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## Introduction and Aims and Methods

Melanoma incidence is predicted to increase by +60% by 2040. Although AJCC Stage I and II tumours represent 91% of all melanomas, all patients diagnosed with early stage tumours are managed as high risk, even though fewer than 20% progress. Furthermore, the 8th edition of AJCC staging criteria are unable to identify subsets of patients with stage I/II melanomas with low risk of disease progression, emphasising the acute need for credible prognostic biomarkers to stratify patient follow-up based on personalised risk.

We have recently identified the combined immunohistochemical expression of epidermal AMBRA1 and Loricrin (AMBLor) overlying AJCC stage I melanomas as a robust prognostic biomarker and valuable pre SLNB test for non-ulcerated tumours (Ellis et al 2020).

The aim of the present multicentre study, was to validate AMBLor as a novel prognostic biomarker for non ulcerated cutaneous AJCC stage I and II melanoma.

Automated immuno-histochemical staining for AMBLor expression was performed using recombinant antibodies to human AMBRA1 and Loricrin (AMLo Biosciences Ltd) in powered retrospective cohorts of formalin fixed paraffin embedded non-ulcerated cutaneous AJCC stage I or II melanomas derived from the Roswell Park Comprehensive Cancer Centre, Buffalo, USA or the Peter McCallum Cancer Centre biobank, Melbourne, Australia (Tables 1 and 2) with semi-quantitative binary scoring analysis and concordance performed by 4 Dermatopathologists. Each cohort was powered to represent rates of metastasis of 10% for AJCC stage I or up to 20% for stage II disease and data correlated with clinical outcome between up to 60 and 287 months.

Melbourne	Number of cases	Buffalo	Number of cases
Age Range (Mean)	19 – 86 (56)	Age Range (Mean)	20-92 (60)
<b>Melanoma Sub-type</b>		<b>Melanoma Sub-type</b>	
Superficial Spreading	109	Superficial Spreading	182
Nodular	27	Nodular	15
Lentigo Maligna Melanoma	11	Lentigo Maligna Melanoma	25
Acral	4	Acral	3
Desmoplastic	4	Desmoplastic	6
Nevoid	0	Nevoid	2
Not otherwise stated	15	Not otherwise stated	8
<b>Overall AJCC stage</b>		<b>Overall AJCC stage</b>	
IA	60	IA	120
IB	68	IB	85
IIA	34	IIA	29
IIB	8	IIB	7

Table 1. Patient demographics for Melbourne and Buffalo cohorts of non-ulcerated AJCC Stage I/II melanomas

## Results 1: Retention of AMBRA1 and/or Loricrin in the epidermis overlying AJCC stage I/II melanoma identifies low risk subsets.

Immunohistochemical staining of AMBLor expression in the epidermis overlying both non-ulcerated cutaneous AJCC I and II melanomas compared to the expression of either AMBRA1 or Loricrin in the marginal (normal) epidermis revealed retained expression of either or both protein markers identified tumours at low risk of disease progression (Figure 2A and 2B). Conversely loss of both AMBRA1 and Loricrin expression in the epidermis overlying either primary AJCC stage I or II melanomas was associated with melanomas at risk of disease progression (Figure 2C).

## Results 2: AMBLor is a prognostic biomarker for AJCC stage I and II melanoma.

Analysis of AMBLor in the mixed cohort of 334 AJCC stage I and 77 non-ulcerated AJCC stage II cutaneous melanomas derived from Buffalo and Melbourne revealed retention of AMBLor in 70 stage I/II tumours was associated with a significantly increased disease free survival of 97% compared to 87% for 341 patients with stage I/II melanomas in which AMBLor was lost (P=0.01; HR 0.20, 95% CI 0.09-0.42, Figure 2a), and with a negative predictive value of 97.14% and assay sensitivity of 95.6% (Figure 2B). Although able to identify genuinely low risk stage I/II melanomas, the low PPV value and assay specificity indicates AMBLor is not however, a suitable test for the identification of patients at high risk of metastasis. In contrast, the low assay specificity and PPV suggests AMBLor identifies tumours with metastatic potential for which surgical removal of the primary tumour may have been a curative event.

## Conclusions

Collectively these data suggest AMBLor as a prognostic biomarker able to identify genuinely low risk subsets of AJCC stage I/II melanomas. Inclusion of AMBLor into clinical pathways may therefore aid the stratification of patients for reduced follow up and/or SLNB, with the potential for significant savings on healthcare resources and improvement in patient anxiety.

