Br J Dermatol 2024; **190**:549–558
https://doi.org/10.1093/bjd/ljad459
Advance access publication date: 25 November 2023

BJD
British Journal of Dermatology
Translational Research

Validation of epidermal AMBRA1 and loricrin (AMBLor) as a prognostic biomarker for nonulcerated American Joint Committee on Cancer stage I/II cutaneous melanoma

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Abstract

Background Combined expression of the autophagy-regulatory protein AMBRA1 (activating molecule in Beclin1-regulated autophagy) and the terminal differentiation marker loricrin in the peritumoral epidermis of stage I melanomas can identify tumour subsets at low risk of metastasis.

Objectives To validate the combined expression of peritumoral AMBRA1 and loricrin (AMBLor) as a prognostic biomarker able to identify both stage I and II melanomas at low risk of tumour recurrence.

Methods Automated immunohistochemistry was used to analyse peritumoral AMBRA1 and loricrin expression in geographically distinct discovery ($n=540$) and validation ($n=300$) cohorts of nonulcerated American Joint Committee on Cancer (AJCC) stage I and II melanomas. AMBLor status was correlated with clinical outcomes in the discovery and validation cohorts separately and combined.

Results Analysis of AMBLor in the discovery cohort revealed a recurrence-free survival (RFS) rate of 95.5% in the AMBLor low-risk group vs. 81.7% in the AMBLor at-risk group (multivariate log-rank, $P<0.001$) and a negative predictive value (NPV) of 96.0%. In the validation cohort, AMBLor analysis revealed a RFS rate of 97.6% in the AMBLor low-risk group vs. 78.3% in the at-risk group (multivariate log-rank, $P<0.001$) and a NPV of 97.6%. In a multivariate model considering AMBLor, Breslow thickness, age and sex, analysis of the combined discovery and validation cohorts showed that the estimated effect of AMBLor was statistically significant, with a hazard ratio of 3.469 (95% confidence interval 1.403–8.580, $P=0.007$) and an overall NPV of 96.5%.

Conclusions These data provide further evidence validating AMBLor as a prognostic biomarker to identify nonulcerated AJCC stage I and II melanoma tumours at low risk of disease recurrence.

What is already known about this topic?

- The combined expression of the autophagy regulatory protein AMBRA1 (activating molecule in Beclin1-regulated autophagy) and the epidermal differentiation marker loricrin (AMBLor) in the tumour microenvironment has recently been identified as a prognostic biomarker in stage I melanoma able to identify patients at low risk of metastasis.

Accepted: 11 November 2023

What does this study add?

- We show that AMBLor is an independent prognostic biomarker in nonulcerated American Joint Committee on Cancer (AJCC) stage I and II melanoma that can stratify patients at low risk of disease recurrence.

What is the translational message?

- The integration of AMBLor into the management of patients with melanoma will facilitate personalized risk stratification and clinical follow-up for patients with AJCC stage I and II melanoma, stratifying patients at low risk of disease recurrence who are unlikely to benefit from sentinel lymph node biopsy, increased intensity of follow-up or adjuvant therapy.

Cutaneous melanoma is the most serious form of skin cancer, with a rapidly rising global incidence. A total of 320 000 new cases of melanoma were recorded worldwide in 2020, and the number of new cases and deaths is projected to increase further by 2040, highlighting the significant public health challenge posed by this malignancy.[1] In the UK alone, melanoma incidence increased by 140% between 1993–1995 and 2016–2018.[2] Management of metastatic melanoma has significantly improved with the advancement of targeted and immune therapies.[3] Emerging evidence suggests that while most people diagnosed with thin melanomas do well, the majority of melanoma deaths occur in patients initially diagnosed with early-stage localized melanoma.[4] With the improving treatment arsenal and increasing number of treatment options, an urgent unmet clinical need exists to identify subsets of patients with early-stage (I or II) melanoma based on personalized risk of disease recurrence.

The prognostic assessment of melanoma is based on the stratification of patients into clinically relevant stage groups, which guides patient surveillance and treatment. The eighth edition of the American Joint Committee on Cancer (AJCC) staging system uses well-established criteria for clinical and pathological grouping, including primary tumour thickness (Breslow depth) and ulceration, the extent of nodal or in-transit and satellite metastasis, the presence of distant metastasis and serum lactate dehydrogenase level.[5] Over 80% of patients are initially diagnosed with early-stage localized disease (AJCC stage I/II),[6–8] and categorized according to primary tumour thickness and the presence or absence of ulceration. These criteria create broad categories of tumour subsets with distinct outcomes that cannot provide a granular individualized prediction of increased risk of recurrence. The reported 10-year melanoma-specific survival for patients with tumours substaged by the current AJCC (eighth edition) staging as stage IA–IIC ranges from 98% to 75%.[5] However, owing to an increased incidence of thin melanomas with a tumour thickness ≤ 1 mm, a high proportion of melanoma deaths arise in patients initially diagnosed with a stage I melanoma.[4] Furthermore, although follow-up time varies, combined AJCC stage I/II melanoma recurrence is now reported at a frequency of 8.6–18%, translating to 5-year recurrence-free survival (RFS) probabilities of 91.2% for patients with stage IA melanomas to 26.5% for those with stage IIC tumours.[9–11] Given the increased incidence and significant risk of recurrence of early-stage melanoma, there is an urgent need for credible prognostic biomarkers to better stratify patients for personalized care. More accurate recurrence risk predictor markers are needed for the clinical management of patients with melanoma to inform the intensity of follow-up and imaging surveillance, and determine the need for adjuvant therapy.[12] Adjuvant immunotherapy has been recommended for stage IIB/C melanoma.[13,14] Although most patients tolerate immunotherapy well, a few patients still succumb to severe immune-related adverse events, and the lifelong implications of side-effects pose a significant problem. Therefore, it is critical to identify patients who are less likely to benefit from adjuvant treatment owing to a low risk of disease recurrence.[15,16]

