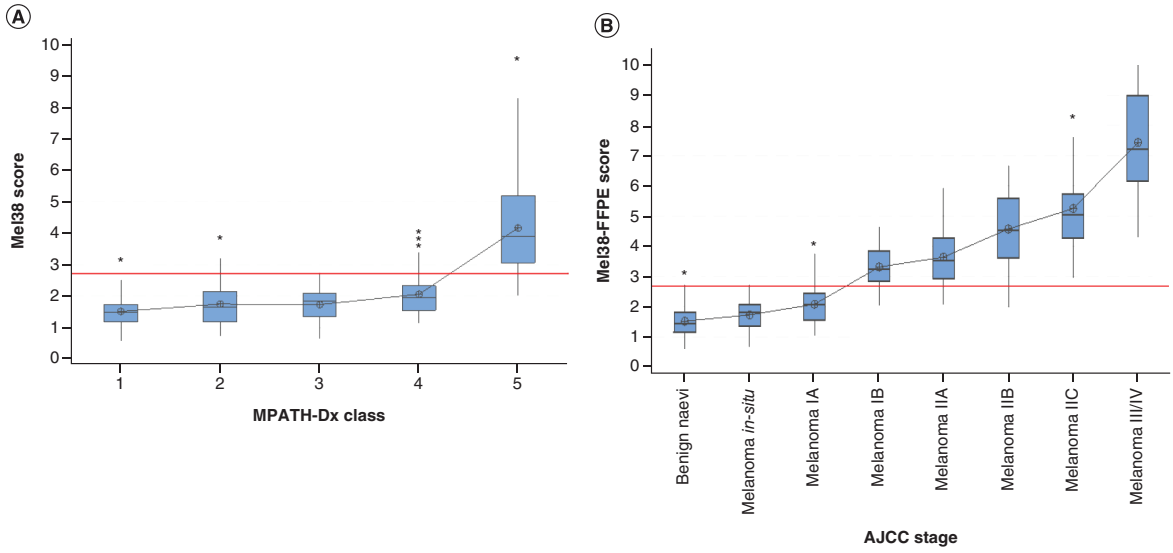

Figure 3. MEL38 scores vs conventional diagnostic categories. (A) Box plot of MEL38 scores of primary melanocytic lesions by MPATH-Dx class. **(B)** Box plot of MEL38 scores of primary melanocytic lesions by AJCC clinical stage (8th Edition). Connecting lines between boxes correspond to mean values. Red horizontal line indicates the MEL38 classification threshold of 2.7 for higher-risk lesions.

or MPATH-Dx class [9]. In both models, the genomic score was significantly different between AJCC stages and MPATH-Dx classes, independent to the other variables in the model ($p < 0.001$).

A separate multivariate analysis of MEL38 scores from invasive melanoma specimens only (n = 128) was performed, including, Breslow depth, tumor cell content, patient age, gender and biopsy site. In this subset, the MEL38 score remained statistically significantly between disease stages ($p = 0.012$), independent of the other variables included in the statistical model, indicating robustness to other factors known to influence miRNA expression.

The MEL38 score is a binary classifier of clinically higher- versus lower-risk melanocytic lesions

While the MEL38 score demonstrates a statistically significant continuous association with malignancy, inspection of the MPATH-Dx versus MEL38 box plot (Figure 3B) shows that the largest difference in genomic scores occurs between MPATH-Dx class IV and V specimens. Notable differences in suggested clinical actions and patient outcome (i.e., 5- and 10-year disease-specific survival rates) are also observed between these two classes, as summarized in Table 3. Further assessment of using the MEL38 score as a binary classification tool to assign higher- (M-PATH Dx class V) versus lower-risk (classes I–IV) lesions was then performed.

ROC analysis on MEL38 scores from 110 MPATH-Dx class V versus 175 class I–IV specimens resulted in AUC of 0.97 (95% CI: 0.95–0.99; $p < 0.001$) (Figure 4A). The binary fitted line plot shown in Figure 4B indicates the probability of MPATH-Dx class V status, according to a biopsied lesion's MEL38 score.