## SUMMARY:

**AMBLor is a novel prognostic biomarker for non ulcerated AJCC stage I and II melanoma**

## Results 1

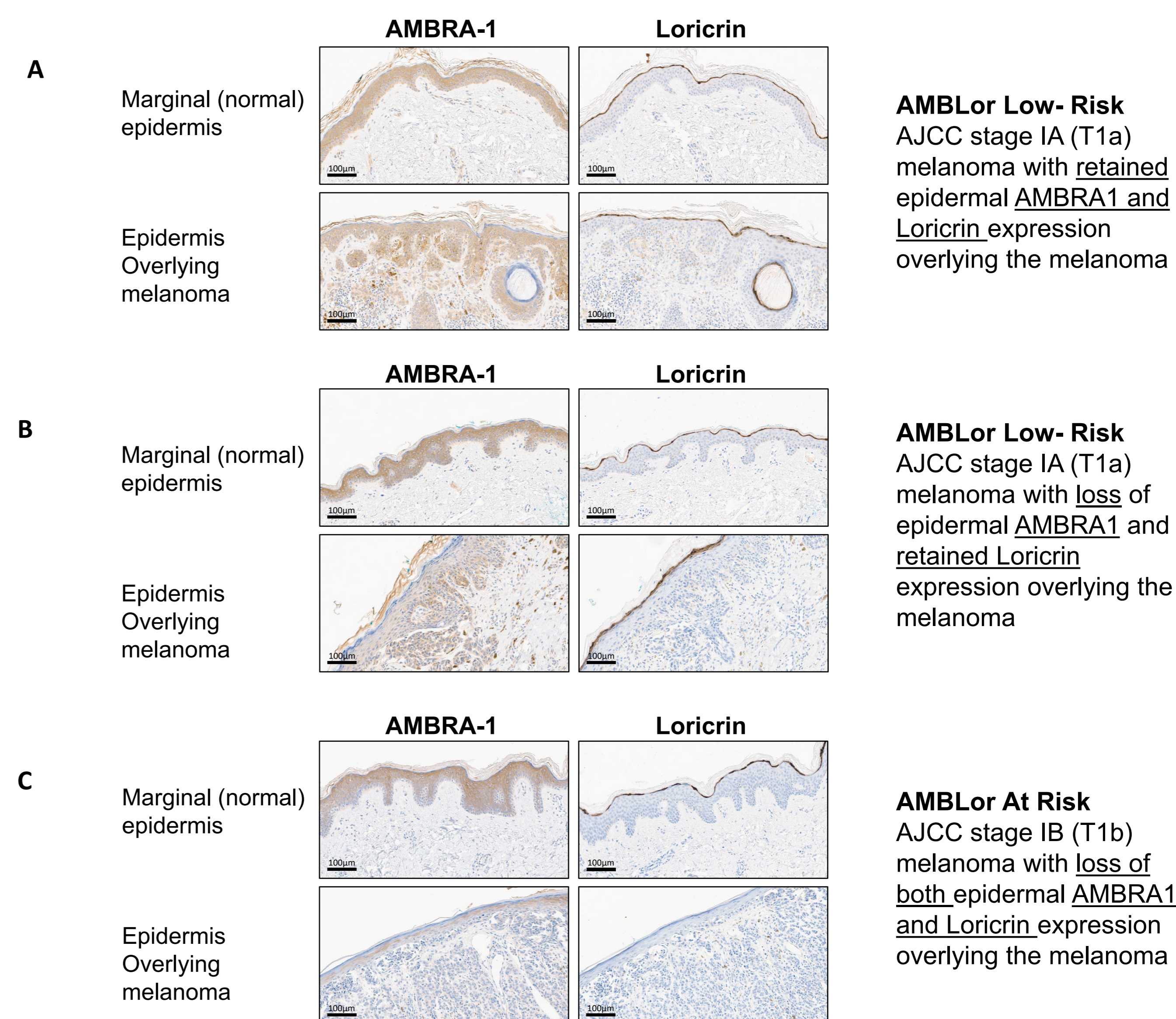
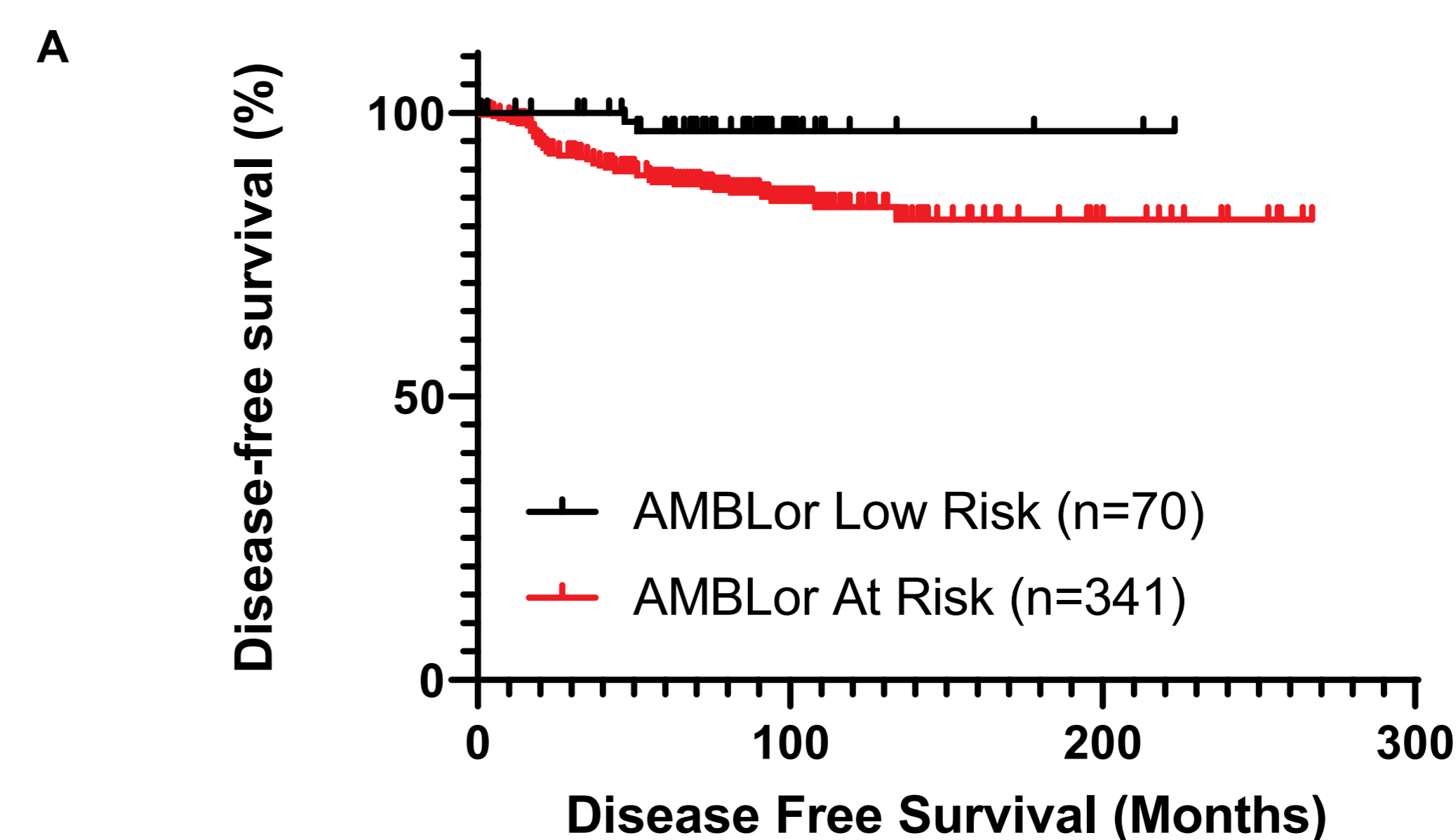