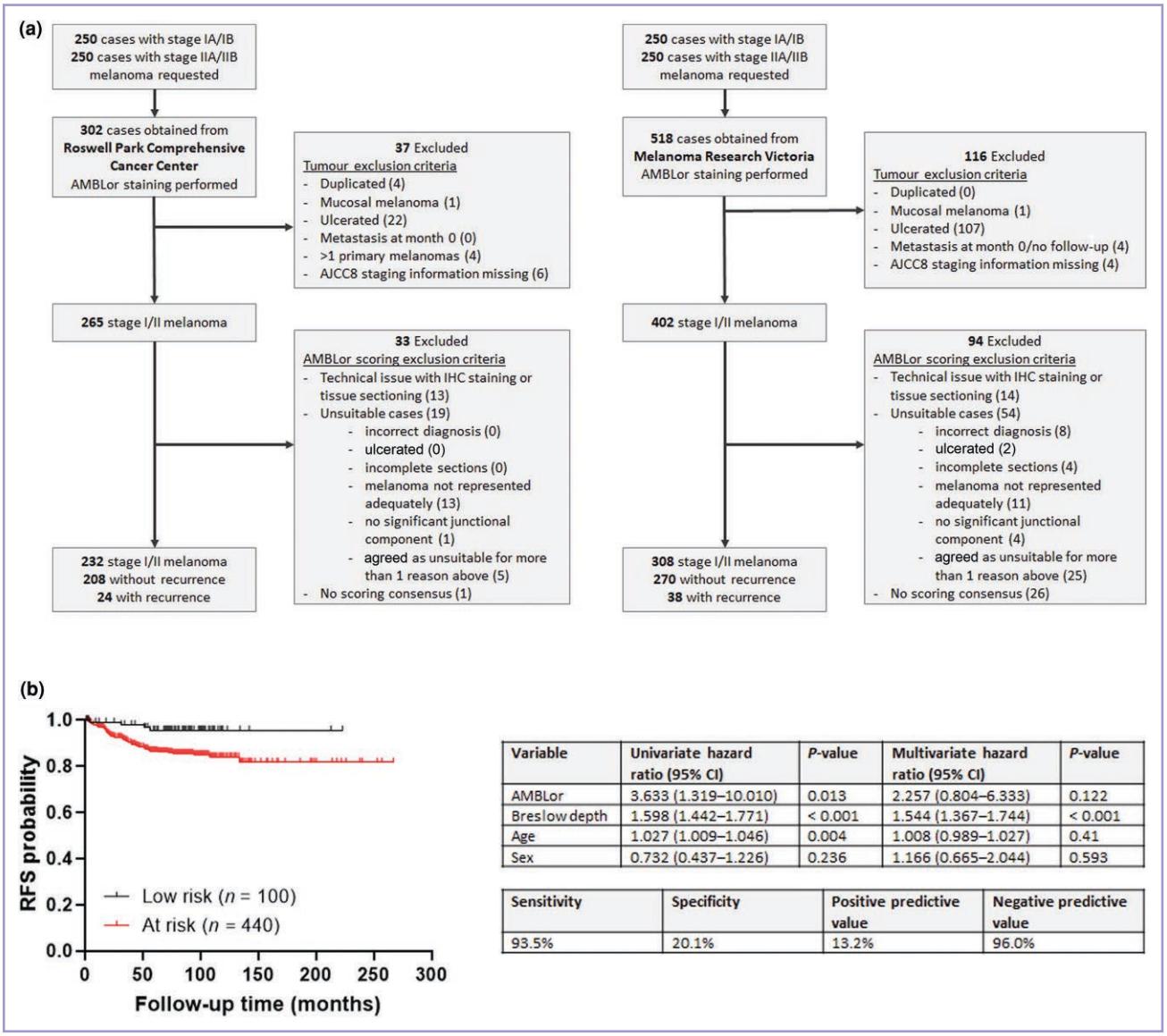
Numerous clinical and histopathological features, such as anatomical site, histological subtype, presence of lymphatic invasion and mitotic rate, are associated with melanoma recurrence and melanoma-specific patient survival.[11,17–20] However, these have not been validated in large-scale prospective studies or shown to augment current AJCC staging criteria. Gene expression profile (GEP) tests have recently gained interest, and a commercial GEP test is considered to improve the prognostic accuracy of AJCC staging.[21] We have previously shown that maintained expression of the autophagy regulatory protein AMBRA1 (activating molecule in Beclin1-regulated autophagy) and the epidermal differentiation marker loricrin in the epidermis overlying nonulcerated stage I melanomas can identify genuinely low-risk tumour subsets with a negative predictive value of 98.3%.[22] The association of tumour secretion of transforming growth factor-β2 with loss of peritumoral AMBRA1 and reduced epidermal integrity underpins a mechanism of melanoma ulceration and supports the prognostic value of this biomarker.[23] This study aimed to validate the ability of AMBLor to identify both stage I and II melanomas at low risk of tumour recurrence.

Patients and methods

Patient cohort selection

The study included four independent collections of AJCC stage I and II melanomas, obtained from four international centres using prospective sampling from existing diagnostic specimen collections. AMBLor biomarker development followed the Cancer Research UK prognostic/predictor biomarker roadmap (assay development stage 2 through to qualification stage 1) and findings were reported according to the REMARK (REporting recommendations for tumour

