Breslow density is a novel prognostic feature in cutaneous malignant melanoma

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AIM

Measure
Breslow density on
ALL MELANOMAS
after today

But

WHY

should I?

MELANOMA PROGNOSIS

Will this patient die of disease?

Clinical
Histological
Biomarkers

Clinical

Age
Sex
Site

Histological

FEATURE

Breslow thickness

Ulcer

Mitotic rate

Microscopic satellites

Regression

TILs

Clark level

LVI / PNI

Growth phase

Clinico-pathological

AJCC stage

Biomarkers

Table 1. Summary of prognostic tissue biomarker studies in melanoma, including detail of discovery and validation studies and outcome prediction for each marker

Biomarker name	Feature associated with poor prognosis	Assay type	FFPE or frozen tissue	Discovery			Validation					Predicts outcome on MVA or UVA	Which variables in multivariate analysis	Outcome	Effect size*	*P* value*	References
				Size	Cohort	TMA	Size	Cohort	TMA	Independent	Same archive as discovery						
Ki67 as alternative to mitotic count	Ki67 ↑	IHC	FFPE	202	Y	N						MVA	B M U	DSS	HR 3.1	0.003	8
Metallothionein	MT ↑	IHC	FFPE	1270	Y	N						MVA	B U S C A G	OS	RR 3.49	<0.001	13
MITF	MITF ↓	IHC	FFPE	63	N	N						MVA	B M U S H	OS	NSp	0.011	14
Wnt5a	Cytoplasmic expression ↑	IHC	FFPE	94	N	N						MVA	B S G A	OS	HR 2.91	0.003	19
BRMS1	BRMS1 ↓	IHC	FFPE	137	N	Y						MVA	A G St	DSS	RR 0.51	0.02	20
MCAM (MUC18)	MCAM ↑	IHC	FFPE	120	N	Y	78	N	Y	Y	Y	MVA	B A U G	SNP	HR 14.8	0.01	21,22
SNF5	SNF5 ↓	IHC	FFPE	88	N	Y						MVA	A G B S U H	OS	RR 5.1	0.01	23
BCL6, Ki67, p16 and p21 multimarker	p21 ↓, p16 ↓, ki-67 ↑, BCL6 ↑	IHC	FFPE	60	Y	Y	72	N	Y	Y	Y	MVA	B, others NSp	OS	NSp	0.001	24
NCOA3, RGS1 and osteopontin multimarker	NCOA3 ↑, RGS1 ↑, Osteopontin ↑	IHC	FFPE	395	Y	Y	141	Y	N	Y	N	MVA	B C U G S A	DSS	RR 1.34	0.01	25
ATF2, p21, β catenin, p16 and fibronectin multimarker	Based on algorithm score	AQUA-IHC	FFPE	192	Y	Y	246	Y	Y	Y	N	MVA	B A G St Mi S	DSS	HR 2.7	0.03	26
MCM4	MCM4 ↑	IHC	FFPE	62	N	Y	176	N	Y	Y	N	MVA	B U A G	OS	HR 4.04	0.01	32
MCM6	MCM6 ↑	IHC	FFPE	62	N	Y	176	N	Y	Y	N	MVA	B U A G	OS	HR 7.42	0.003	32

Table 1. (*Continued*)

Biomarker name	Feature associated with poor prognosis	Assay type	FFPE or frozen tissue	Discovery			Validation					Predicts outcome on MVA or UVA	Which variables in multivariate analysis	Outcome	Effect size*	*P* value*	References
				Size	Cohort	TMA	Size	Cohort	TMA	Independent	Same archive as discovery						
Osteopontin	Osteopontin ↑	DASL	FFPE	156	Y	Y	198	N	Y	Y	N	MVA	A G S (NS when B included)	RFS	HR 1.67 (1.24)	0.006 (0.32)	34
miRNA expression signature	Expression pattern	miRNA expression array	FFPE	59	N	N						MVA	St	OS	HR 3.16	0.0029	35
miR-15b	MiR15-b ↑	miRNA RT-PCR	FFPE	128	N	N						MVA	A B U	OS	HR 0.41	0.013	36
Gene expression subtype	Gene expression subtype	Expression array	Frozen	57	N	N	44	N	N	Y	Y	UVA		OS	NSp	0.04	38
INK4A	INK4A ↓	RT-PCR	FFPE & frozen	86	N	N						UVA (NS on MVA)		OS	NSp	0.006	39
9p21.3 gene dosage	9p21.3 ↓	MPLA	FFPE	75	N	N						UVA		Relapse	NSp	0.04	40

*Based on validation set data where available.

A, Age; B, Breslow thickness; C, Clark's level; DASL, cDNA-mediated annealing, selection, extension and ligation, DSS, disease-specific survival; G, gender, FFPE, formalin-fixed paraffin-embedded; H, histological subtype; HR, hazard ratio; IHC, immunohistochemistry; M, mitoses; Met, metastasis; Mi, microsatellite lesions; miRNA, microRNA; MPLA, multiplex ligation-dependent probe amplification; MVA, multivariate analysis; N, no; NA, nuclear area; NS, not significant; NSp, not specified; OS, overall survival; RFS, relapse-free survival; RR, relative risk; S, site; SNP, sentinel node positivity; St, American Joint Committee on Cancer stage; TMA, tissue microarray; U, ulceration; UVA, univariate analysis; Y, yes.

Moore, Histopath 2012

Biomarkers

TABLE 4. Protein Biomarkers With Independent Prognostic Significance

Marker	Function	Staining
AP-2 (alpha)[96,97]	Transcription factor	High level of AP-2 expression in the cytoplasm relative to the nucleus correlates with poor prognosis and the loss of nuclear
	52-kd DNA-binding protein	AP-2 expression is associated with malignant transformation and progression of melanoma[96]
	Self-sufficiency in growth signals	Decreased AP-2 expression independently associated with elevated risk of subsequent metastatic of stage I cutaneous malignant melanoma[97]
ATF-2[98]	Transcription factor	In primary cutaneous melanomas, strong nuclear staining and weak cytoplasmic staining was an independent poor outcome predictor
	Self-sufficiency in growth signals	
NCOA3[99]	Steroid receptor coactivator family member	Expression was associated with increased SLN metastases, reduced relapse-free and disease-specific survival
	Stimulates transcriptional activity in a hormone-dependent fashion by direct binding to nuclear receptors	NCOA3 was shown to be a stronger disease-specific survival predictor than all other variables, including tumor thickness.
	Self-sufficiency in growth signals	
PRKCA[100]	Belongs to the epithelial–mesenchymal transition group	Increased cytoplasmic expression in melanoma cells
	Regulates cell growth and progression	Predicts melanoma metastasis independent of Breslow index
	Self-sufficiency in growth signals	
Bcl-2[101]	Evasion of apoptosis	High expression was associated with a better outcome in the entire cohort and among metastatic specimens only
		Expression was higher in primary than in metastatic melanomas
Survivin[102]	Inhibitor of apoptosis protein family	Nuclear expression is associated with disease recurrence and poor survival in patients with stage I and II melanoma
CEACAM-1[103]	Required for the intercellular adhesion and subsequent signal transduction events	28 of 40 patients with CEACAM1-positive primary melanomas developed metastatic disease, compared with only 6 of 60 patients with CEACAM1-negative melanomas.
	Tissue invasion and metastasis	Highly significant association between CEACAM1 expression and metastasis
CXCR4[104]	Seven-domain transmembrane chemokine receptor recently implicated in cancer metastasis	Expression in melanoma cells correlated with unfavorable prognosis and correlated with a decreased median disease-free and overall survival.
	Tissue invasion and metastasis	
CD44[105]	Cell surface glycoprotein	Reduced CD44 expression associated with short recurrence-free survival and unfavorable prognosis in stage I cutaneous melanoma
	Tissue invasion and metastasis	
MCAM[106,107]	Adhesion molecule	Expression was an independent prognostic indicator inversely correlated with patient survival
	Mediates interactions between melanoma cells and between melanoma cells and endothelial cells	5-yr Survival was 92% for MCAM-negative patients compared with 40% for MCAM-positive patients.
	Tissue invasion and metastasis	MCAM expression was a stronger prognostic indicator than Breslow thickness.
L1-CAM[108]	Adhesion molecule	Overexpression associated with metastasis in malignant melanoma
	Binds to integrin alpha5-beta3	
	Tissue invasion and metastasis	
MMP-2[109,110]	Tissue invasion and metastasis	MMP-2 overexpression (>20% of malignant cells positive) was an independent prognostic marker for melanoma related death
		10-yr Disease-specific survival rate was only 51% in patients with MMP-2 overexpression compared with 79% in patients with a primary melanoma with low expression for MMP-2
OPN osteopontin[111]	Glycoprotein expressed by various tissues and cells—tissue invasion and metastasis	Expression was associated with reduced disease-specific and recurrence-free survival and was significantly predictive of SLN metastasis and burden
Tenascin-C[112]	Tissue invasion and metastasis	In primary cutaneous melanoma, absence of tenascin-C expression in the stroma of invasion fronts and within melanoma cells seems to be related to a more benign disease behavior with a lower risk of developing metastases

Abbas, A J Dermatopathol 2014

Can we discover new biomarkers using only H&E sections?

What can be measured

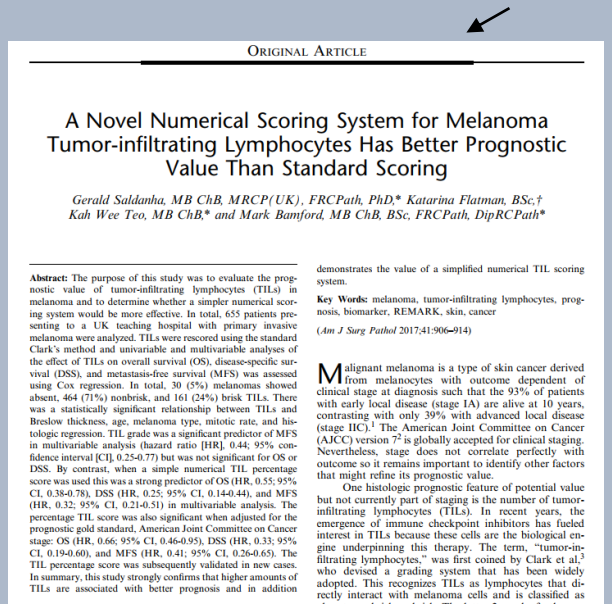
… and is simple, quick and cheap?

H&E biomarker candidates

Histological correlates of molecular pathology – UV damage / BRAFoma

Cellular infiltrates – neutrophils / eosinophils

Enhancement of existing histological biomarkers – TILs / regression/Ulcer / BT



ORIGINAL ARTICLE

A Novel Numerical Scoring System for Melanoma Tumor-infiltrating Lymphocytes Has Better Prognostic Value Than Standard Scoring

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Abstract: The purpose of this study was to evaluate the prognostic value of tumor-infiltrating lymphocytes (TILs) in melanoma and to determine whether a simpler numerical scoring system would be more effective. In total, 655 patients presenting to a UK teaching hospital with primary invasive melanoma were analyzed. TILs were rescored using the standard Clark's method and univariable and multivariable analyses of the effect of TILs on overall survival (OS), disease-specific survival (DSS), and metastasis-free survival (MFS) was assessed using Cox regression. In total, 30 (5%) melanomas showed absent, 464 (71%) nonbrisk, and 161 (24%) brisk TILs. There was a statistically significant relationship between TILs and Breslow thickness, age, melanoma type, mitotic rate, and histologic regression. TIL grade was a significant predictor of MFS in multivariable analysis (hazard ratio [HR], 0.44; 95% confidence interval [CI], 0.25-0.77) but was not significant for OS or DSS. By contrast, when a simple numerical TIL percentage score was used this was a strong predictor of OS (HR, 0.55; 95% CI, 0.38-0.78), DSS (HR, 0.25; 95% CI, 0.14-0.44), and MFS (HR, 0.32; 95% CI, 0.21-0.51) in multivariable analysis. The percentage TIL score was also significant when adjusted for the prognostic gold standard, American Joint Committee on Cancer stage: OS (HR, 0.66; 95% CI, 0.46-0.95), DSS (HR, 0.33; 95% CI, 0.19-0.60), and MFS (HR, 0.41; 95% CI, 0.26-0.65). The TIL percentage score was subsequently validated in new cases. In summary, this study strongly confirms that higher amounts of TILs are associated with better prognosis and in addition demonstrates the value of a simplified numerical TIL scoring system.

Key Words: melanoma, tumor-infiltrating lymphocytes, prognosis, biomarker, REMARK, skin, cancer

(*Am J Surg Pathol* 2017;41:906–914)

Malignant melanoma is a type of skin cancer derived from melanocytes with outcome dependent of clinical stage at diagnosis such that the 93% of patients with early local disease (stage IA) are alive at 10 years, contrasting with only 39% with advanced local disease (stage IIC).[1] The American Joint Committee on Cancer (AJCC) version 7[2] is globally accepted for clinical staging. Nevertheless, stage does not correlate perfectly with outcome so it remains important to identify other factors that might refine its prognostic value.