Inspection of the ROC data and application of the Youden index revealed that a MEL38 score of ≥2.7 was the optimal classification threshold for classifying a specimen as higher risk; defined as an MPATH-Dx class V or more advanced primary lesion [35]. This threshold corresponded to a true positive rate of 89% (i.e., sensitivity; 95% CI: 82–94) and true negative rate of 94% (i.e., specificity; 95% CI: 89–97). These data suggest that using MEL38 with a threshold of ≥2.7 to identify class V/higher-risk lesions would result in an underdiagnosis rate of 8.4%, compared with the reported rate of 27% for conventional pathology alone [11].

ROC analysis was also performed for MEL38 scores and AJCC staging. An AUC of 0.96 (95% CI: 0.95–0.99) was computed, as shown in Figure 4C. A binary fitted line plot was generated to visualize the probability of a ≥stage 1b lesion versus MEL38 (Figure 4D).

MEL38 profiling of Spitz nevi shows similarity to both benign & invasive melanoma

Spitz nevi are an uncommon type of melanocytic nevus, often histologically similar to melanoma and regarded as a challenging subcategory of melanocytic skin lesions to diagnose. MEL38 analysis was performed on 20 specimens

Table 3. MPATH-Dx classes, mean MEL38 scores and associated clinicopathological variables, including suggested clinical actions.

MPATH-Dx class:	I	II	III	IV	V
MEL38 score (mean)	1.5	1.7	1.7	2.1	5.2
MEL38 risk group	Low risk				High risk
T stage	N/A	N/A	0	T1a	T1b or more
Perceived risk for progression	Very low risk	Low risk	Higher risk	Substantial risk for local/regional progression	Greatest risk for regional and/or distant metastases
5/10-year melanoma-specific survival (%)†	100/100	100/100	100/100	98/96	≤93/≤89
Suggested clinical action‡	Follow-up as necessary	Narrow but complete excision (<5 mm)	Complete excision with at least 5 mm but <10 mm margins	Wide local excision with ≥10 mm margins	Wide local excision with ≥10 mm margins. Consideration of sentinel lymph node biopsy, adjuvant therapy
Clinical action likely to require referral	No				Yes

†Melanoma specific survival figures based on AJCC melanoma staging and outcome data corresponding to the T-stage associated with each MPATH-Dx class.
‡Suggested clinical actions assume representative sampling and positive margins.
NA: Not applicable.
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of Spitz nevi submitted for routine histopathologic analysis from individuals ranging from 2 to 30 years old. As can be observed in Figure 1, the expression profile of the Spitz nevi exhibits similarity to both benign and malignant lesions. Inspection of individual miRNAs in this figure reveals several that appear to have Spitz-specific patterns of expression, including hsa-mir-424-5p, hsa-mir-301a-3p and hsa-mir-1537-3p.

One-way ANOVA of the MEL38 scores in Spitz nevi versus other nevi subtypes revealed a statistically significant difference ($p < 0.001$), with mean scores of 2.7 (95% CI: 2.5–3.0) versus 1.5 (95% CI: 1.4–1.7), respectively. Pairwise comparisons between Spitz and other nevi subtypes were also statistically significant. When analyzed using a multivariate general linear model, incorporating patient age, gender and nevi subtype, the MEL38 scores of the Spitz nevi were only significantly higher than the compound nevi subtype ($p = 0.015$). This suggests that the younger ages of the Spitz nevi patients, compared with the rest of the cohort, may have an influence on the MEL38 expression profile.