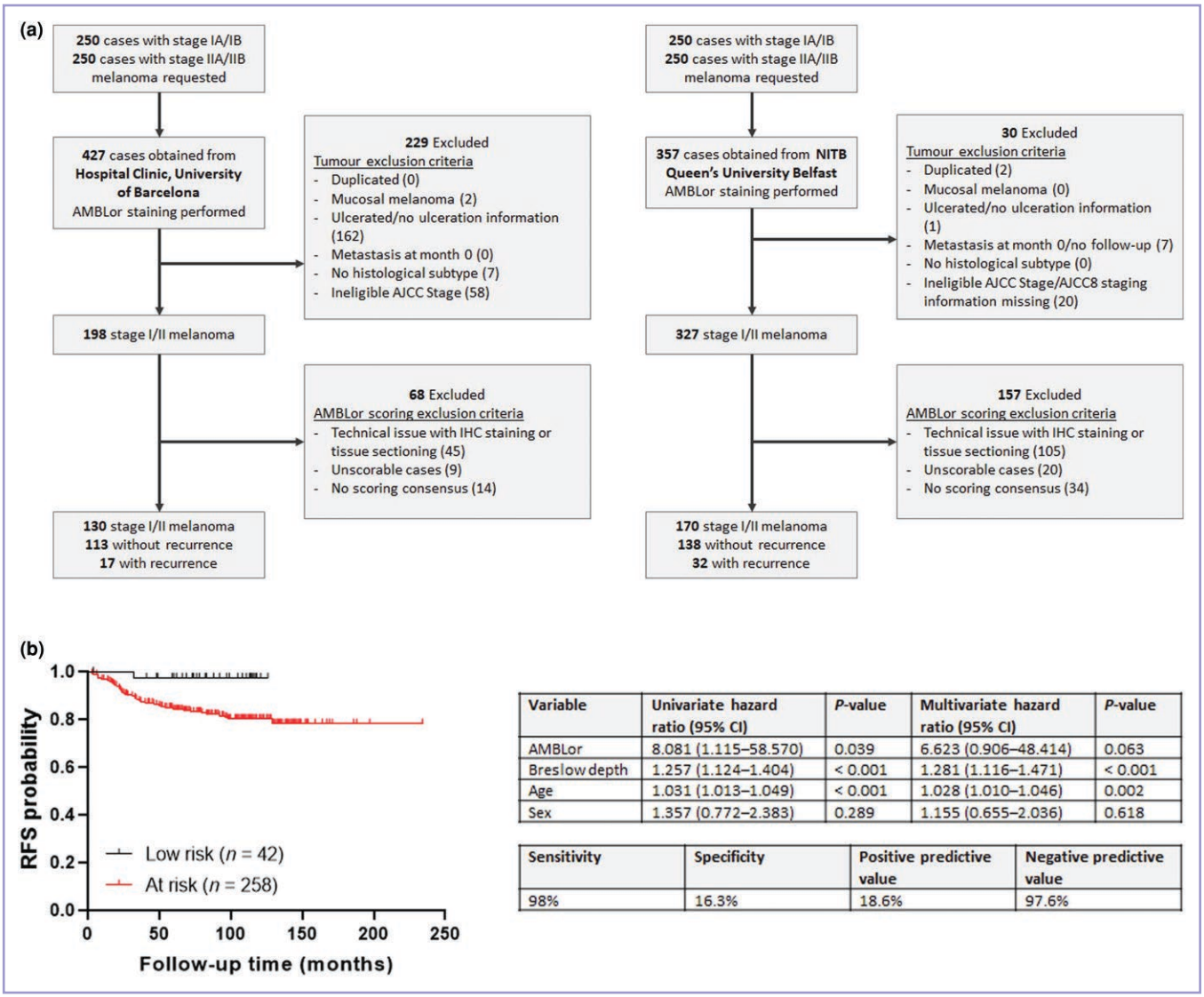
MARKer prognostic studies) guidelines.[24] Retrospective analysis was performed using a discovery cohort, derived from Melanoma Research Victoria (Melbourne, Australia; $n=308$) and the Roswell Park Comprehensive Cancer Center (Buffalo, NY, USA; $n=232$) collections, and a validation cohort derived from the Hospital Clinic Barcelona (University of Barcelona, Spain; $n=130$) and the Northern Ireland Tissue Bank (Belfast, UK; $n=170$)[25] collections. Patients without evidence of tumour recurrence who had <3 years of follow-up were included ($n=86$). Patients who were duplicated in the database, had multiple primary melanomas, an initial diagnosis of stage III/IV melanoma, ulcerated melanoma, mucosal melanoma, melanoma *in situ*, where there was incomplete AJCC eighth edition staging information or where AMBRA1/loricrin scoring criteria were not met were excluded (Figures 1, 2). Patients were clinically staged using AJCC eighth edition guidelines.[5] Where sentinel lymph node biopsy (SLNB) data were available, patients were given a final pathological stage. Where patient staging information was provided using AJCC seventh edition guidelines, patients were restaged using Breslow depth and ulceration status according to AJCC eighth edition criteria. Based on a hazard ratio (HR) of 4.04, as reported for a comparable cohort,[22] power calculations indicated that – in order to obtain 90% power to detect a HR of at least this size at the $P=0.05$ level in a univariate log-rank test, assuming a 10% dropout rate and recurrence rate of 10% in the population – a total cohort of at least 244 individuals would be



Variable	Univariate hazard ratio (95% CI)	*P*-value	Multivariate hazard ratio (95% CI)	*P*-value
AMBLor	3.633 (1.319–10.010)	0.013	2.257 (0.804–6.333)	0.122
Breslow depth	1.598 (1.442–1.771)	< 0.001	1.544 (1.367–1.744)	< 0.001
Age	1.027 (1.009–1.046)	0.004	1.008 (0.989–1.027)	0.41
Sex	0.732 (0.437–1.226)	0.236	1.166 (0.665–2.044)	0.593

Sensitivity	Specificity	Positive predictive value	Negative predictive value
93.5%	20.1%	13.2%	96.0%

Figure 1 AMBRA1 (activating molecule in Beclin 1-regulated autophagy) and loricrin (AMBLor) status is associated with tumour recurrence in an early stage I/II melanoma discovery cohort. (a) Flow diagram showing melanoma sample selection, exclusion criteria and proportion of patients with melanoma recurrence in the discovery cohort. (b) AMBLor levels in the peritumoral epidermis of nonulcerated American Joint Committee on Cancer (AJCC) eighth edition (AJCC8) stage I/II melanomas were defined as 'maintained' or 'lost'. Kaplan–Meier survival plots show the recurrence-free survival (RFS) probabilities as a function of follow-up time (months) for patients stratified into 'low-risk' or 'at-risk' AMBLor groups. RFS is shown by Kaplan–Meier survival curves for a univariate model based on AMBLor status, with assay performance and univariate and multivariate Cox regression analysis of RFS. CI, confidence interval; IHC, immunohistochemistry.



Variable	Univariate hazard ratio (95% CI)	*P*-value	Multivariate hazard ratio (95% CI)	*P*-value
AMBLor	8.081 (1.115–58.570)	0.039	6.623 (0.906–48.414)	0.063
Breslow depth	1.257 (1.124–1.404)	< 0.001	1.281 (1.116–1.471)	< 0.001
Age	1.031 (1.013–1.049)	< 0.001	1.028 (1.010–1.046)	0.002
Sex	1.357 (0.772–2.383)	0.289	1.155 (0.655–2.036)	0.618

Sensitivity	Specificity	Positive predictive value	Negative predictive value
98%	16.3%	18.6%	97.6%

Figure 2 AMBRA1 (activating molecule in Beclin 1-regulated autophagy) and loricrin (AMBLor) status is associated with tumour recurrence in an early stage I/II melanoma validation cohort. (a) Flow diagram showing melanoma sample selection, exclusion criteria and proportion of patients with melanoma recurrence for the validation cohort. (b) AMBLor levels in the peritumoral epidermis of nonulcerated American Joint Committee on Cancer (AJCC) eighth edition (AJCC8) stage I/II melanomas were defined as 'maintained' or 'lost'. Kaplan–Meier survival plots show the recurrence-free survival (RFS) probabilities as a function of follow-up time (months) for patients stratified into 'low-risk' or 'at-risk' AMBLor groups. RFS is shown by Kaplan–Meier survival curves for a univariate model based on AMBLor status, with assay performance and univariate and multivariate Cox regression analysis of RFS. CI, confidence interval; IHC, immunohistochemistry; NITB, Northern Ireland Tissue Bank.

required. To achieve sufficient power from geographically diverse samples, patients from different tissue collections/institutions were combined to create the discovery ($n=540$; Figure 1) and validation ($n=300$; Figure 2) cohorts.