One histologic prognostic feature of potential value but not currently part of staging is the number of tumor-infiltrating lymphocytes (TILs). In recent years, the emergence of immune checkpoint inhibitors has fueled interest in TILs because these cells are the biological engine underpinning this therapy. The term, "tumor-infiltrating lymphocytes," was first coined by Clark et al,[3] who devised a grading system that has been widely adopted. This recognizes TILs as lymphocytes that directly interact with melanoma cells and is classified as

From biomarker discovery to practice

The long road to biomarker translation

Scientific validity = discovery

Analytical validity

Clinical validity

Clinical utility

The long road to biomarker translation - BD

Scientific validity = discovery

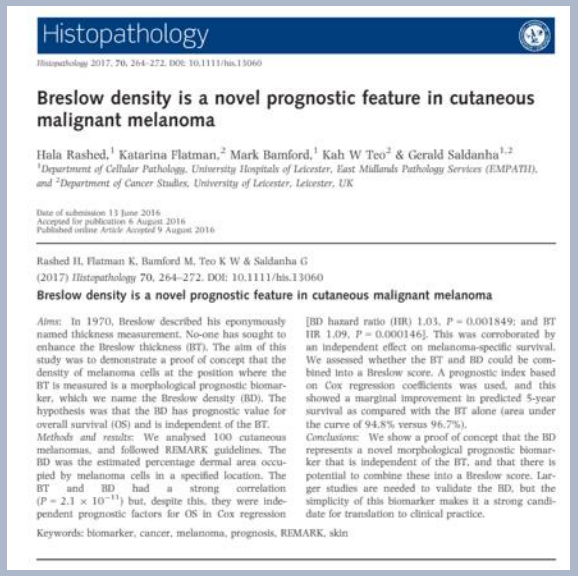
Analytical validity

Clinical validity

Clinical utility

Today

Future studies



Histopathology

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Breslow density is a novel prognostic feature in cutaneous malignant melanoma

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Breslow density is a novel prognostic feature in cutaneous malignant melanoma

Aims: In 1970, Breslow described his eponymously named thickness measurement. No-one has sought to enhance the Breslow thickness (BT). The aim of this study was to demonstrate a proof of concept that the density of melanoma cells at the position where the BT is measured is a morphological prognostic biomarker, which we name the Breslow density (BD). The hypothesis was that the BD has prognostic value for overall survival (OS) and is independent of the BT.
Methods and results: We analysed 100 cutaneous melanomas, and followed REMARK guidelines. The BD was the estimated percentage dermal area occupied by melanoma cells in a specified location. The BT and BD had a strong correlation ($P = 2.1 \times 10^{-11}$) but, despite this, they were independent prognostic factors for OS in Cox regression [BD hazard ratio (HR) 1.03, $P = 0.001849$; and BT HR 1.09, $P = 0.000146$]. This was corroborated by an independent effect on melanoma-specific survival. We assessed whether the BT and BD could be combined into a Breslow score. A prognostic index based on Cox regression coefficients was used, and this showed a marginal improvement in predicted 5-year survival as compared with the BT alone (area under the curve of 94.8% versus 96.7%).
Conclusions: We show a proof of concept that the BD represents a novel morphological prognostic biomarker that is independent of the BT, and that there is potential to combine these into a Breslow score. Larger studies are needed to validate the BD, but the simplicity of this biomarker makes it a strong candidate for translation to clinical practice.

Keywords: biomarker, cancer, melanoma, prognosis, REMARK, skin

BRESLOW DENSITY: WHAT IS IT?

Enhance Breslow thickness

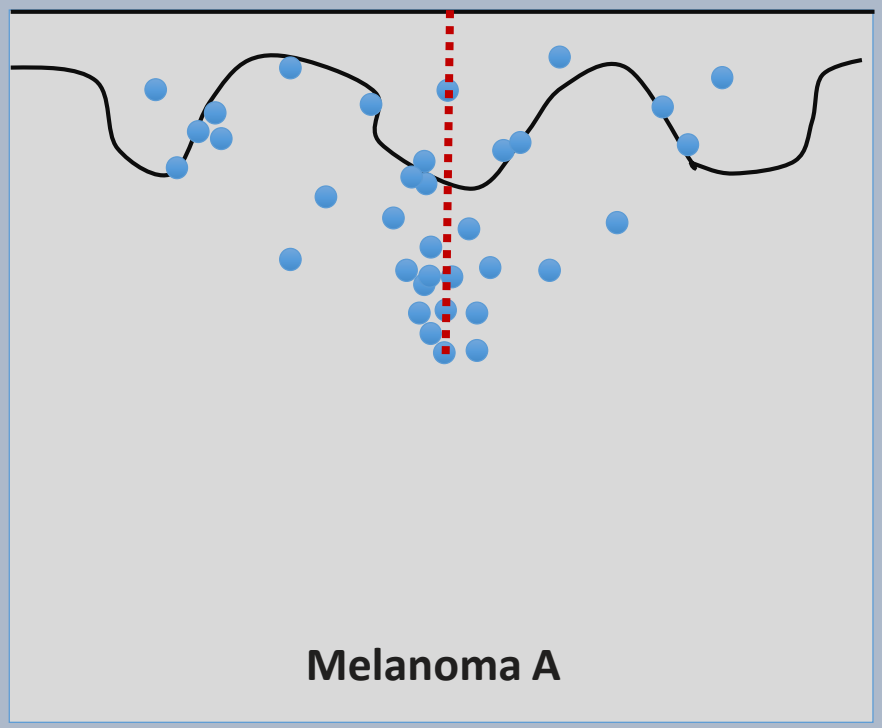
A one dimensional crude surrogate for invasive melanoma cell burden

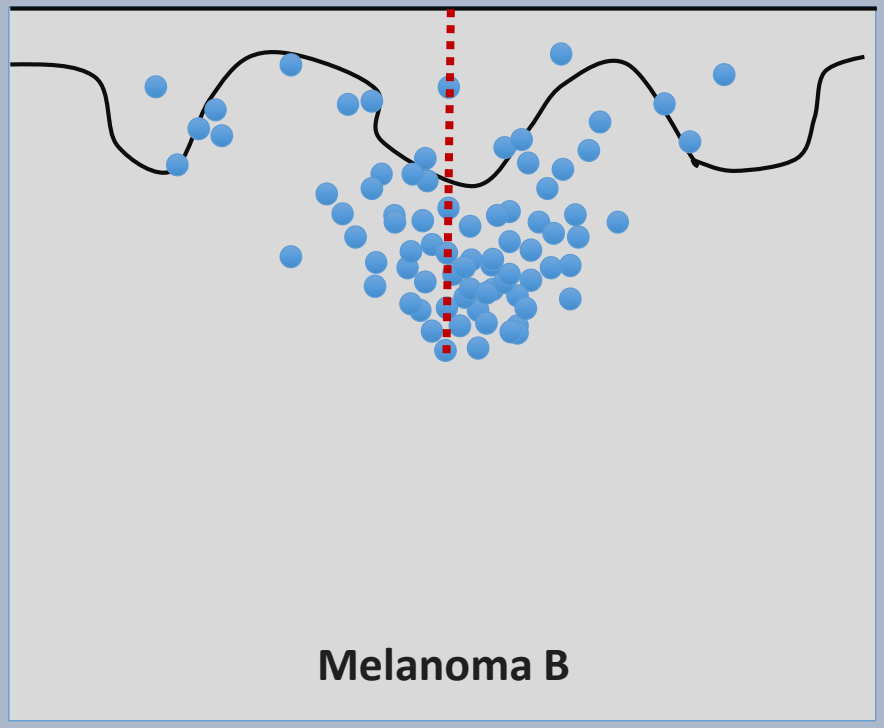
BUT

A H&E section has 2 dimensions so why not take advantage of this?

Breslow density – the concept

1 dimension



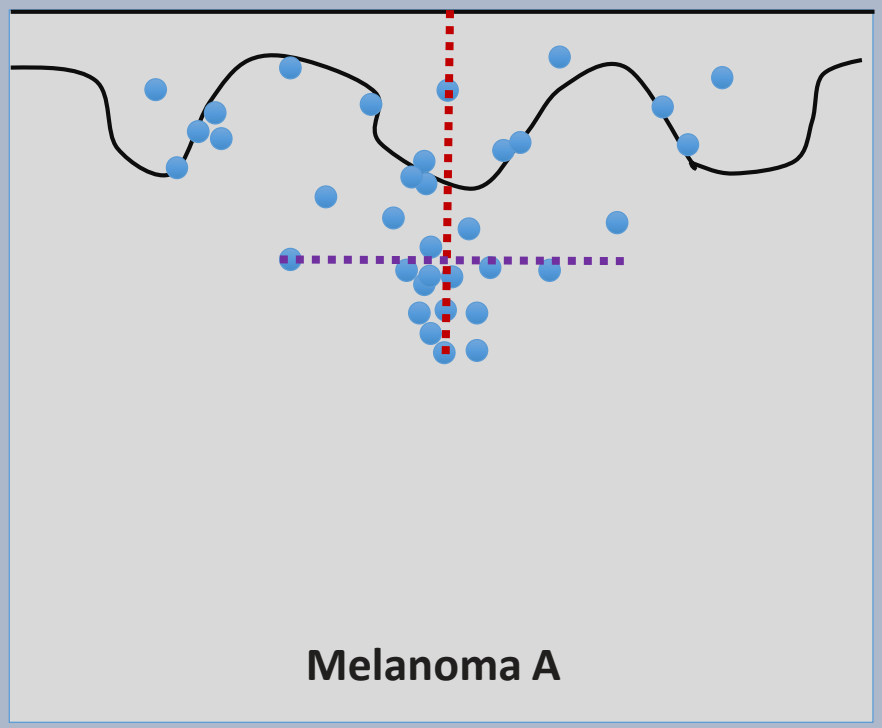


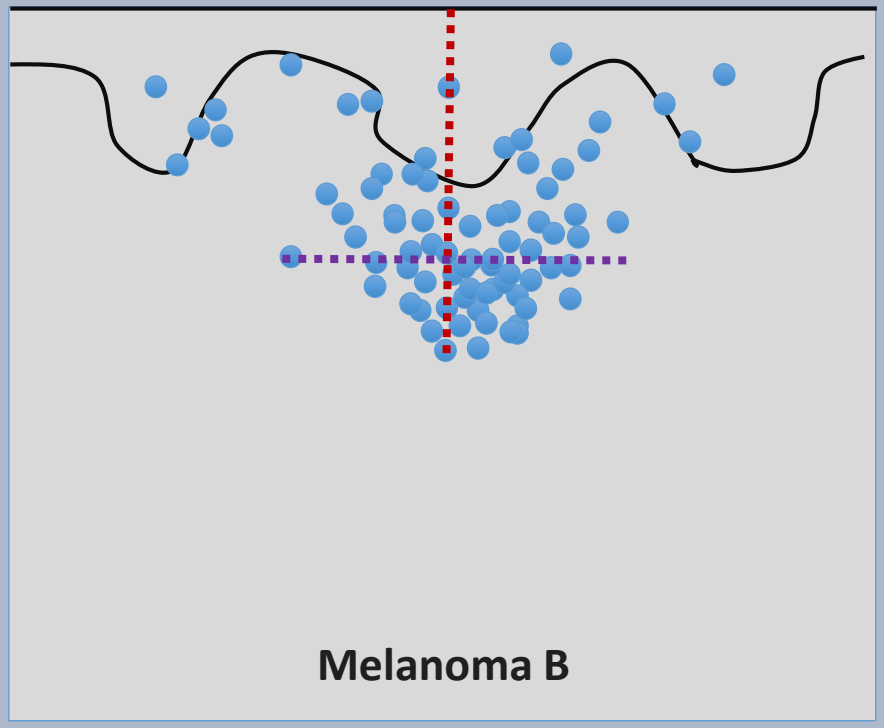
Prognosis

Melanoma A = Melanoma B

Breslow density – the concept

2 dimensions



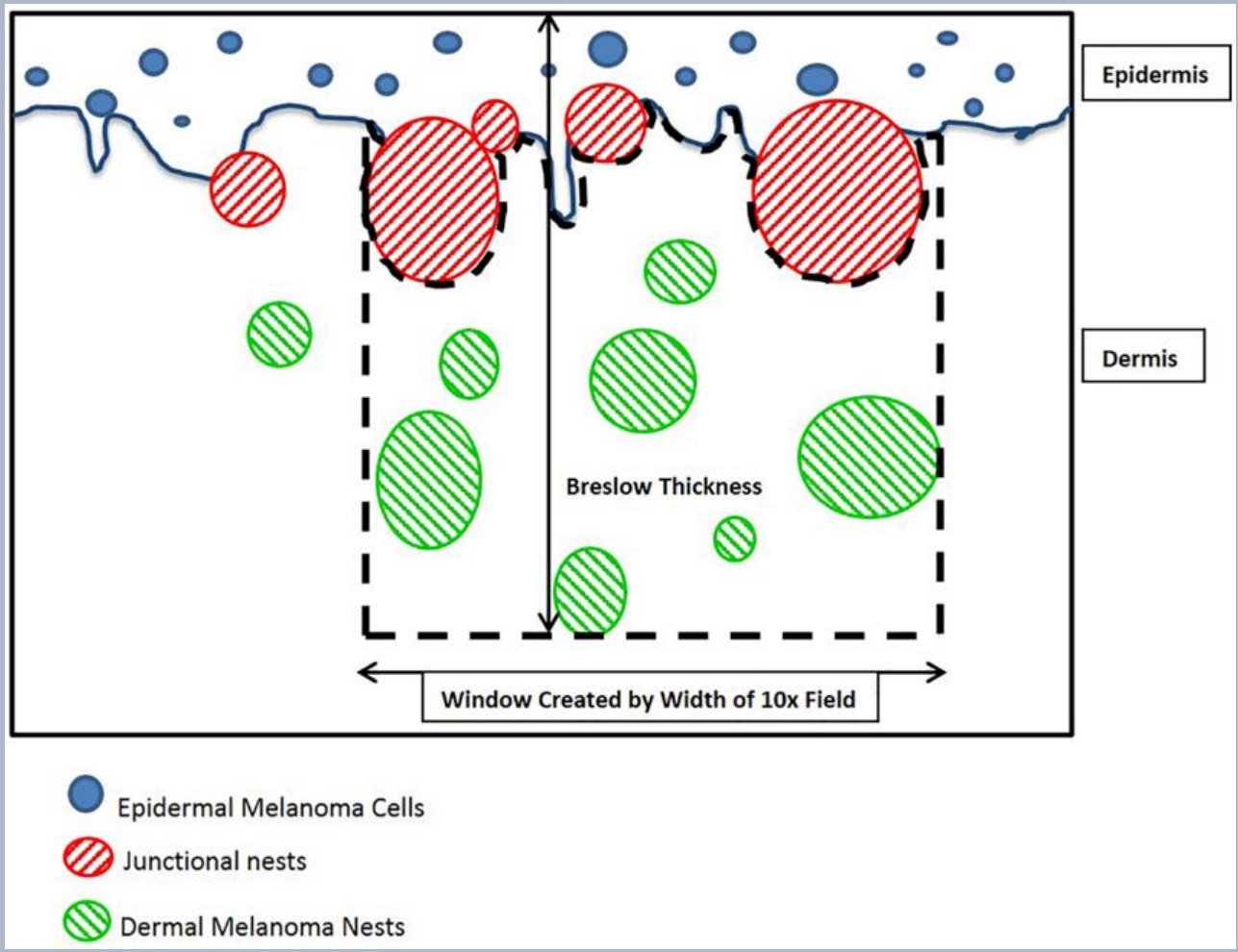


Prognosis

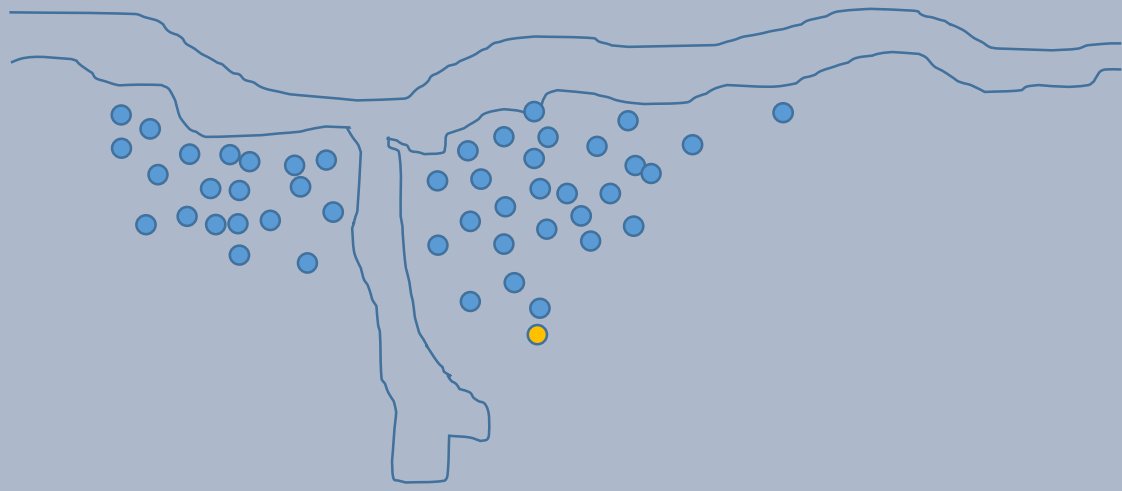
Melanoma A **>** Melanoma B

BRESLOW DENSITY: HOW IS IT MEASURED?