The MEL38 score exhibits low technical variability between replicates & in longitudinal control sample analysis

RNA from 30 nevi and 30 melanoma samples was pooled, aliquoted into 3 µl volumes and analyzed over a period of 7 (nonsequential) weeks. The MEL38 scores of each pool are shown longitudinally in Figure 5A. The mean score for the nevi control pool is 2.1 (standard deviation: 0.25) and 6.5 (standard deviation: 0.15) for the invasive melanoma pool. Additional control sample data will be generated over time and monitored to assess technical noise using Levey Jennings plots.

To assess the reproducibility of the genomic score throughout its dynamic range (i.e., 0–10), RNA from 89 samples was re-analyzed and compared with the original MEL38 score. As shown in Figure 5B, there is high consistency between replicate scores, with an intraclass correlation coefficient of 0.96, (95% CI: 0.94–0.98) and no deviation from linearity (Cusum test, $p = 0.62$). These results demonstrate that the MEL38 signature exhibits a high degree of longitudinal stability and technical reproducibility.

Discussion

Melanoma is a heterogeneous, progressive disease. It originates from melanocytic skin lesions and progresses through a series of well-documented stages of malignancy. In this study we describe the performance of a novel genomic biomarker for melanoma in a different specimen type (FFPE biopsy tissue) from that in which it was discovered (i.e. plasma). To our knowledge, this is the first study to demonstrate that a circulating series of miRNAs also exhibit disease-specific levels of expression in solid tissue. By comparing the 38-miRNA signature to relevant clinicopathological and specimen variables, its potential to contribute both accurate and reproducible information to a patient's diagnostic picture is shown.