Figure 1. Epidermal AMBRA1 and/or Loricrin expression identifies low versus at risk AJCC stage I melanoma subsets.

Representative photomicrographs of AMBRA1 and Loricrin (AMBLor) expression in the normal marginal epidermis or the epidermis overlying **A** and **B**: low risk AJCC stage IA (T1a) melanomas or **C**: an at risk AJCC stage IB (T1b) melanoma. Scale bar = 100µm

## Results 2



Test	Disease recurrence within 5 years		No disease recurrence within 5 years		Total
	Positive	Negative	Positive	Negative	
Positive	True Positive = 44	False Positive = 297	False Negative = 2	True Negative = 68	341
Negative	False Negative = 2	True Negative = 68	True Positive = 44	False Positive = 297	70
Total	46	365	46	365	411

Figure 2. AMBLor is a prognostic biomarker for non ulcerated cutaneous AJCC stage I and II melanoma

**A.** Disease free survival (DFS, %, up to 287 months) for 70 AMBLor low risk or 341 AMBLor at risk patients with non ulcerated AJCC stage I/II cutaneous melanomas derived from Buffalo, USA and Melbourne, Australia **B.** Contingency table indicating AMBLor test effectiveness; assay sensitivity 95.6%, assay specificity 18.6%, PPV 11.2% and NPV 97.14%