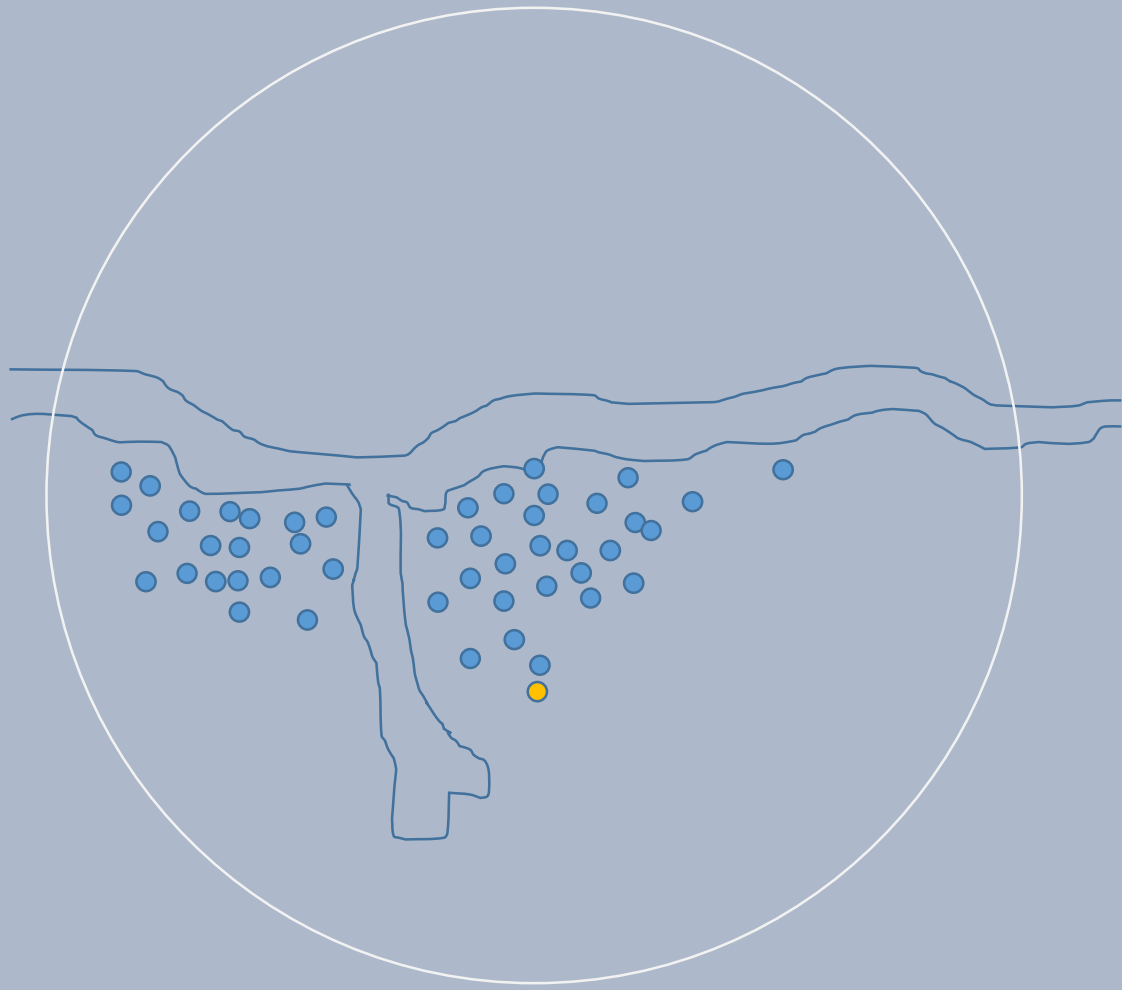
How can Breslow density be measured?



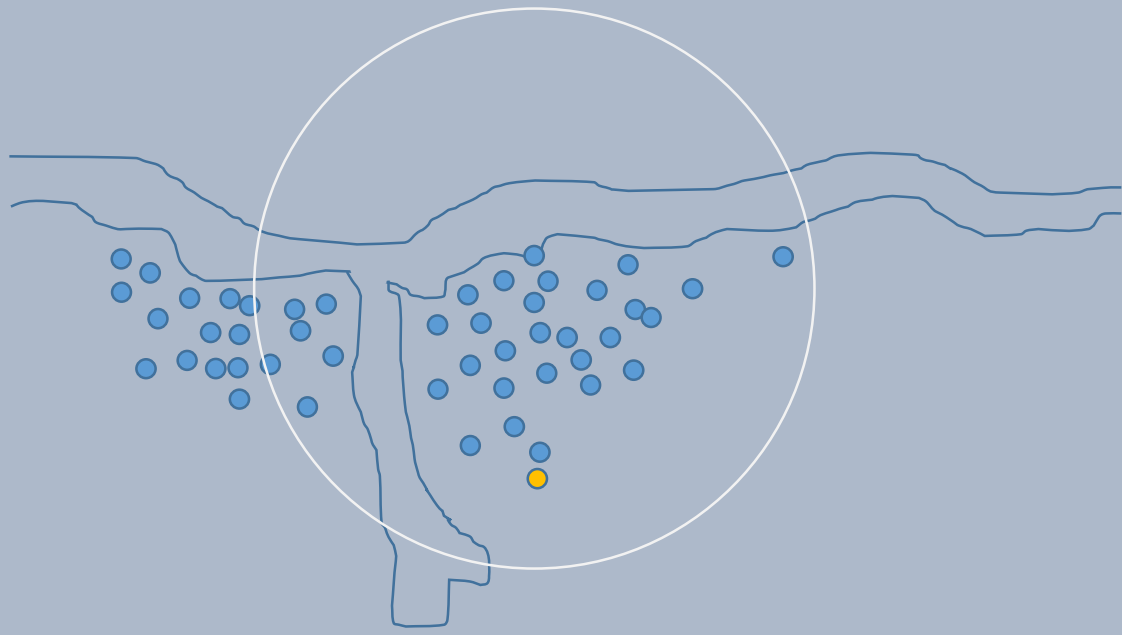
1. During routine reporting, find deepest cell



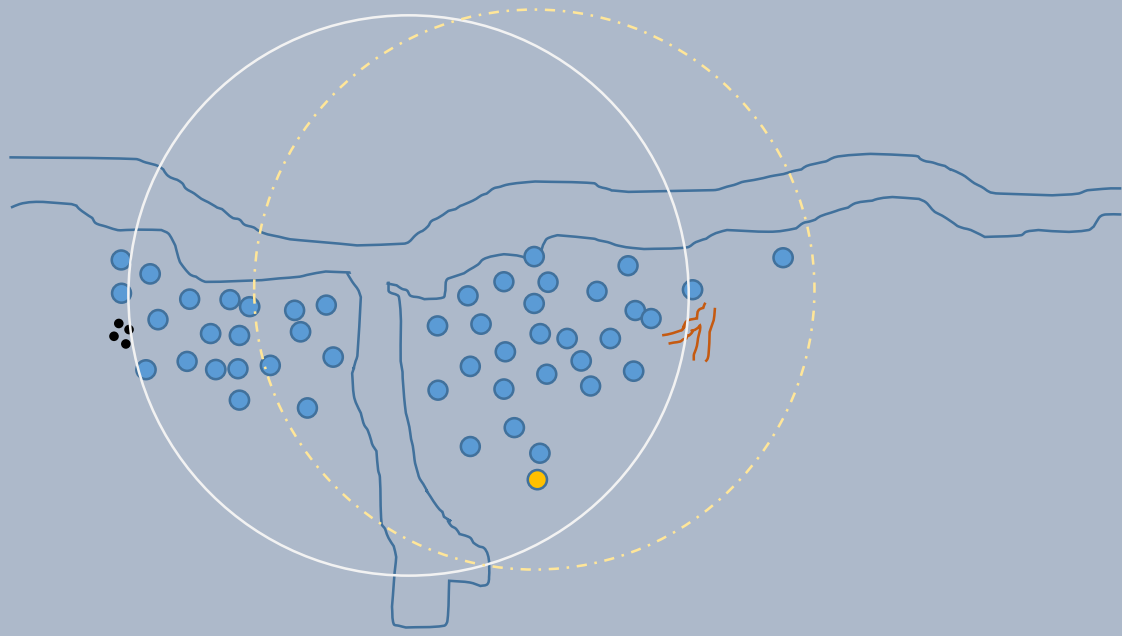
1. During routine reporting, find deepest cell
2. With scanning lens, centre the field on deepest cell



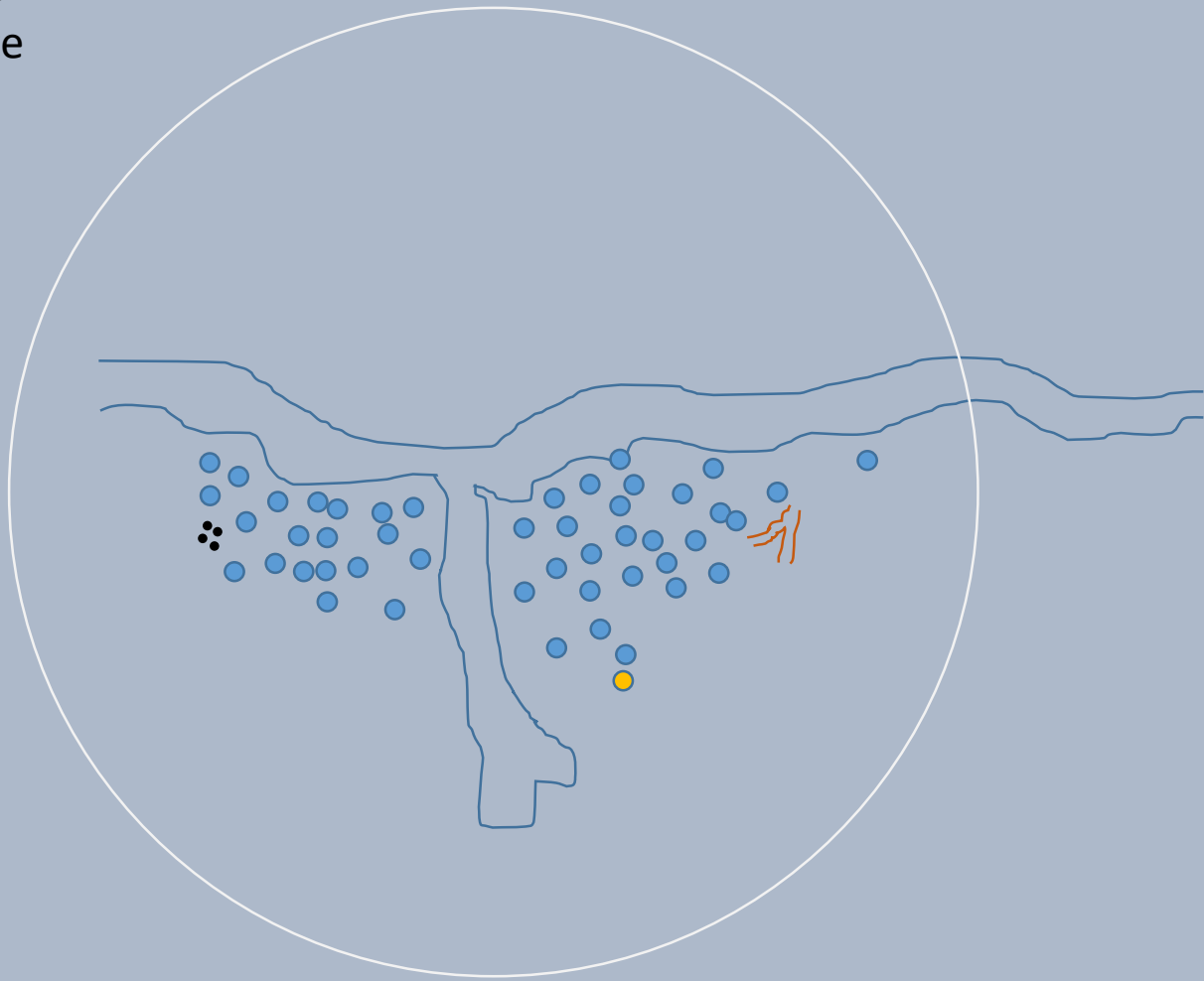
1. During routine reporting, find deepest cell
2. With scanning lens, centre the field on deepest cell
3. Switch to x10 lens