Immunohistochemistry analysis of AMBRA1 and loricrin

Consecutive 4-μm sections prepared from formalin-fixed paraffin-embedded tissue blocks were subjected to automated immunohistochemical analysis using proprietary antibodies for AMBRA1 and loricrin (AMLo Biosciences, Newcastle upon Tyne, UK). Immunohistochemistry (IHC) was performed using a Ventana Benchmark ULTRA automated IHC staining instrument (Roche Tissue Diagnostics, Oro Valley, AZ, USA). Briefly, slides were deparaffinized and antigen retrieval was performed using ULTRA Cell Conditioning (ULTRA CC1; Roche Diagnostics, Mannheim, Germany) for 36 min. Primary antibodies were diluted in Da Vinci Green Diluent (PBC-PD900L; Cell Path, Powys, UK) to a final concentration of 0.34 mg mL^{-1} for AMBLor anti-AMBRA1 and 14 mg mL^{-1} for AMBLor antiloricrin and incubated for 32 min at 36 °C. Primary antibody binding was detected with an ultraView DAB IHC Detection Kit (Ventana Medical Systems). IHC was performed at Newcastle Cellular Pathology (Newcastle Hospitals NHS Foundation Trust) using United Kingdom Accreditation Service-approved proprietary primary antibodies (discovery cohort), or at HistoCyte Laboratories using a manufactured AMBLor® test kit (AMLo Biosciences; validation cohort). Following

the quality control assessment of immunostaining, slides were scanned using a Leica Aperio AT2 (Leica Biosystems, Wetzlar, Germany) at × 40 original magnification to produce high-resolution digital images.

AMBRA1 and loricrin scoring

Slides were examined and scored independently by two consultant cellular pathologists (out of P. Barrett, N.S., A.H., P.S. and M.R.) who were blinded to patient outcomes. Reported scores had consensus agreement between at least two pathologists, with the appointment of a third adjudicator if necessary. The presence of a normal staining pattern for AMBRA1 (little expression in the basal layer with increasing intensity towards the granular layer) and loricrin (continuous band of intense cytoplasmic staining in the granular layer) in normal skin adjacent to the primary melanoma was used as an internal positive control with a normal skin section used as an additional batch-positive control. AMBRA1 and loricrin were scored as 'maintained' or 'lost'. 'Maintained' AMBRA1 was defined as equivalent to that in the adjacent epidermis; 'lost' AMBRA1 was defined as a reduction in staining intensity in any area of epidermis overlying the melanoma. 'Maintained' loricrin was defined as a continuous line expression in the granular layer; 'lost' loricrin was defined as at least one gap greater than a single cell length (Figure 3). We excluded patients where there were technical issues, if unsuitable cases were identified or if scoring consensus was not agreed (Figures 1,2).

Statistical analysis

RFS was calculated from the time of diagnosis of the primary melanoma to the time of first locoregional or distant metastasis (including local recurrence, in-transit and satellite metastasis, involvement of regional lymph nodes or distant metastasis). Patients were censored at the time of the last follow-up or death due to any cause. To represent RFS, Kaplan–Meier survival curves for a univariate model based on AMBLor status were generated using PRISM version 9 (GraphPad, La Jolla, CA, USA). Survival analyses were performed in R (version 4.2.1),[26] using the function 'coxph()' from the survival package. Overall RFS significance, as displayed in the Kaplan–Meier plot, was assessed by the log-rank (score) test from a multivariate Cox model considering AMBLor status, Breslow thickness, age and sex as covariates. Individual coefficients (i.e. HR estimates from both a full additive multivariate model and individual univariate models including only that predictor) are presented along with 95% confidence intervals (CIs) in Figures 1, 2 and 4. *P*-values for these coefficients were obtained from the *z*-test of difference from zero.

Results

Cohort characteristics

Clinical and histopathological characteristics of the four independent, geographically distinct tissue collections used for AMBLor analysis are provided in Tables 1–4. Mean patient age at diagnosis, Breslow depth and distribution of histological subtype were similar between the collections. Most melanomas were AJCC stage I, with 13.8% and 21.1% of patients with stage II disease in the Buffalo and Melbourne collections, and 36.9% and 41.2% of patients with stage II disease in the Barcelona and Belfast collections, respectively. These collections included 111 cases with melanoma recurrence in total, in 10.3–18.8% of patients in each collection, consistent with previously reported recurrence rates.[10]

We have previously shown that 'maintained' expression of AMBRA1 and/or loricrin in the peritumoral epidermis was able to identify a subgroup of patients with nonulcerated stage I melanoma with a truly low risk of tumour recurrence with a negative predictive value (NPV) of 98.3%.[22] Here we aimed to evaluate the clinical utility of combined AMBRA1/loricrin expression as a prognostic biomarker in both AJCC stage I and II nonulcerated melanoma. IHC detection of AMBRA1 and loricrin was performed in a discovery cohort of stage I/II melanomas using proprietary primary antibodies, followed by external assessment in a geographically distinct validation cohort using an AMBLor test kit. Patients were defined as having a 'low-risk' AMBLor status if AMBRA1 and/or loricrin expression was 'maintained', while patients were defined as having an 'at-risk' AMBLor status if there was a decrease in peritumoral AMBRA1 expression *and* a break in the continuous expression of loricrin in the epidermis. The term 'at-risk' was used for this group as the risk of tumour recurrence was assumed to be equivalent to that predicted by AJCC eighth edition staging.

Evaluation of AMBLor as a prognostic indicator

Analysis of RFS with a median follow-up time of 83 months in the discovery cohort showed a reduced RFS in the 440 AJCC stage I/II melanomas stratified as AMBLor at-risk vs. the 100 melanomas stratified as low-risk (estimated RFS 81.7% vs. 95.5%; log-rank $P < 0.001$). While the individual contribution of AMBLor in the multivariate model considering AMBLor, Breslow thickness, age and sex did not reach statistical significance in the discovery cohort [HR 2.257, 95% CI 0.804–6.333; $P = 0.12$ (Figure 1b)], in a univariate model we observed a significant HR [HR 3.633, 95% CI 1.319–10.010, $P = 0.01$ (Figure 1b)]. Further, as a guide to clinical utility, diagnostic performance statistics showed that the proportion of patients who experienced tumour recurrence correctly identified by AMBLor status (test sensitivity) was 93.5%, while the post-test probability of AMBLor's ability to identify patients with a low risk of tumour recurrence (NPV) was 96.0%.