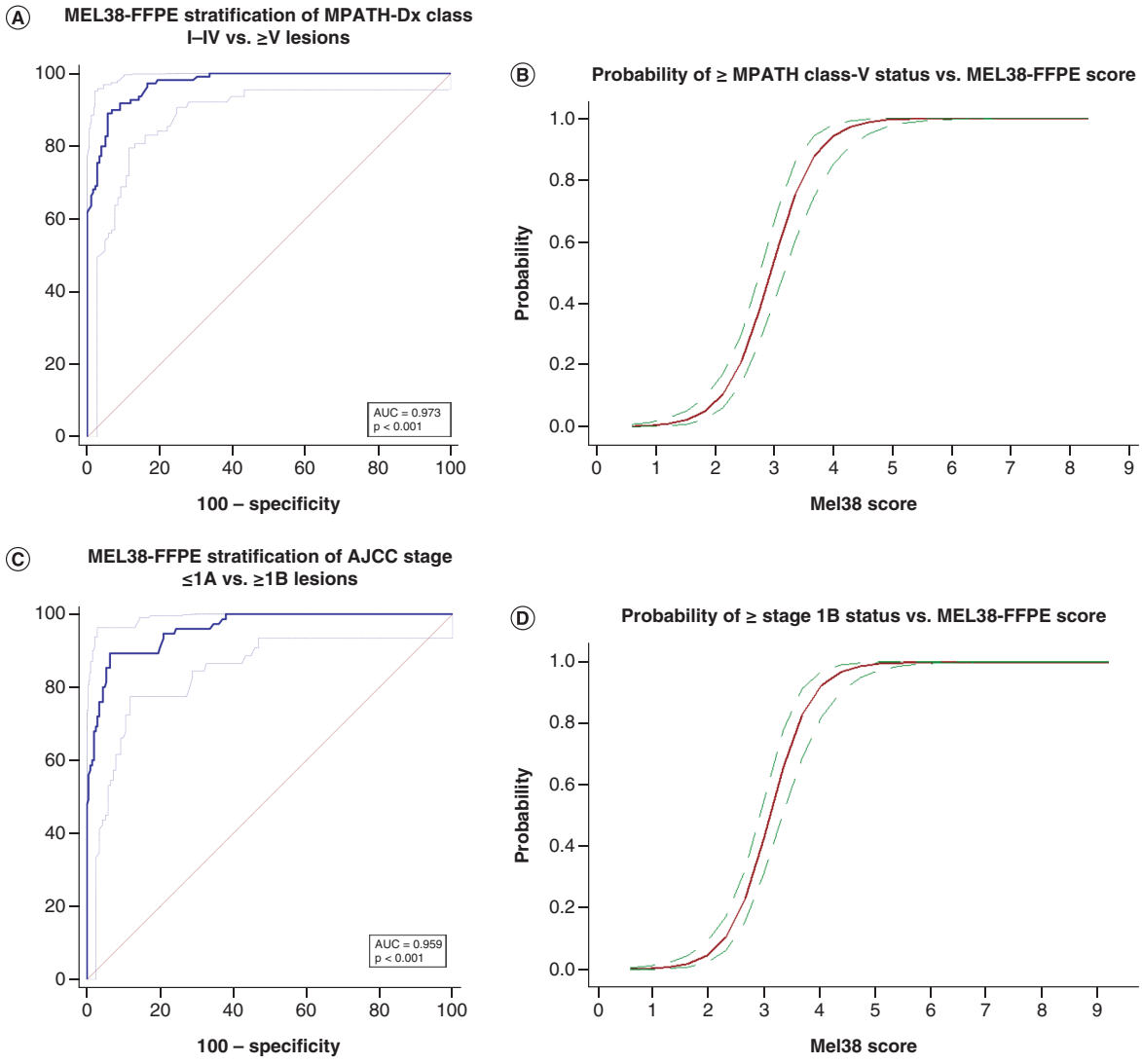

Figure 4. Sensitivity and specificity analysis of MEL38. (A) Receiver operator curve analysis of MEL38 scores from primary melanocytic skin lesions versus MPATH-Dx classes I–IV versus class V. AUC: 0.97; 95% CI: 0.95–0.99. **(B)** Binary fitted line plot from logistic regression analysis of MEL38 scores versus probability of MPATH-Dx class V melanoma. **(C)** Receiver operator curve analysis of MEL38 scores from primary melanocytic skin lesions versus AJCC stage ≤1A versus ≥1B. AUC: 0.96; 95% CI: 0.95–0.99. **(D)** Binary fitted line plot from logistic regression analysis of MEL38 scores versus probability of MPATH-Dx class V melanoma.
AUC: Area under the curve.

The cohort presented in this study was designed to reflect the clinical continuum of melanoma and allowed the miRNA expression data generated to be analyzed in relation to a spectrum of disease, from benign nevi to metastatic melanoma, as well as a binary condition, by classifying specimens into MPATH-Dx classes. In the former context, the MEL38 score exhibits a strong positive correlation with the progressive stages of melanoma, as represented by the intra-class correlation (ICC) coefficient (0.85). When analyzed as a binary condition, based on diagnostic classes with substantially different recommended follow-up actions and estimated 5/10-year survival rates, a MEL38 threshold of ≥2.7 was able to identify the higher-risk melanomas with high sensitivity and specificity.

A selection of Spitz nevi specimens was included in this study due to their histological similarity with melanoma. Despite the fact they are usually diagnosed in younger patients, misdiagnosis of melanoma as a Spitz nevus is an ongoing challenge [14]. Hierarchical clustering showed the expression profile of this subtype to have similarities to both benign and malignant disease, which was also reflected by their MEL38 scores. To date very few genomic studies of Spitz nevi have been performed; only 56 out of 47,333 (0.1%) melanocytic lesion profiles in the NCBI