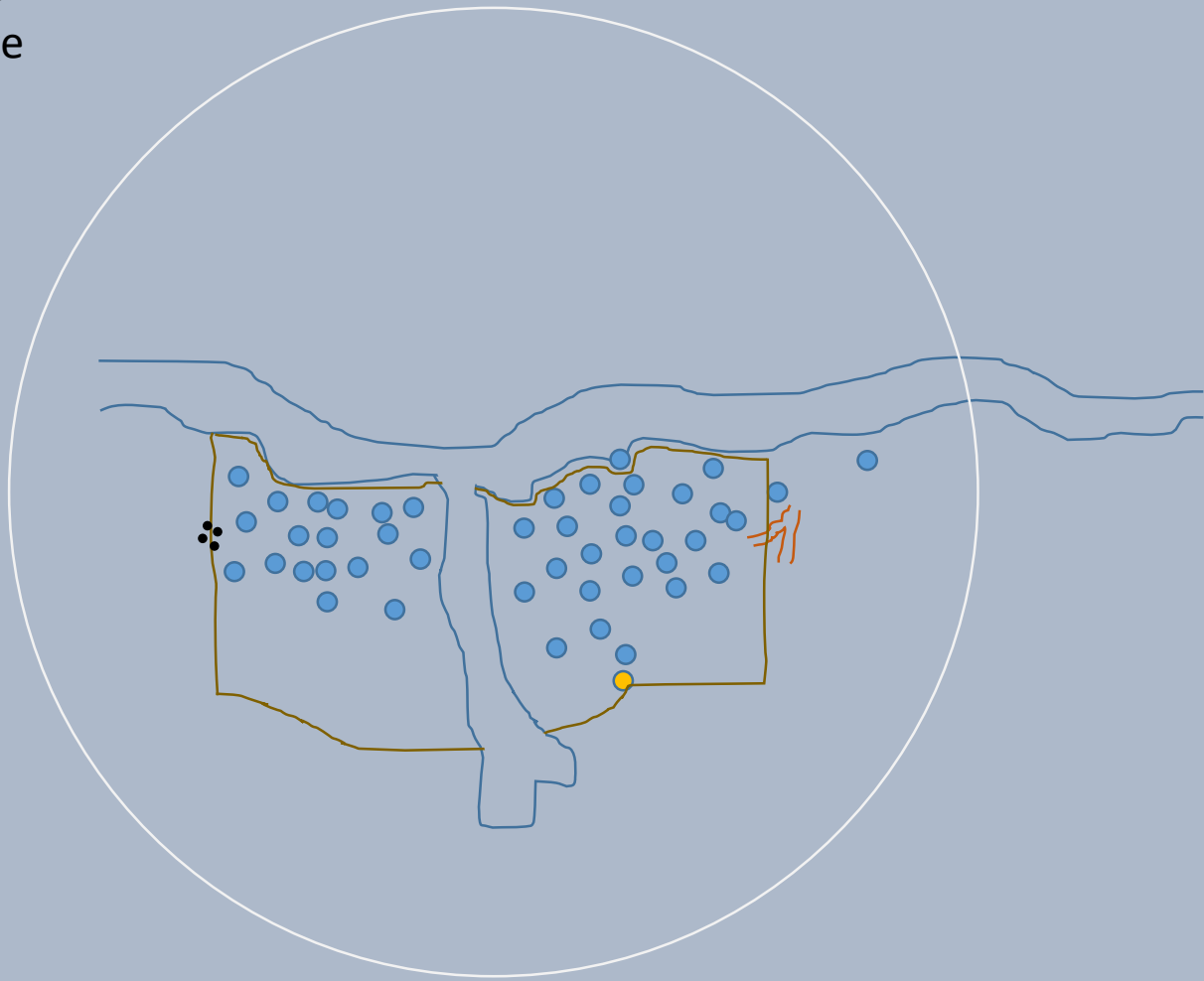
1. During routine reporting, find deepest cell
2. With scanning lens, centre the field on deepest cell
3. Switch to x10 lens
4. Adjust field to maximise BD while still including deepest cell. Use any landmarks to delineate lateral edge of BD window



1. During routine reporting, find deepest cell
2. With scanning lens, centre the field on deepest cell
3. Switch to x10 lens
4. Adjust field to maximise BD while still including deepest cell. Use any landmarks to delineate lateral edge of BD window
5. Switch back to scanning lens



1. During routine reporting, find deepest cell
2. With scanning lens, centre the field on deepest cell
3. Switch to x10 lens
4. Adjust field to maximise BD while still including deepest cell. Use any landmarks to delineate lateral edge of BD window
5. Switch back to scanning lens
6. Mentally construct crude BD window using landmarks and estimate BD% (~20%)



1. During routine reporting, find deepest cell
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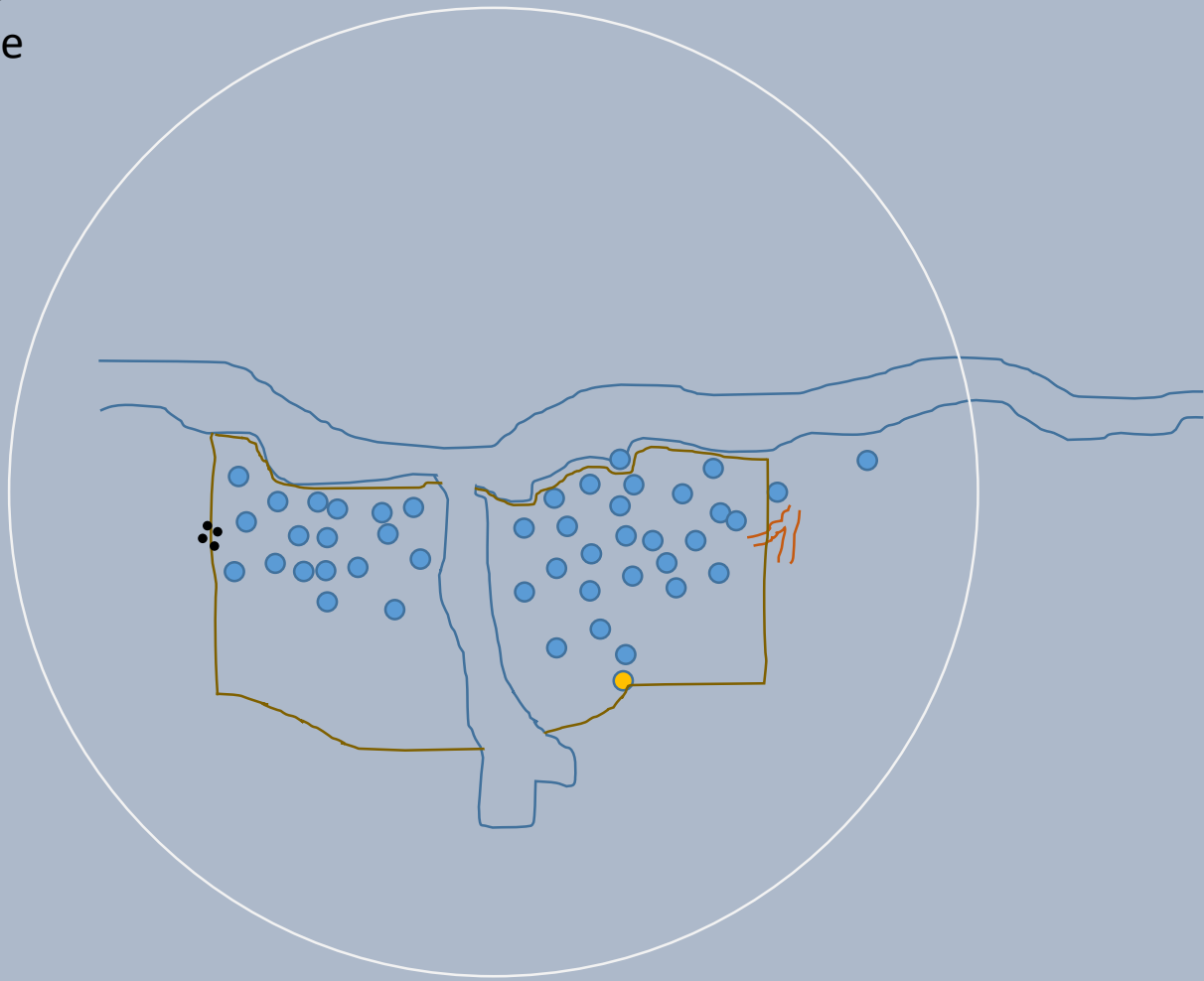
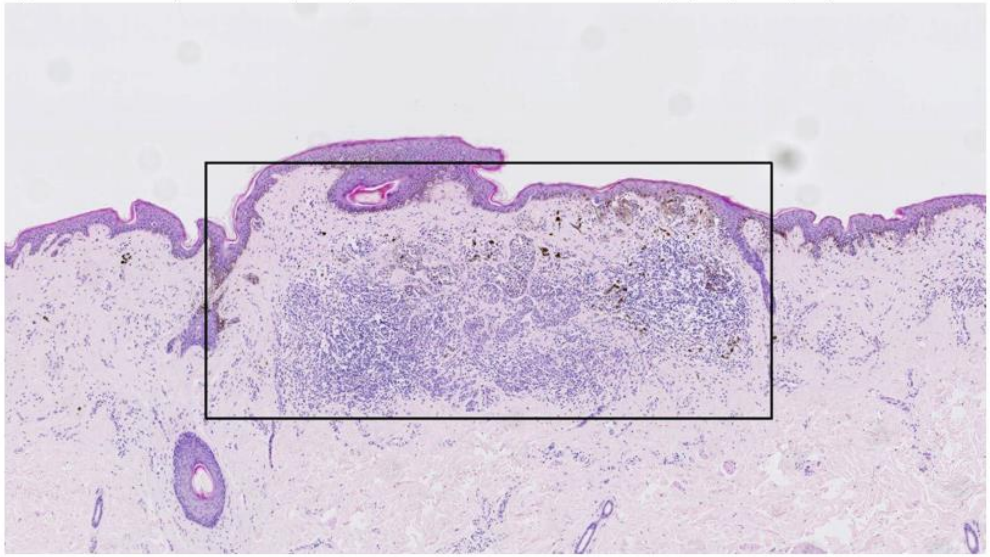
30 seconds

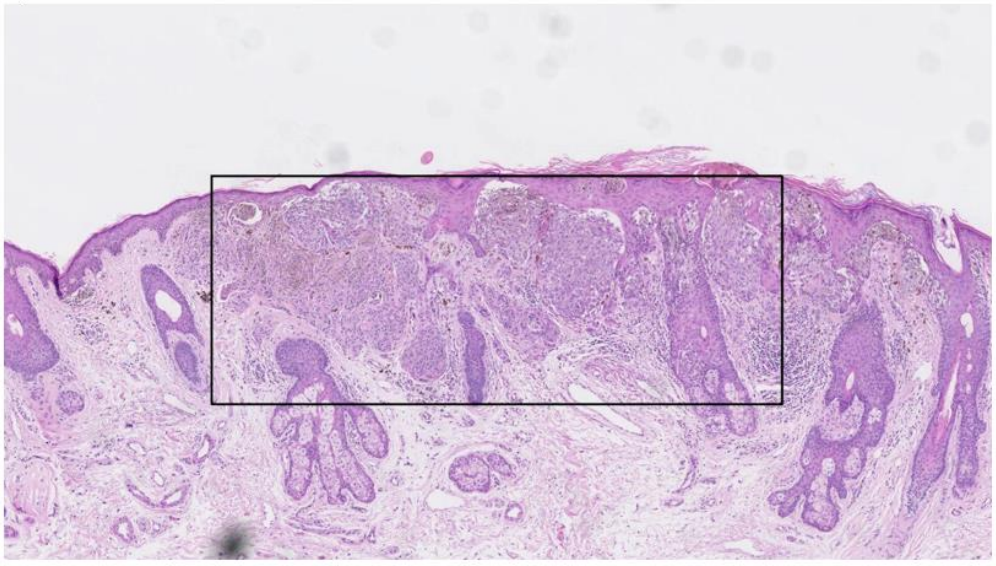


How can Breslow density be measured?

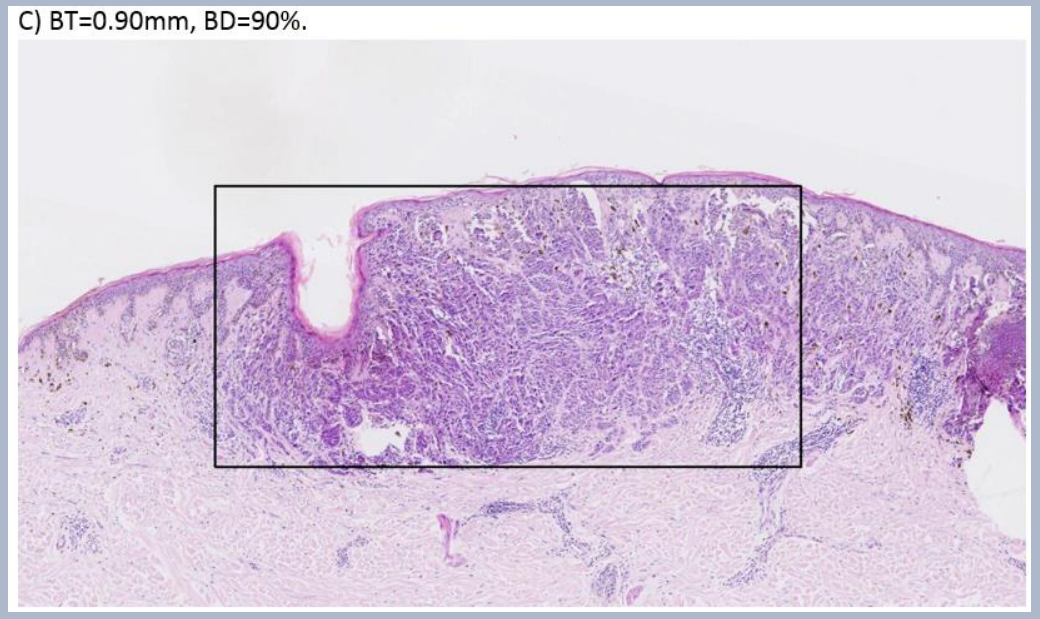
A) BT=0.80mm, BD=35% (many cells are tumour-infiltrating lymphocytes).