Analysis of RFS with a median follow-up time of 99 months in an independent, geographically distinct validation cohort revealed a reduced RFS in the 258 AJCC stage I/II melanomas stratified as AMBLor at-risk vs. the 42 melanomas stratified as low-risk (estimated RFS 78.3 vs. 97.6%; log-rank $P < 0.001$). As with the discovery cohort, while in a multivariate model the estimated HR associated with AMBLor did not reach statistical significance [HR 6.623, 95% CI 0.906–48.414; $P = 0.063$; Figure 2b], considering a univariate model for AMBLor did reveal a significant HR estimate [HR 8.081, 95% CI 1.115–58.570; $P = 0.04$ (Figure 2b)]. In this cohort, the test sensitivity was 98.0% and the NPV was 97.6%

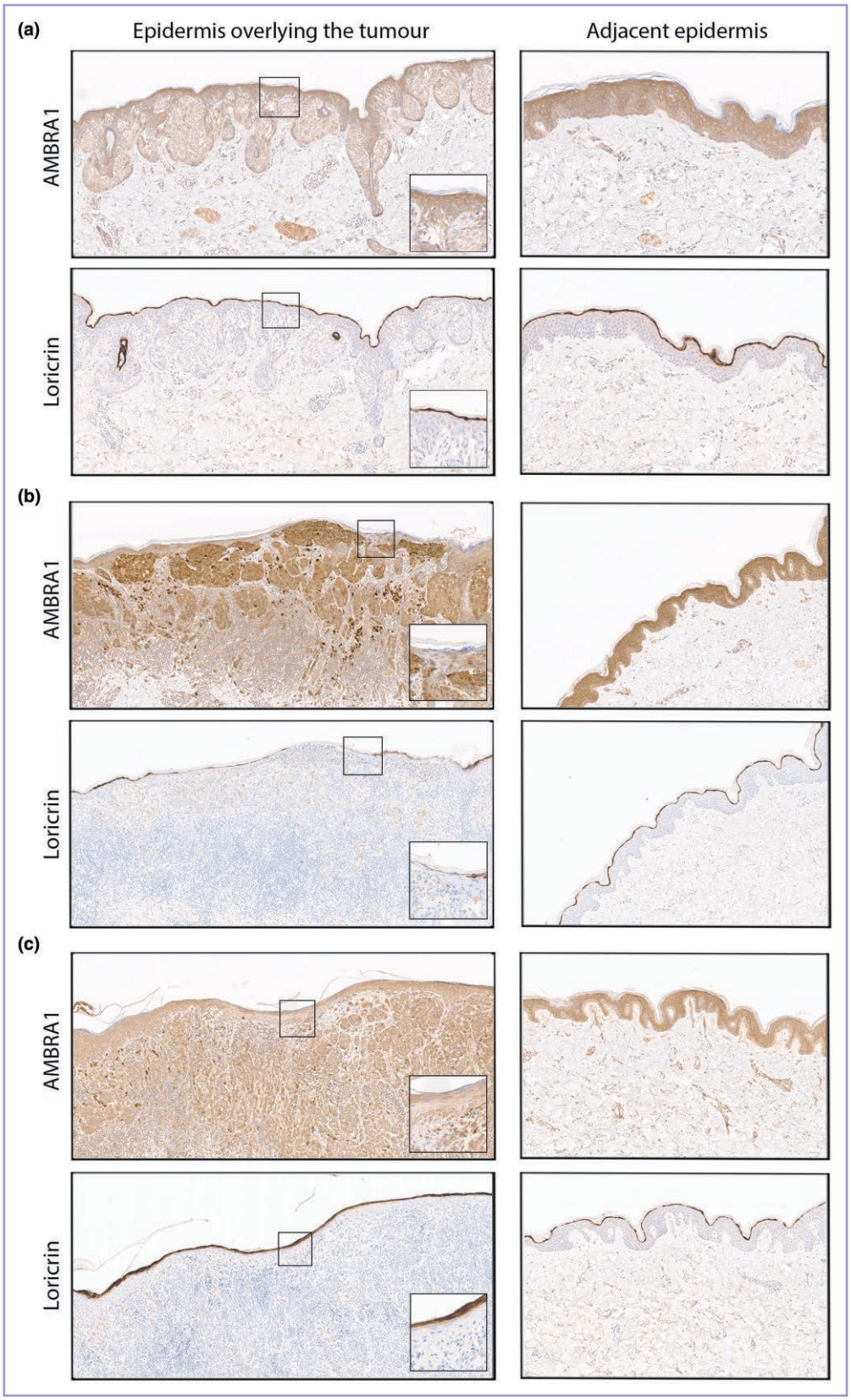
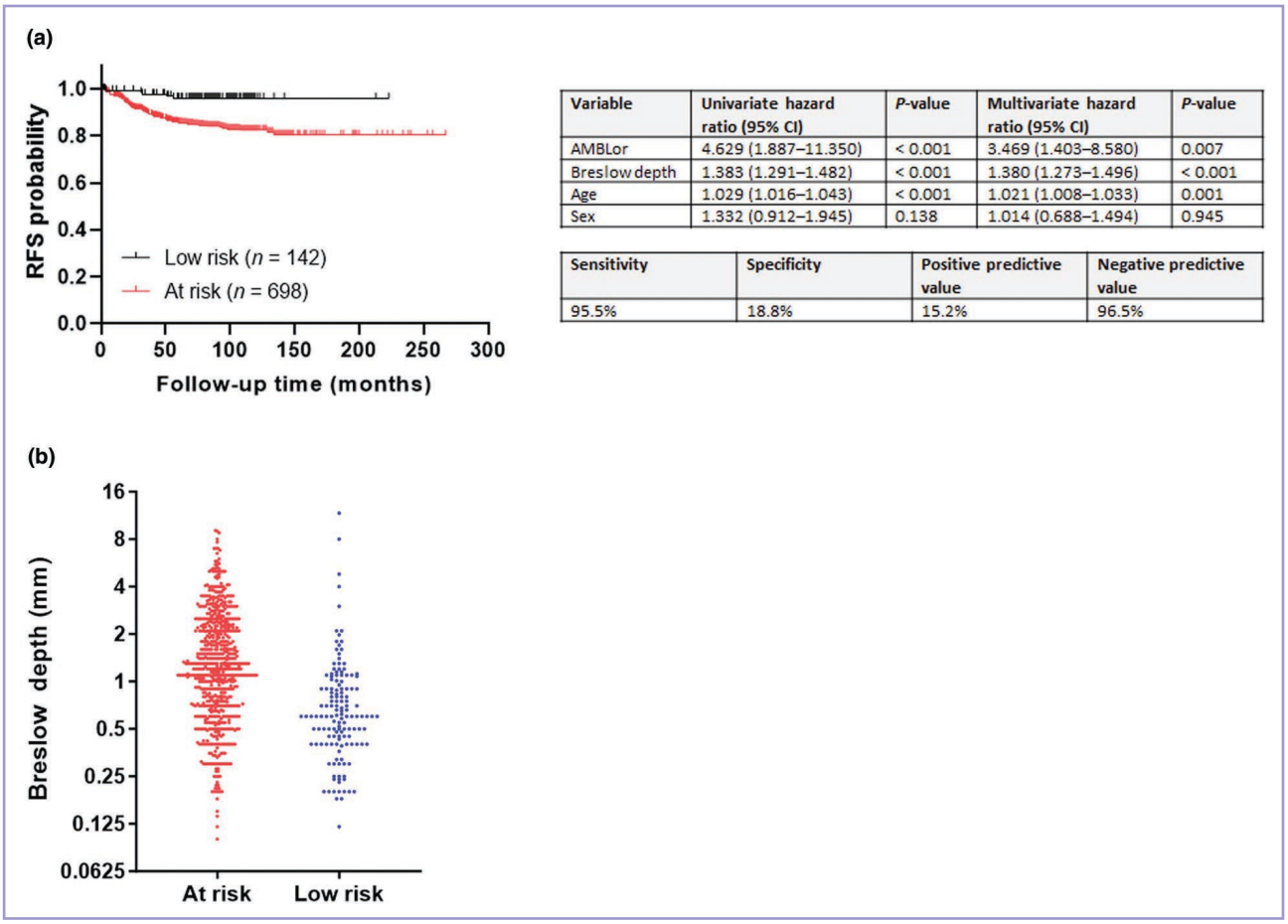