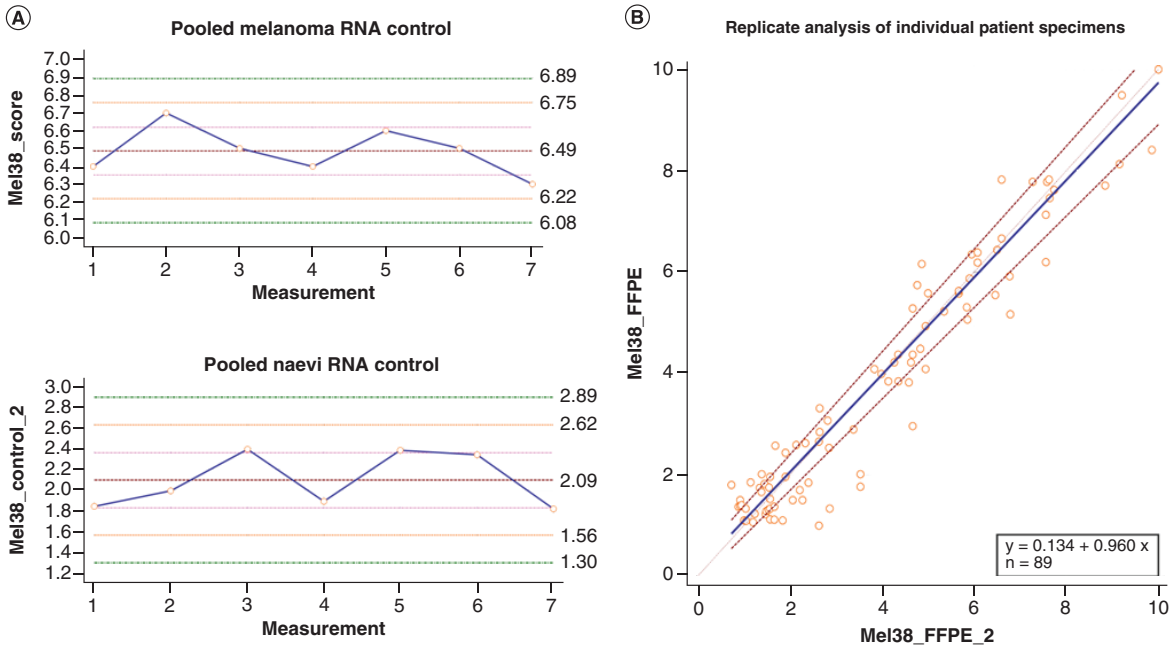

Figure 5. MEL38 technical reproducibility. (A) Longitudinal analysis of control sample MEL38 scores. Dashed horizontal lines corresponding to 1s, 2s and 3s Westguard rules indicated [36]. **(B)** Passing and Bablok regression of replicate MEL38 analyses. No significant deviation from linearity is detected (Cusum test p = 0.62) and intraclass correlation of 0.96 (0.94–0.98) reflects the signatures high degree of reproducibility.

Gene Expression Omnibus are of spitzoid type [37]. This observation, albeit based on a limited sample size, may indicate there are subsets of Spitz nevi, which may benefit from additional treatment or monitoring.

Technical reproducibility of MEL38 signature was demonstrated by the low level of variation observed for both a melanoma and nevi control RNA pool, tested repeatedly over several months. Low variation and strong linearity were observed between a series of samples covering the full dynamic range of the score, which were analyzed in duplicate. These technical data are in line with those of other well-validated genomic signatures used for individual patient management. Finally, the demonstrated reproducibility of the MEL38 score analyses exceeds that of conventional pathology-based diagnoses, where almost a third of specimens may receive a different MPATH-Dx class when rereviewed by the same pathologist [11].

Limitation of this study is the small numbers of less common melanoma and nevi subtypes, such as amelanotic melanoma. Future work will focus on specific subtypes of melanoma and noncancerous skin lesions which present challenges to accurately diagnose using conventional methods. A further limitation is not directly associating the genomic score to patient outcome or BRAF/NRAS status, due to a lack of access to the molecular and clinical variables necessary for these types of analyses. However, in a previous *in silco* study of previously published melanoma mRNA expression data, a significant association between MAPK pathway genes, melanoma-specific survival and the MEL38 signature was described [27].