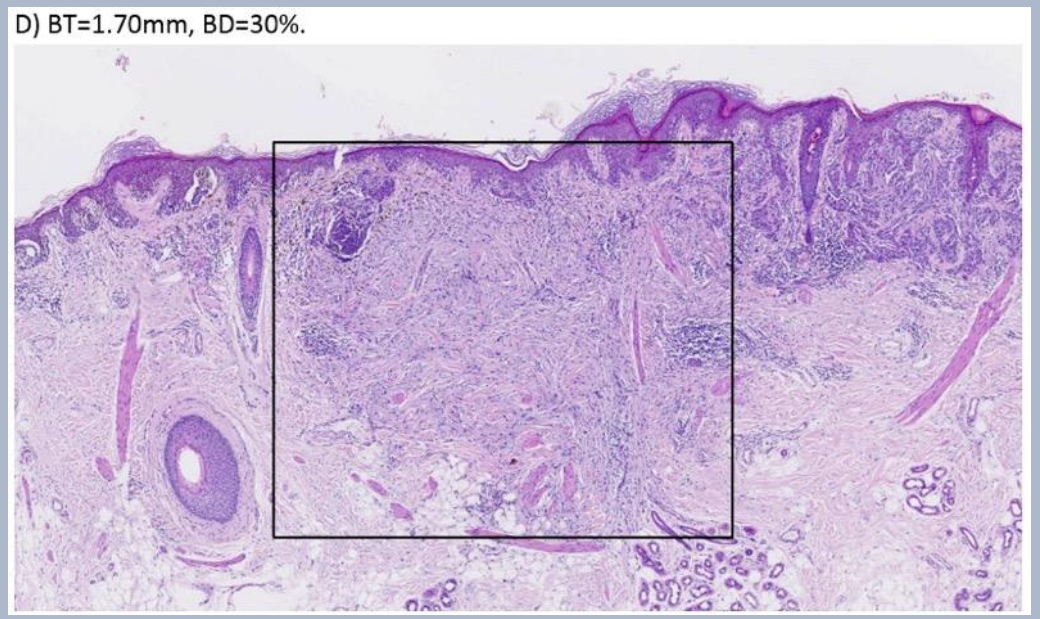


B) BT=0.80mm, BD=60%.

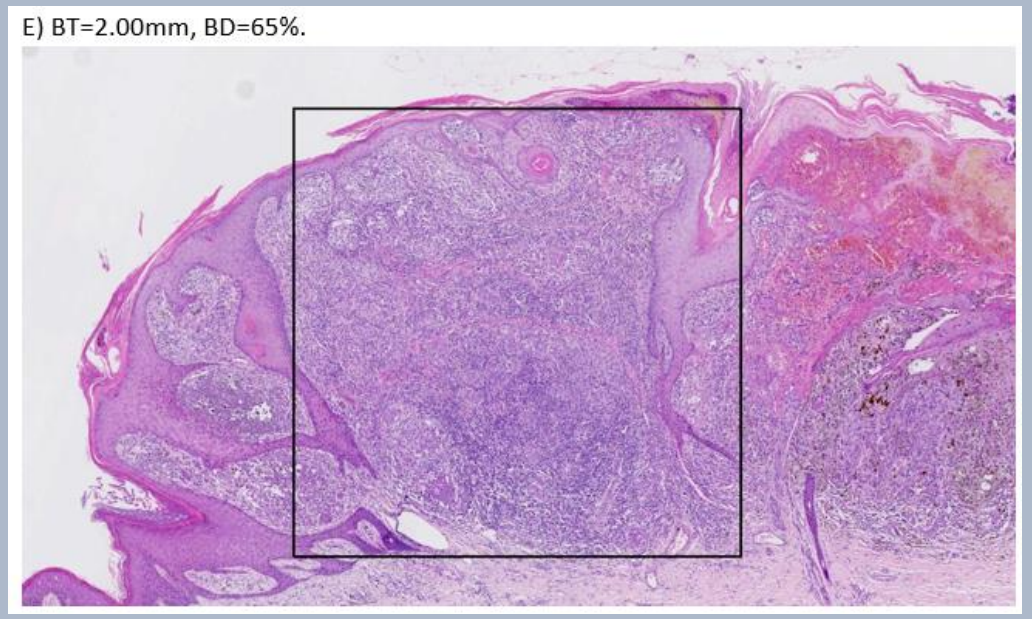


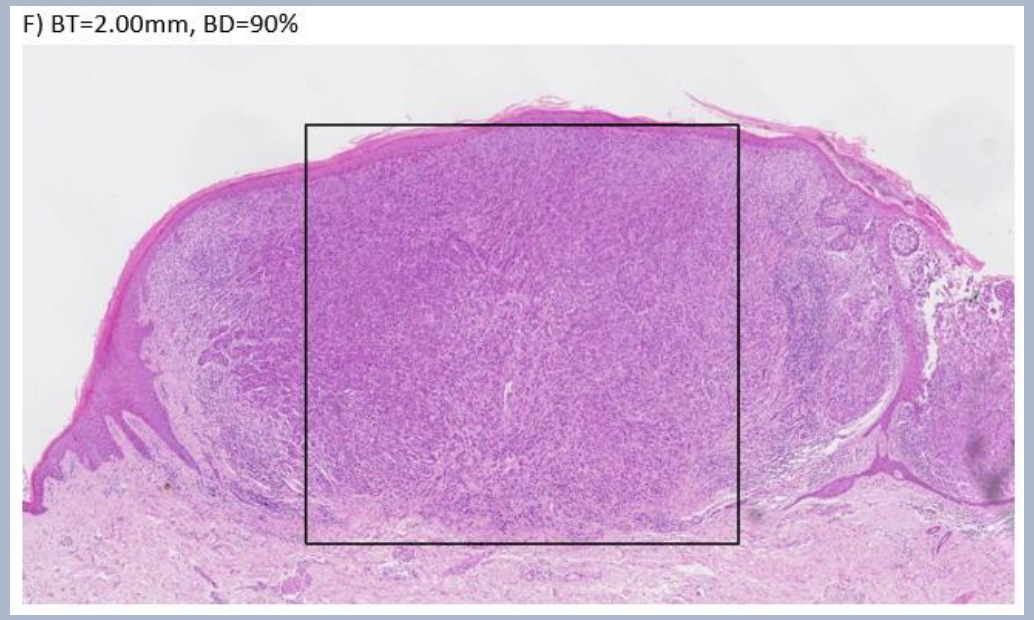
How can Breslow density be measured?



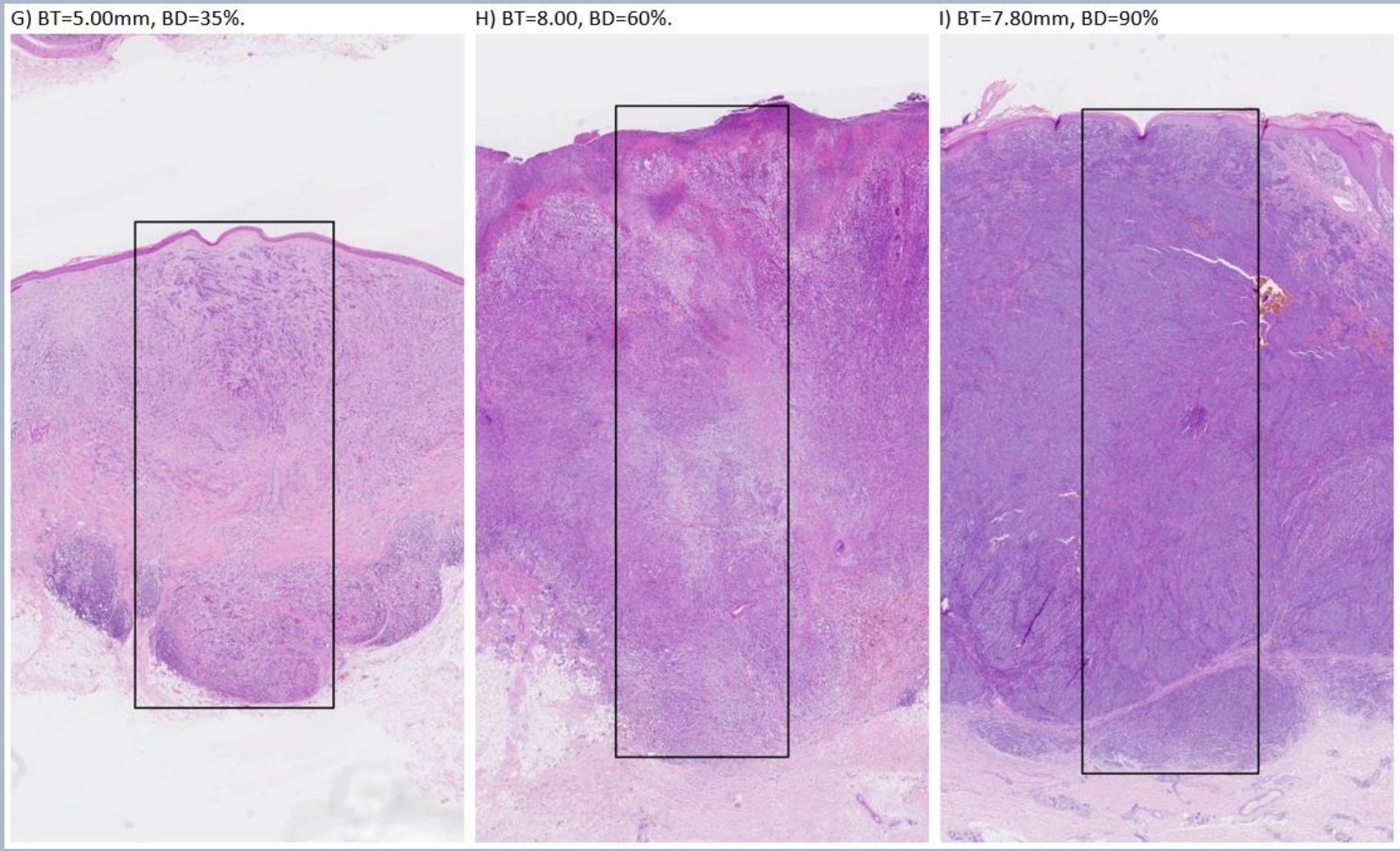


How can Breslow density be measured?





How can Breslow density be measured?



BRESLOW DENSITY: Analytically valid?

Accuracy?