Figure 3 AMBRA1 (activating molecule in Beclin 1-regulated autophagy) and loricrin (AMBLor) AMBLor immunohistochemistry staining patterns. Representative photomicrographs of AMBRA1 and loricrin immunohistochemical staining in the epidermis overlying the melanoma and adjacent normal epidermis. (a) AMBRA1 and loricrin were scored as 'maintained', (b) AMBRA1 and loricrin were scored as 'lost' (note the single-cell loricrin gaps present in the adjacent epidermis) and (c) AMBRA1 was scored as 'lost' and loricrin scored as 'maintained' (original magnification × 10).



Variable	Univariate hazard ratio (95% CI)	*P*-value	Multivariate hazard ratio (95% CI)	*P*-value
AMBLor	4.629 (1.887–11.350)	< 0.001	3.469 (1.403–8.580)	0.007
Breslow depth	1.383 (1.291–1.482)	< 0.001	1.380 (1.273–1.496)	< 0.001
Age	1.029 (1.016–1.043)	< 0.001	1.021 (1.008–1.033)	0.001
Sex	1.332 (0.912–1.945)	0.138	1.014 (0.688–1.494)	0.945

Sensitivity	Specificity	Positive predictive value	Negative predictive value
95.5%	18.8%	15.2%	96.5%

Figure 4 AMBRA1 (activating molecule in Beclin 1-regulated autophagy) and loricrin (AMBLor) status is associated with tumour recurrence in the combined discovery and validation cohort. (a) AMBRA1 and loricrin levels in the peritumoral epidermis of nonulcerated American Joint Committee on Cancer (AJCC) eighth edition stage I/II melanomas were defined as 'maintained' or 'lost'. Kaplan–Meier survival plots show the recurrence-free survival (RFS) probabilities as a function of follow-up time (months) for patients stratified into 'low-risk' or 'at-risk' AMBLor groups. RFS is shown by Kaplan–Meier survival curves for a univariate model based on AMBLor status, with assay performance and univariate and multivariate Cox regression analysis of RFS. (b) Melanoma Breslow depth in patients stratified into 'low-risk' or 'at-risk' AMBLor groups. CI, confidence interval.

Table 1 Roswell Park Comprehensive Cancer Center (Buffalo Medical Group) tissue collection ($n=232$)

Age (years), mean (range)	60 (20–92)
Breslow depth (mm), mean (range)	1.13 (0.12–8.8)
Sex	
Male	132
Female	100
Melanoma subtype	
Superficial spreading	178
Nodular	13
Lentigo maligna melanoma	24
Acral	2
Desmoplastic	5
Naevoid	2
Not otherwise stated	8
Overall AJCC stage	
IA	117
IB	83
IIA	26
IIB	6

Data are provided as *n* unless otherwise stated. AJCC, American Joint Committee on Cancer.

Table 2 Melanoma Research Victoria tissue collection ($n=308$)

Age (years), mean (range)	56 (18–92)
Breslow depth (mm), mean (range)	1.4 (0.18–8.0)
Sex	
Male	170
Female	138
Melanoma subtype	
Superficial spreading	219
Nodular	35
Lentigo maligna melanoma	20
Acral	5
Desmoplastic	7
Naevoid	0
Not otherwise stated	22
Overall AJCC stage	
IA	117
IB	126
IIA	51
IIB	14

Data are provided as *n* unless otherwise stated. AJCC, American Joint Committee on Cancer.

Table 3 Hospital Clinic Barcelona tissue collection ($n=130$)

Age (years), mean (range)	56 (14–91)
Breslow depth (mm), mean (range)	2.1 (0.9–9.0)
Sex	
Male	65
Female	65
Melanoma subtype	
Superficial spreading	91
Nodular	16
Lentigo maligna melanoma	8
Acral	7
Not otherwise stated	8
Overall AJCC stage	
IA	2
IB	80
IIA	37
IIB	11

Data are provided as *n* unless otherwise stated. AJCC, American Joint Committee on Cancer.