It is important to highlight the proposed use of MEL38 as a complementary molecular biomarker to conventional histopathology is not intended to replace current diagnostic practices. Genomic testing, microscopic and immunohistochemical assessments of cellular/tissue structure all add unique and sometimes overlapping data points that contribute to a complete diagnostic picture. Incorporating genomic profiling into diagnostic workups may be useful for understanding future clinical events, including treatment reactions that are inconsistent with the original diagnosis and stage of disease. Combining diagnostic modalities is becoming standard of care for many cancer types including breast (e.g., Oncotype DX, MammaPrint and EndoPredict) and prostate (Oncotype DX, Prolaris and ProstaVysion), both of which have seen promising reductions in mortality over recent years, in part due to increased diagnostic precision and molecular disease subtyping. Clinical adaptation of MEL38 has the potential to add novel, robust and personalized genomic information to the diagnostic picture of patients with clinically suspicious melanocytic lesions.

Conclusion

Successful treatment of melanoma begins with accurate evaluation of melanocytic skin lesions. The consequences of over or underdiagnosis could be avoided by using complementary diagnostic techniques. The MEL38 genomic signature, originally discovered in plasma and now validated in solid tissue, demonstrates a high degree of clinical accuracy and technical reproducibility. These factors make it suitable for use as an adjunct diagnostic biomarker in the clinical setting. By providing physicians with the opportunity to offer patients a genomic 'second opinion' of an excised lesion, it is hoped that diagnostic precision and accuracy can be improved and a greater understanding of genotype/phenotype differences in melanoma can be gained.

Summary points

- Early and accurate diagnosis of melanoma is crucial for achieving optimal outcomes. Studies show that major diagnostic discordances in melanoma histopathology may occur in over 25% of cases, which would result in a change of patient management in 18% of cases.
- MEL38 is a genomic signature of melanoma originally discovered in patient plasma, now additionally validated as a robust biomarker of disease status in solid biopsy tissue.
- The area under the curve of the signature for differentiating between MPATH Dx class I–IV versus class V lesions is 0.97, and between AJCC stage 0–1a and AJCC stage 1b–4 is 0.96.
- The MEL38 score, computed by applying a machine-learning algorithm to a specimen's microRNA profile, ranges from 0 to 10 and is correlated with melanoma progression.
- The score can be evaluated as continuous variable or binary classifier, with scores of 2.7 or greater indicating a high likelihood of malignancy and more intensive suggested clinical actions.
- When analyzed in multivariate models including age, gender, histological subtype, Breslow depth, tumor cell percentage, clinical stage or MPATH-Dx class, the MEL38 score remained statistically significant.
- MEL38 analysis of Spitz nevi revealed an intra-subtype profile, with elements of the profile in common to both benign nevi and invasive melanoma.
- Technical reproducibility studies were performed using repeated analysis of individual patient samples and pooled RNA from low- and high-risk lesions, resulting in an assessment of MEL38 score stability over time.
- Incorporation of MEL38 into the diagnostic workflow of clinically suspicious melanocytic lesions has the potential to improve outcomes by increasing diagnostic accuracy and precision, thereby reducing patient anxiety and medicolegal risks involved in melanoma diagnosis.

Author contributions

R Van Laar, M Saad, I Winship, C Uzzell and A Landgren contributed to study design and management. S King, A Landgren and R McCoy contributed to laboratory work. C Uzzell and A Landgren contributed to pathology review. R Van Laar and S Fereday contributed to data analysis. All the authors contributed to manuscript drafting and review.

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Financial & competing interests disclosure

R Van Laar is an employee and shareholder in Geneseq Biosciences. I Winship is a board member and shareholder in Geneseq Biosciences. S Fereday is consultant to Geneseq Biosciences. S King, R McCoy, M Saad, C Uzzell and A Landgren are employees of Australian Clinical Labs. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The study was approved by Australian Clinical Labs internal medical advisory board and satisfies criteria for use of human tissue by diagnostic pathology companies as outlined by the Australian Government's National Health and Medical Research Council.

Data sharing statement

Data available upon request.

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Papers of special note have been highlighted as: • of interest; •• of considerable interest

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