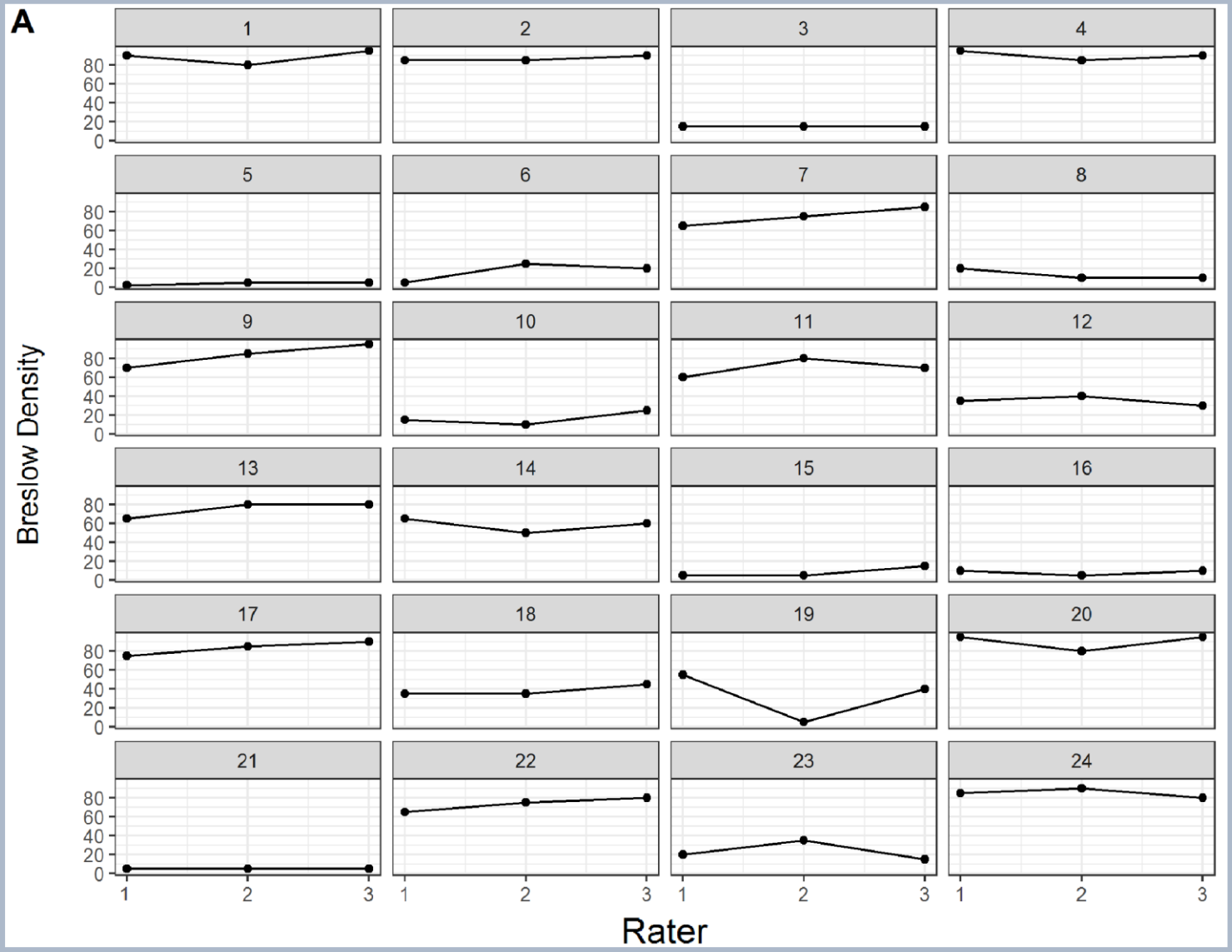
Image analysis vs observer estimate

ICC

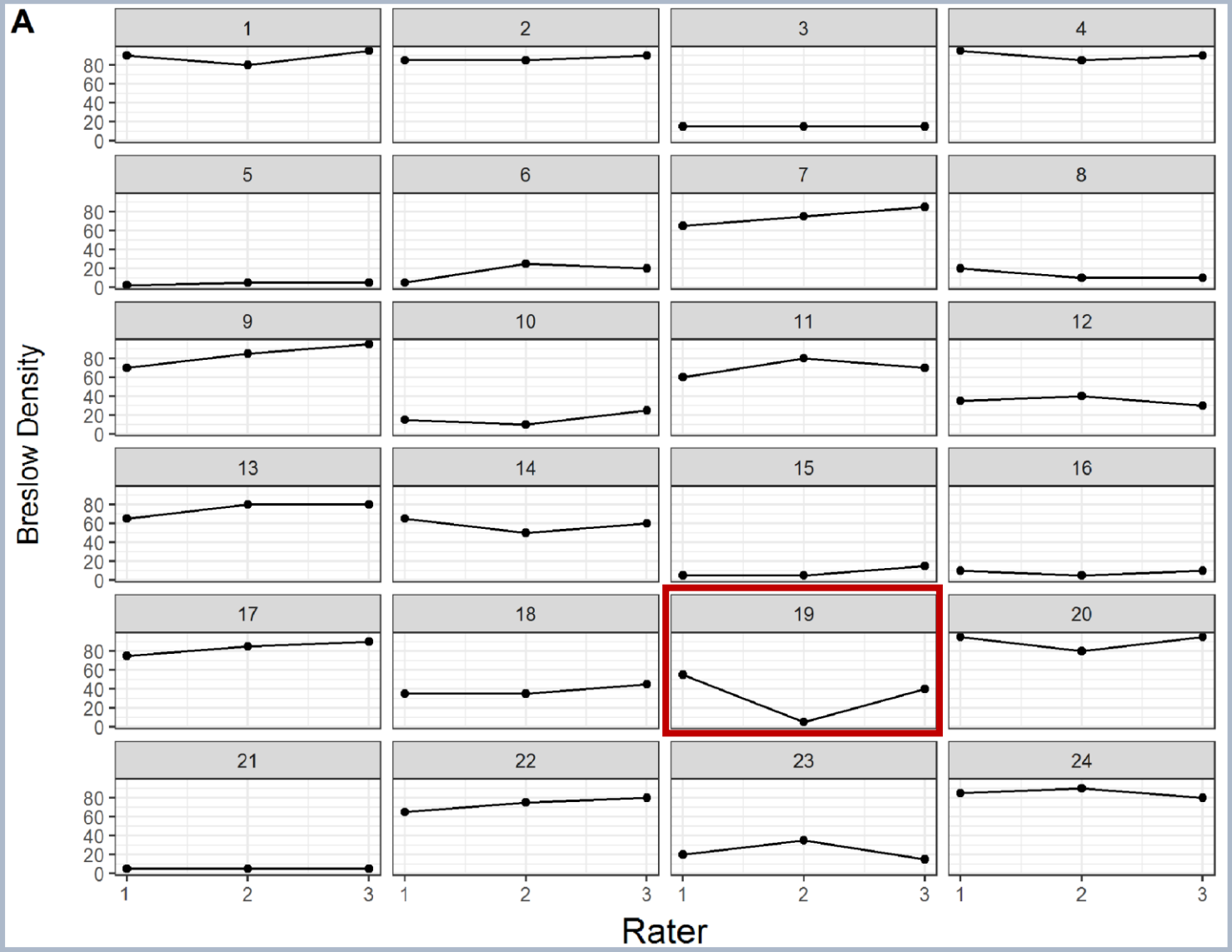
0.84

Precision

ICC
0.93



Precision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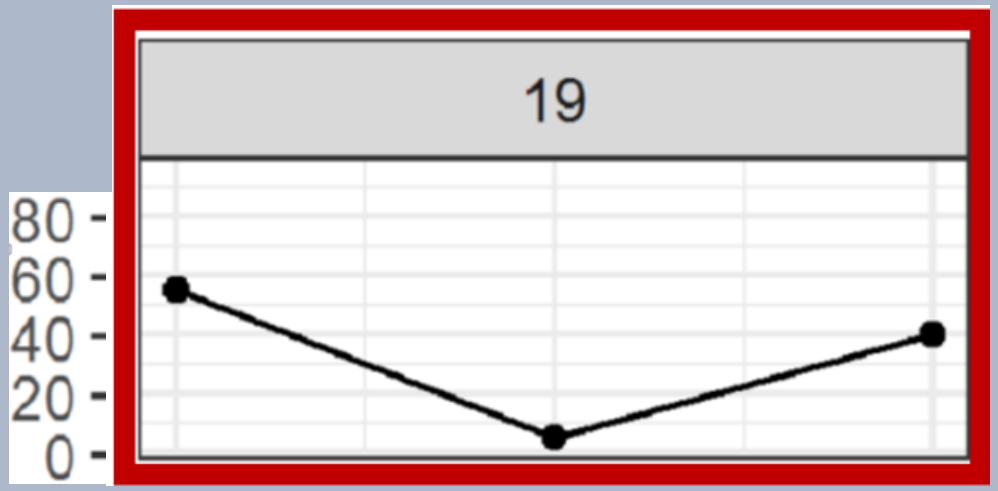
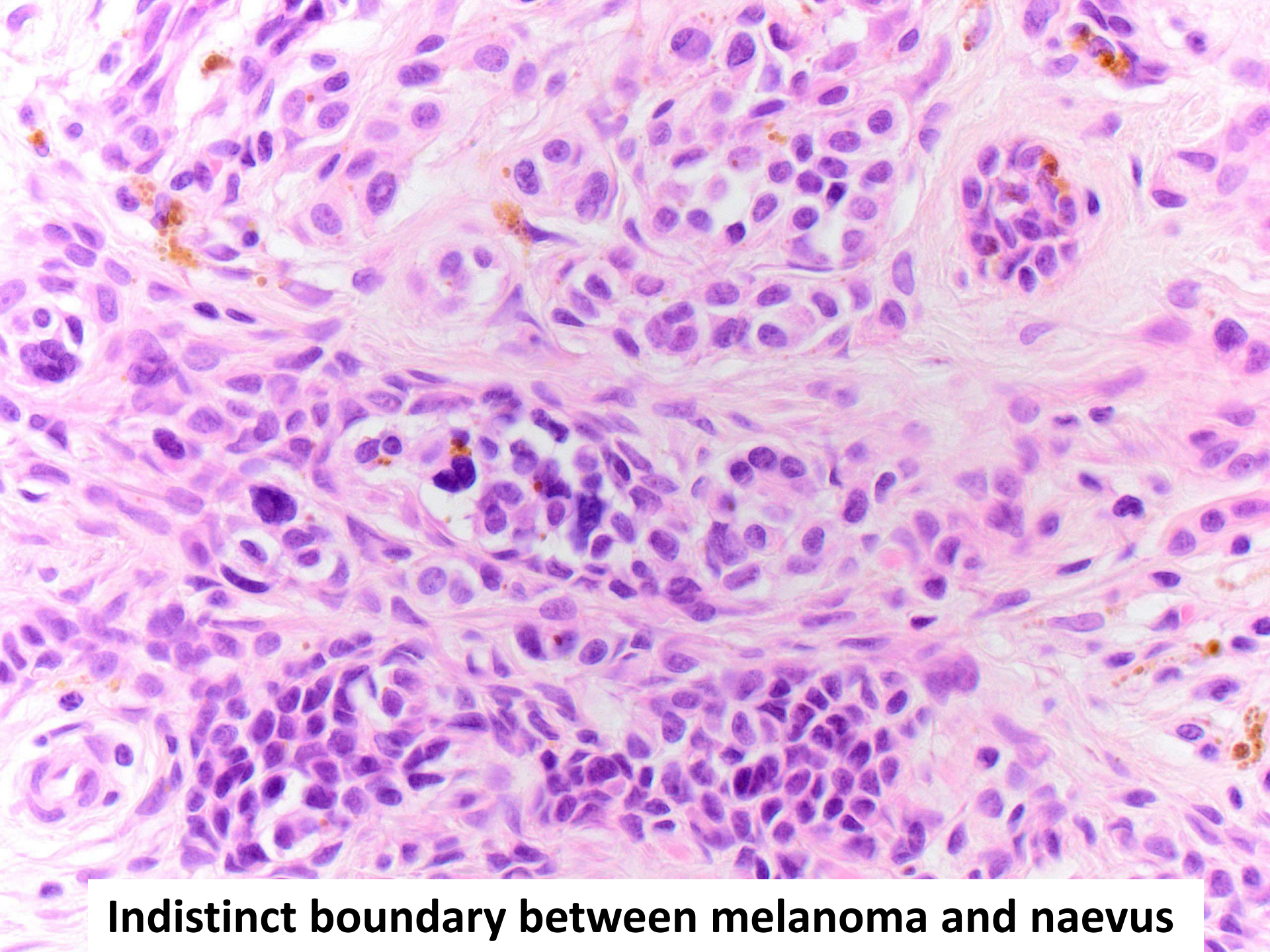
Breslow Density
0
20
40
60
80
1
2
3
Rater

Precision





Indistinct boundary between melanoma and naevus

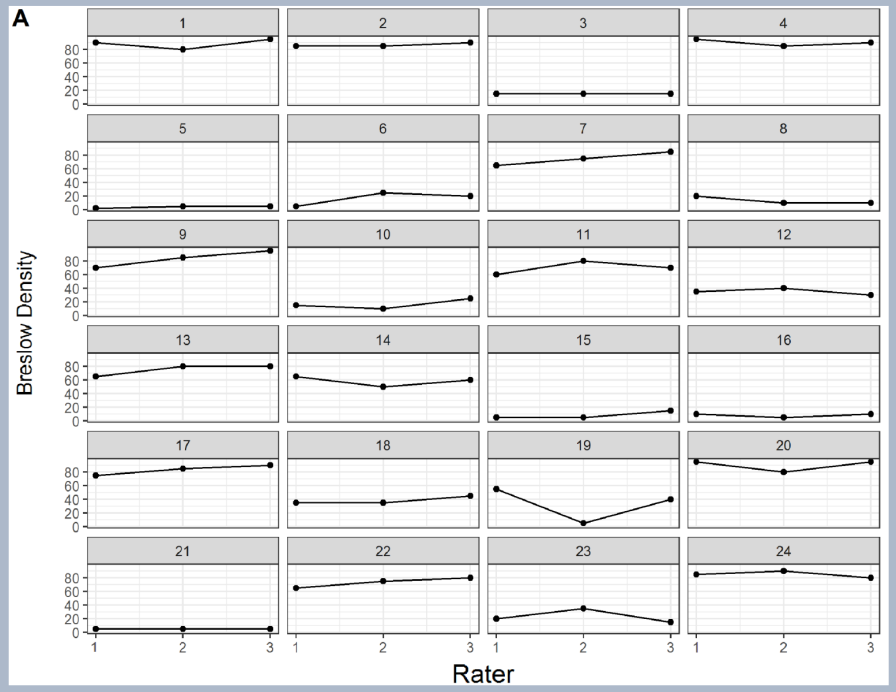
Precision

Indistinct boundary between melanoma and naevus

Inflamed melanomas

Expansile junctional nests

Precision



Mean score v Pathologist with only written instruction

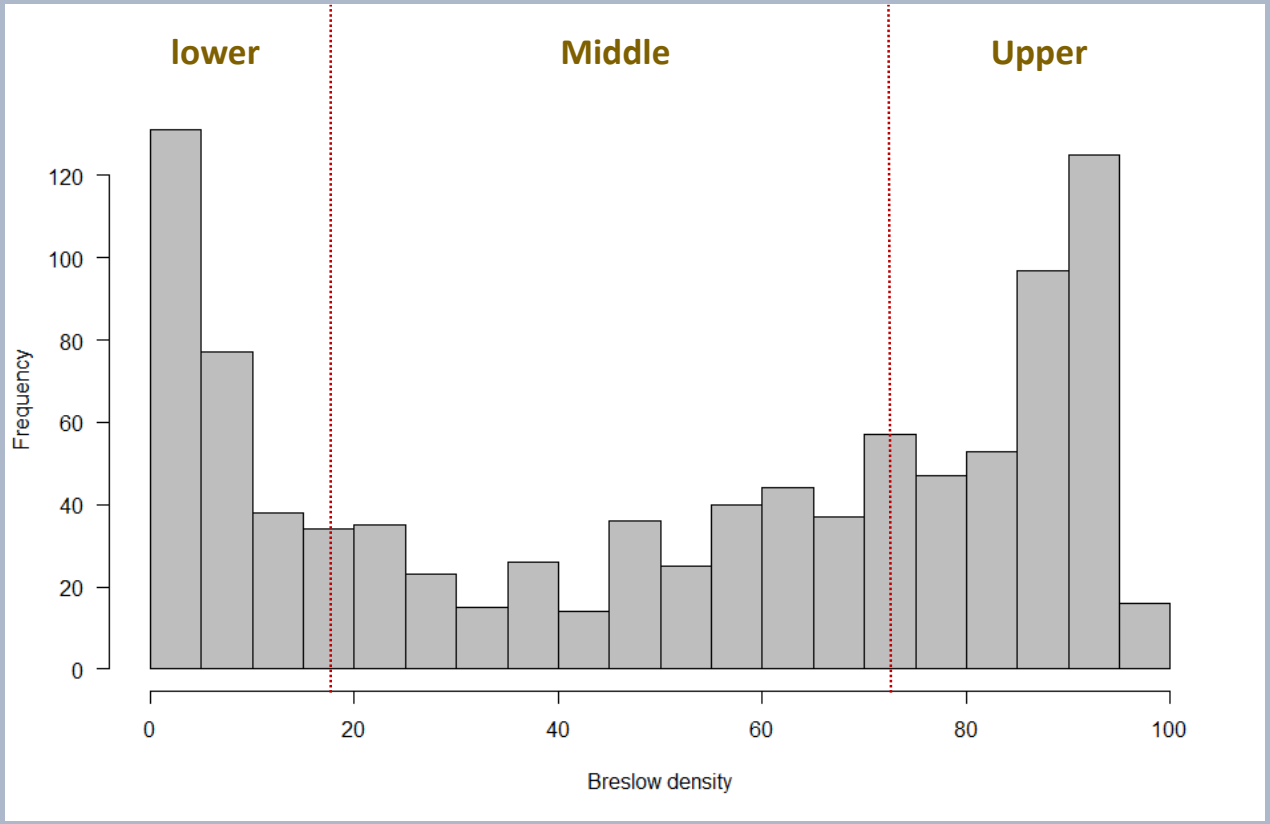
ICC 0.93

BRESLOW DENSITY: IS IT PROGNOSTIC?