The observation of statistically significant HR effects in univariate analyses in both cohorts and the fact that, in both cases, the combined effects of significant terms in the multivariate analyses were less than the significant HR estimated in the associated univariate model [discovery cohort $1.544 < 3.633$; validation cohort $(1.028 + 1.281) < 8.081$] suggested that the additional power obtained from combining the two cohorts may provide more accurate estimates. Indeed, multivariate analysis of the combined discovery and validation cohorts revealed a significantly reduced RFS in 698 melanomas stratified as AMBLor at-risk compared with 142 melanomas stratified as low-risk (estimated RFS 80.4% vs. 96.2%; log-rank $P<0.001$). Further, the estimated effect of AMBLor in this combined cohort dataset in a multivariate model was statistically significant [HR 3.469, 95% CI 1.403–8.580; $P=0.007$ (Figure 4a)]. Breslow thickness also made a significant contribution to the effect in this multivariate model (HR 1.380, 95% CI 1.273–1.496; $P<0.001$), with AMBLor identifying low-risk tumours over a range of Breslow depths (Figure 4b), including those ≥ 0.8 mm, which is the threshold for consideration of SLNB based on AJCC eighth edition recommendations and current National Institute for Health and Care Excellence (NICE) guidelines.[25,26] In the combined discovery and validation cohorts, test sensitivity was 95.5% and the NPV was 96.5%.

Table 4 Northern Ireland Tissue Bank tissue collection ($n=170$)

Age (years), mean (range)	60 (20–98)
Breslow depth (mm), mean (range)	1.9 (0.1–11.7)
Sex	
Male	80
Female	90
Melanoma subtype	
Superficial spreading	112
Nodular	32
Lentigo maligna melanoma	16
Acral	7
Not otherwise stated	3
Overall AJCC stage	
IA	55
IB	45
IIA	57
IIB	13

Data are provided as *n* unless otherwise stated. AJCC, American Joint Committee on Cancer.

Discussion

Despite advances in melanoma treatment,[3] melanoma remains the leading cause of skin cancer mortality. Cutaneous melanoma incidence rates are increasing worldwide; melanoma is now one of the most common cancers in younger individuals in North America, Australia and Europe.[1,27] Approximately 80% of patients with melanoma are diagnosed with AJCC stage I or II melanoma [7,8,28] and while mortality rates in early-stage melanomas are relatively low,[5] as most patients are diagnosed with early-stage disease, the majority of melanoma deaths now originate from this group.[4,29] Furthermore, analysis of $>17\,500$ patients with early-stage melanoma reported 10-year recurrence rates of 10% for stage IA, rising to 20% for stage IB and 37% for stage IIA, to 44% for stage IIB and 59% for stage IIC disease,[10] with poorer melanoma-specific survival rates than published AJCC eighth edition data,[5] highlighting the clinical need to further stratify patients according to the risk of disease recurrence to inform treatment decisions. Current AJCC staging categorizes patients according to risk using pathology data to predict melanoma-specific survival; however, staging lacks granularity for accurate prediction of individual melanoma recurrence and mortality risk. Therefore, credible prognostic biomarkers are needed to refine individual risk prediction and guide precision medicine in the management of melanoma. In this study, we provide further evidence validating AMBLor as a biomarker that can identify nonulcerated AJCC stage I and II melanoma tumours at low risk of recurrence.

Under current NICE guidance, patients with stage IIB/C melanoma are eligible for adjuvant therapy,[14] while in patients with stage IB or IIA melanoma, SLNB remains an important prognostic tool to inform therapy eligibility for SLNB-positive patients and follow-up time and imaging surveillance in SLNB-negative patients. In the present study, AMBLor identified a subset of patients with nonulcerated stage I or II melanoma at low risk of disease recurrence, with a NPV of 96.5%. Importantly, AMBLor retained the ability to identify low-risk patient subsets independently of Breslow depth, consistent with previous findings in AJCC stage I melanoma.[22] Detection of low-risk tumours with a Breslow depth ≥ 0.8 mm (the current threshold for consideration of SLNB) provides further support for AMBLor as a pre-SLNB test to identify patients unlikely to benefit from SLNB, increased imaging surveillance or adjuvant therapy.

There is considerable interest in the use of prognostic biomarkers to improve risk stratification above AJCC eighth edition staging for patients with melanoma. A 31-GEP assay can stratify patients with AJCC stage I and II melanoma into low- and high-risk groups for recurrence and SLNB positivity,[30–32] and improve AJCC eighth edition stratification in patients with stage I–IIA melanoma.[33] Refinement of the 31-GEP test using a continuous risk score combined with clinical and pathological features has facilitated an individualized risk assessment for disease recurrence and melanoma-specific death in patients with stage I–III melanoma, identifying low-risk patients who may forgo SLNB and patients who

are at high risk and may benefit from early intervention.[34] A model combining clinicopathological features and an 8-GEP (CP-GEP) can refine the risk of disease recurrence in patients with stage I–IIA melanoma,[35] while an 11-GEP test is an independent predictor of melanoma-specific survival in patients with stage I–III disease.[36,37] These models identify an independent contribution of molecular information to risk stratification and provide evidence for the combined use of molecular and clinicopathological characteristics as an optimum strategy to improve prognostication. Furthermore, prospective studies have shown that incorporation of the 31-GEP test into the assessment of stage I–II melanoma changed the clinical management for many of these patients.[21] To date, no studies have assessed how and whether changes in management based on these tests meaningfully change melanoma outcomes, and the cost-effectiveness of these tests has not yet been determined.

The AMBLor test is a simple IHC assay that can integrate easily into the standard melanoma care pathway, and its prognostic validity is supported by identification of the biologic mechanism connecting the loss of peritumoral AMBRA1 to tumour ulceration.[23] Health professionals report a very positive view on the contribution of this test to the risk stratification of patients with melanoma, while patients viewed the main benefit as provision of reassurance if classified as low risk.[38] Furthermore, modelling of a low-cost prognostic test in the management of stage I/II melanoma identified the potential utility of such a test in focusing National Health Service resources on patients at high risk of disease recurrence while excluding low-risk patients from higher-intensity follow-up and adjuvant treatments with potential side-effects and considerable healthcare costs.[39]

A limitation of this study was its size, which did not allow for stage or substage analysis, making it difficult to provide evidence of prognostic ability above existing AJCC eighth edition staging. While we have previously demonstrated the prognostic ability of AMBLor to identify patients at low risk of disease recurrence in patients with stage IB melanoma,[22] additional studies in more defined retrospective and prospective cohorts are warranted to fully evaluate AMBLor and its association with patient outcomes, to further establish clinical utility. In summary, our study provides evidence that AMBLor is an independent prognostic biomarker for nonulcerated early-stage melanoma and can stratify patients at low risk of disease recurrence who are not likely to benefit from SLNB, increased follow-up intensity or adjuvant therapy, and aid the personalized management of patients with melanoma.