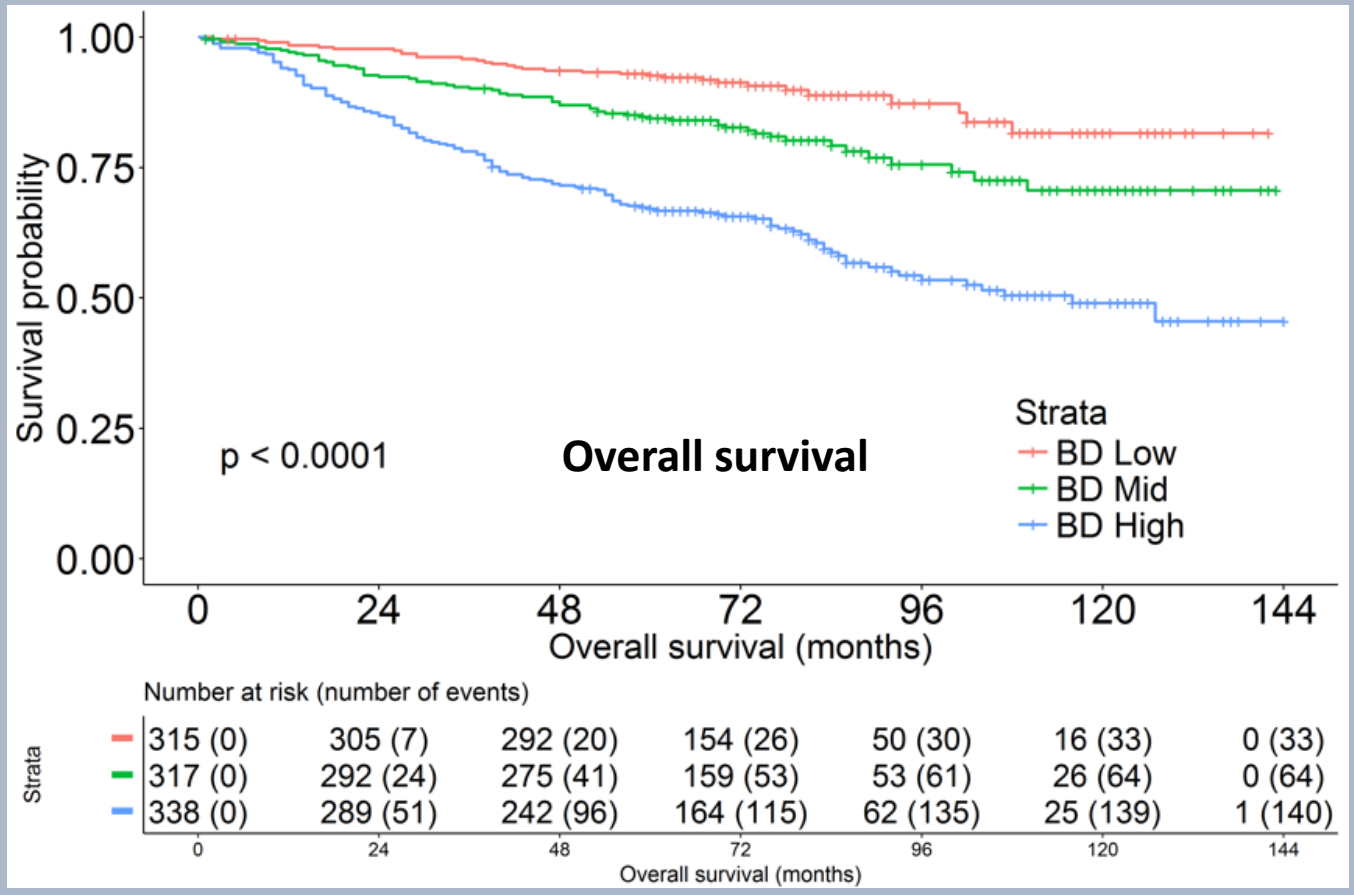
Break Breslow density into 3 risk groups