Acknowledgements

This study was supported by a National Institute for Health Research (NIHR) Invention for Innovation (i4i) grant: Innovative Prognostic Test for Early-Stage Cutaneous Melanoma (AMLo) (project reference 20993, Principal Investigator P.E.L.) in association with AMLo Biosciences Ltd. The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care. We are grateful to all patients from Barcelona (Spain), Belfast (UK), Buffalo (NY, USA) and Victoria (Australia) who donated their tissue to sample repositories in each centre for their support in these studies. For tissue samples from Melanoma Research Victoria (MRV), Australia, the authors acknowledge the MRV lead investigators Grant McArthur, Victoria Mar, Damien Kee and Craig Underhill. Samples received from the Northern Ireland Tissue Bank (NITB) in Belfast, UK, were supported by funds the NITB received from the HSC Research and Development Division of the Public Health Agency in Northern Ireland. We are grateful for the excellent logistical and technical support provided by the Pathology Network Shared Resource and the Clinical Data Delivery and Honest Broker Services of Roswell Park Comprehensive Cancer Center and the Dermatopathology Laboratory of Buffalo Medical Group. We also thank Dr Max Robinson, consultant histopathologist at Newcastle Hospitals NHS Foundation Trust, for AMBLor scoring.

Funding sources

This study (study design, data collection, data analysis and manuscript preparation) was supported by a National Institute for Health and Care Research (NIHR) Invention for Innovation (i4i) grant: Innovative Prognostic Test for Early-Stage Cutaneous Melanoma (AMLo) (project reference 20993, principal investigator, P.E.L.) and AMLo Biosciences Ltd.

Conflicts of interest

P.E.L. is the chief scientific officer, P.S. is chief histopathologist and M.L. is chief executive officer for the Newcastle University spinout company AMLo Biosciences Ltd. J.L., G.R. and D.S. are/were employees of and J.L.A is a consultant to AMLo Biosciences.

Data availability

The data underlying this article will be shared upon reasonable request to the corresponding author.

Ethics statement

Full ethical approval was obtained for these studies through the Newcastle University Dermatology Biobank (REC REF 19/NE/004_Lovat).

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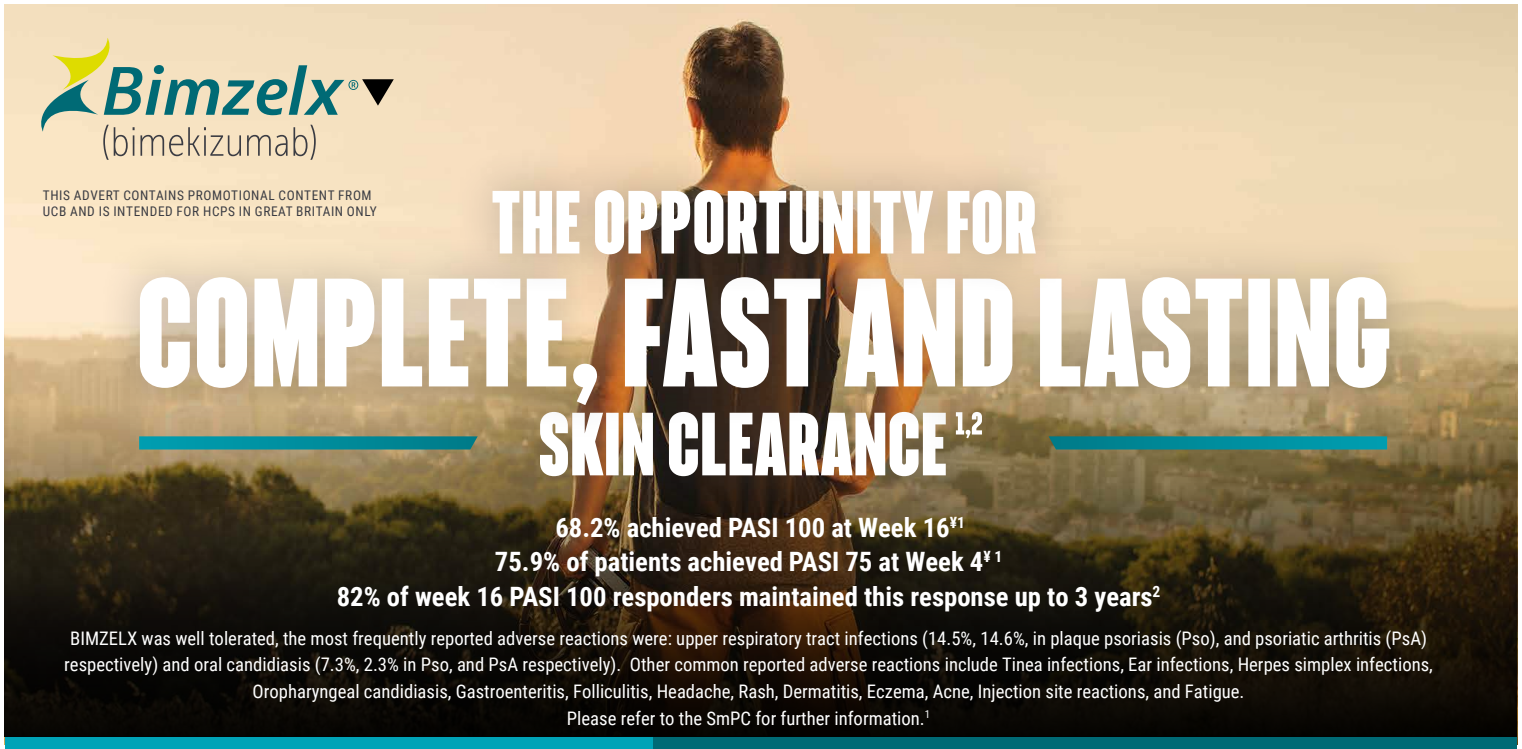

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For patients with plaque psoriasis (including psoriatic arthritis with coexistent moderate to severe psoriasis) and a body weight ≥ 120 kg who did not achieve complete skin clearance at week 16, 320 mg every 4 weeks after week 16 may further improve treatment response. Consider discontinuing if no improvement by 16 weeks of treatment. Renal or hepatic impairment: No dose adjustment needed. Elderly: No dose adjustment needed. Administer by subcutaneous injection to thigh, abdomen or upper arm. Rotate injection sites and do not inject into psoriatic plaques or skin that is tender, bruised, erythematous or indurated. Do not shake pre-filled syringe or pre-filled pen. 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