970 patients from Leicester 2004-2009

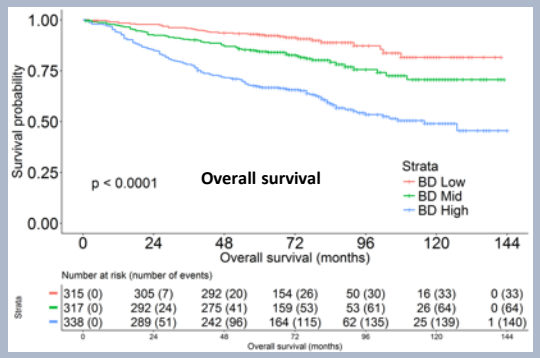
BD Histogram

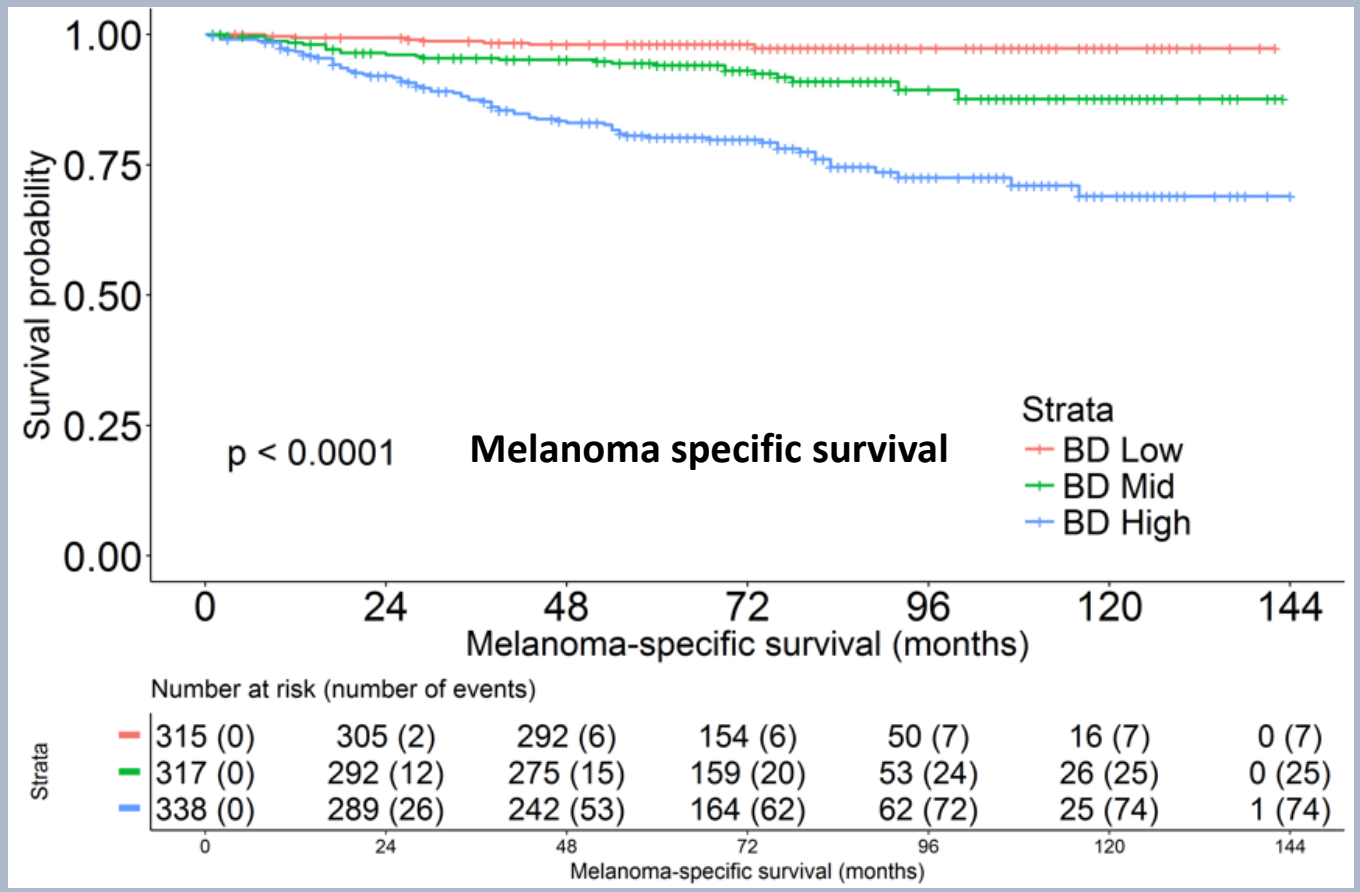


Survival curves for BD risk groups

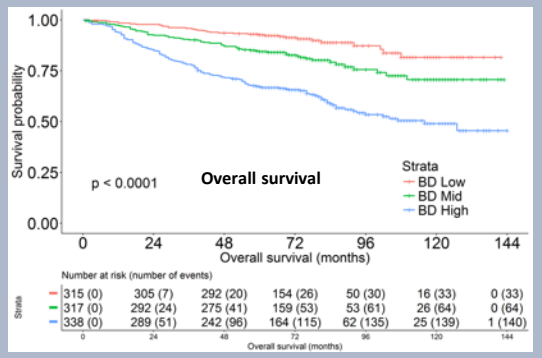


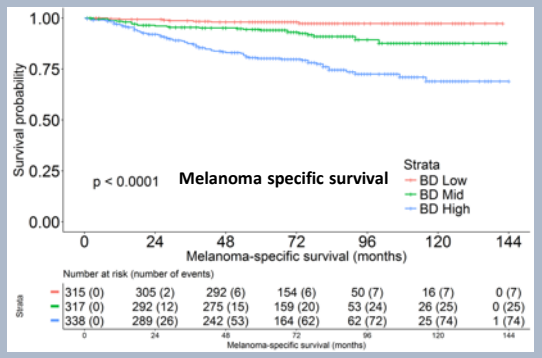
Survival curves for BD risk groups

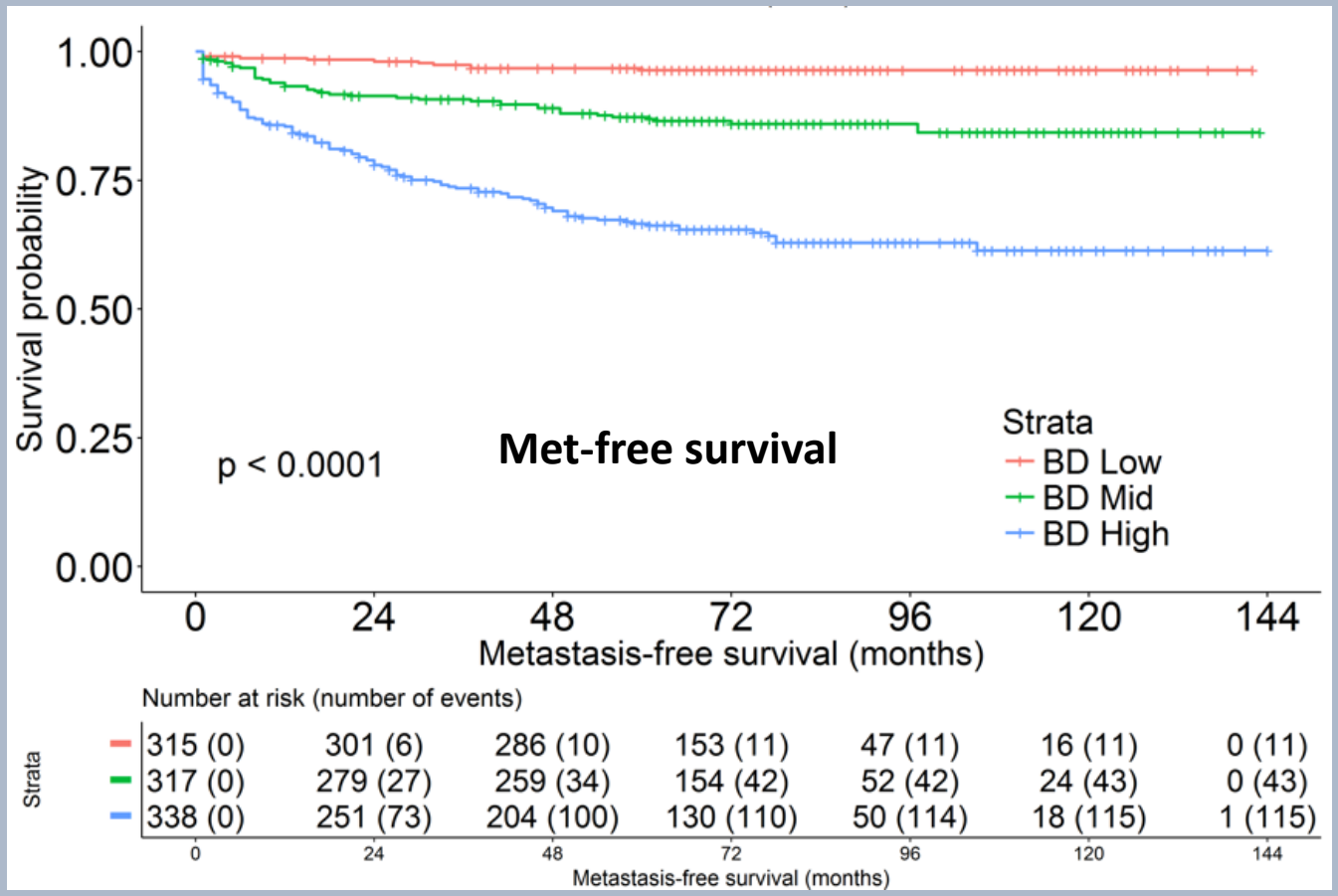




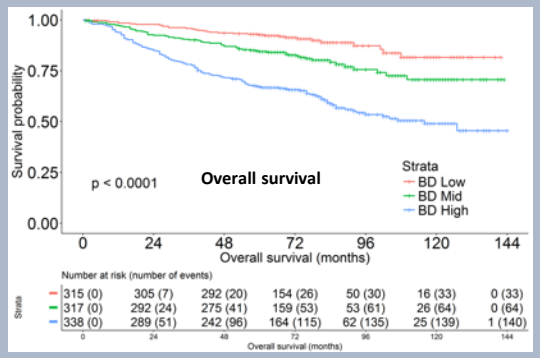
Survival curves for BD risk groups

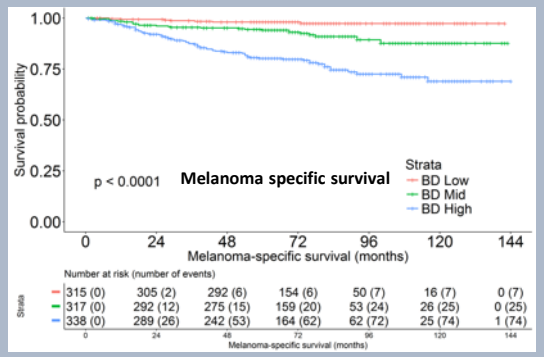


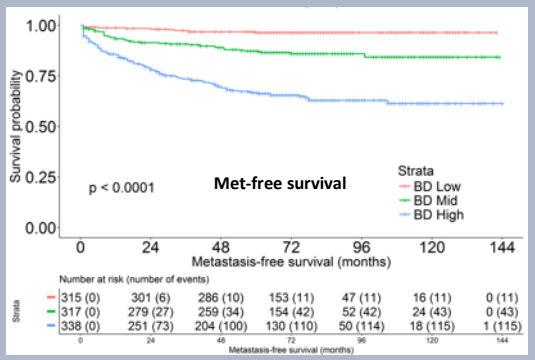




Survival curves for BD risk groups

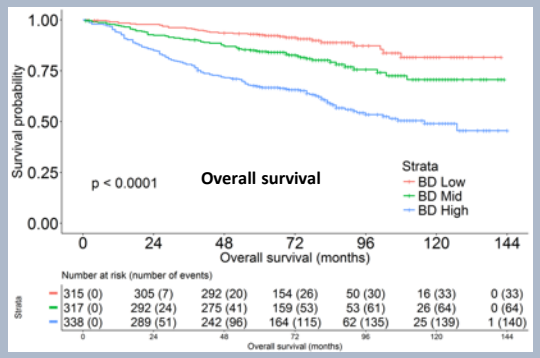


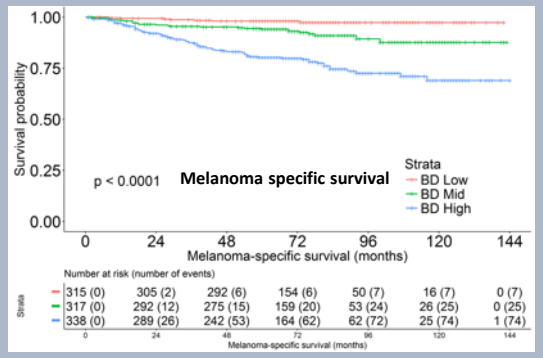


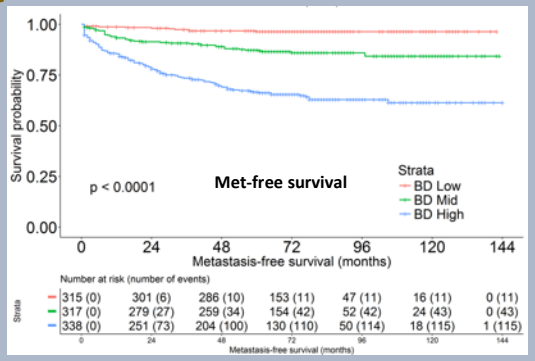


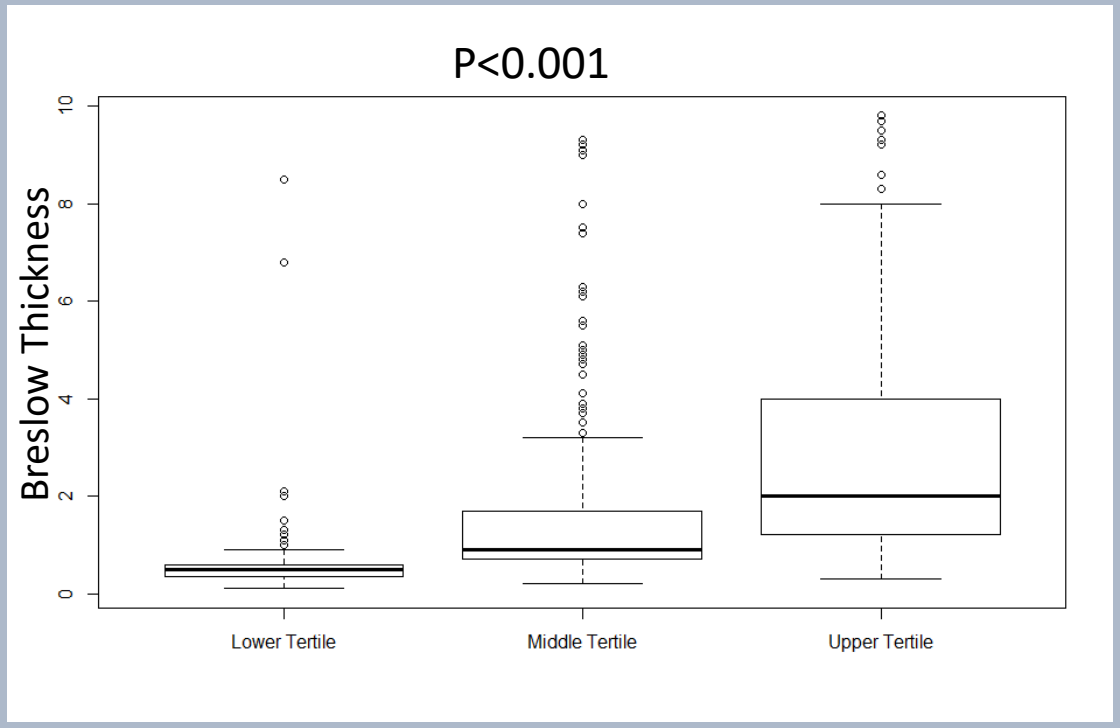
How do we know survival curves aren't confounded?

Survival curves for BD risk groups

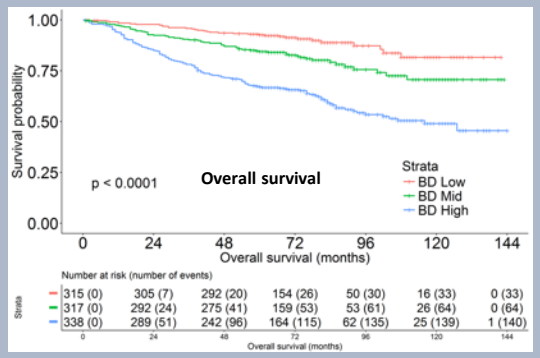


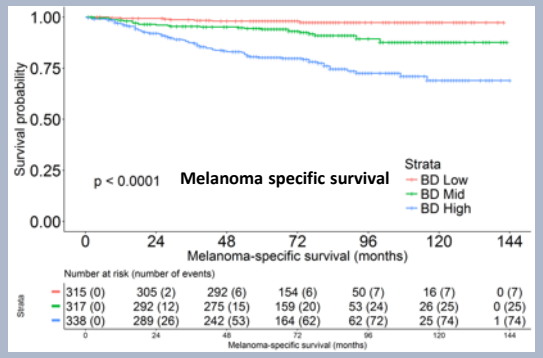


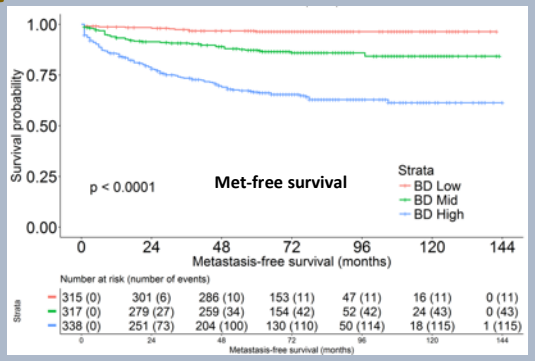


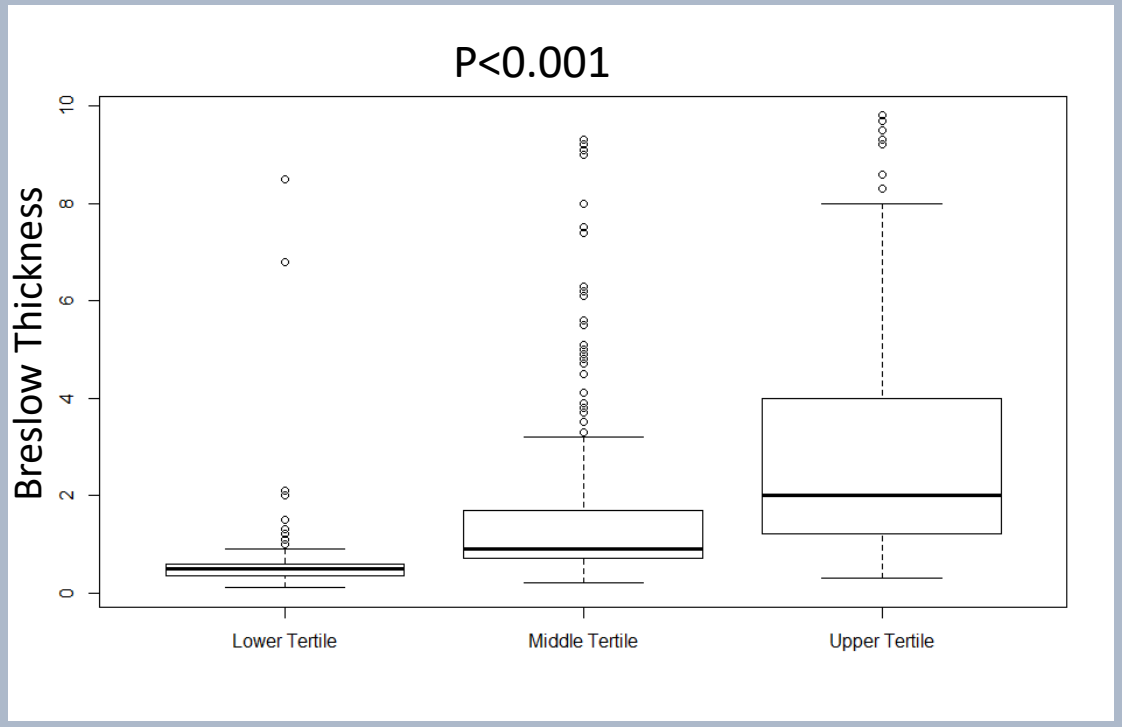


Survival curves for BD risk groups









BD was associated with:

Age at diagnosis

Melanoma type

Ulceration

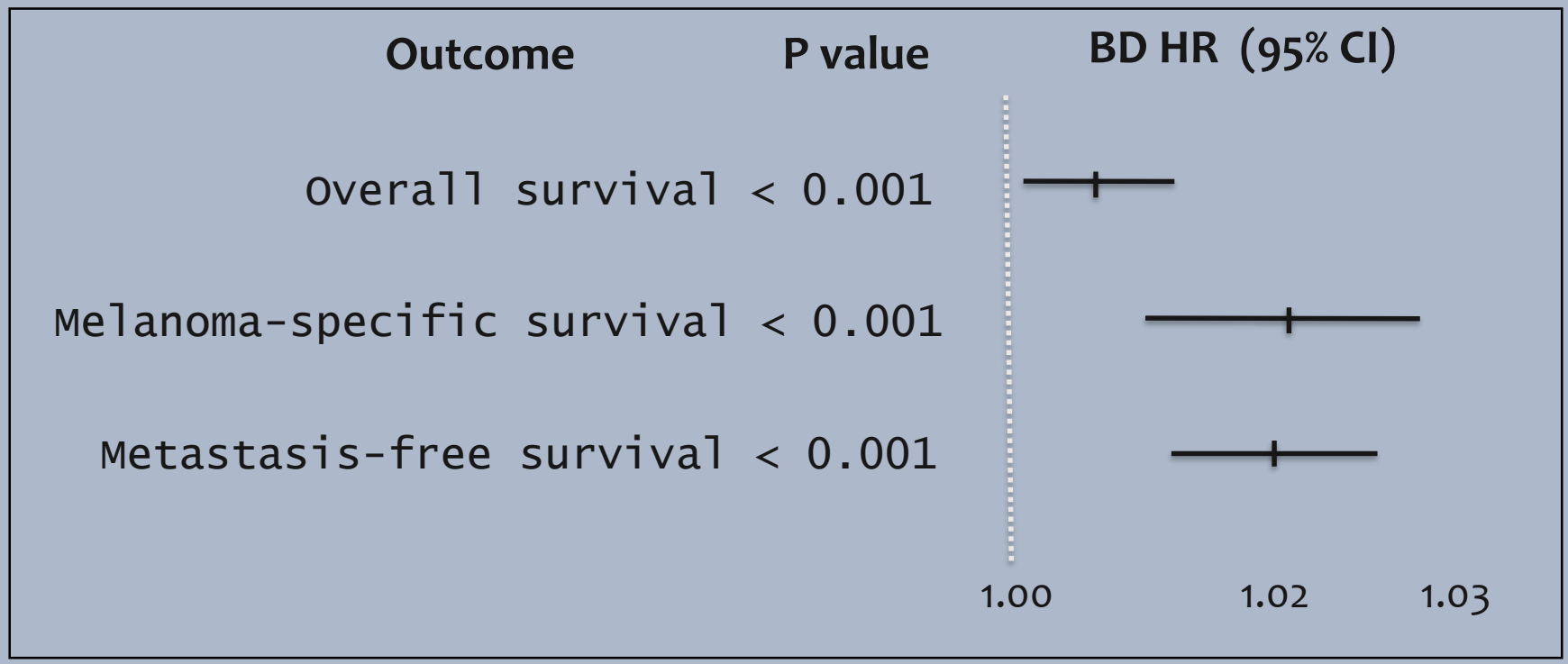
Mitotic count

Microscopic satellites

AJCC version 7 stage at diagnosis

BD adjusted for

Breslow, Ulcer, Mitoses, Age, Sex,
Site, Microsatellites



BD - the story so far

01

BD adds 2nd dimension to BT

02

BD is quick, easy and cheap to measure

03

BD accuracy comparable to image analysis & precision is excellent

04

BD is a powerful prognostic feature

BRESLOW DENSITY: FROM THEORY TO PRACTICE

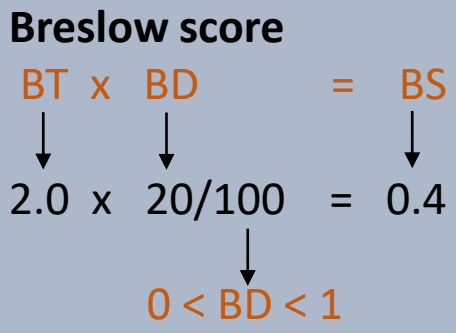
How can we use BD?

1. AJCC staging feature

2. AJCC staging adjunct

Could BD be used as AJCC8 adjunct?

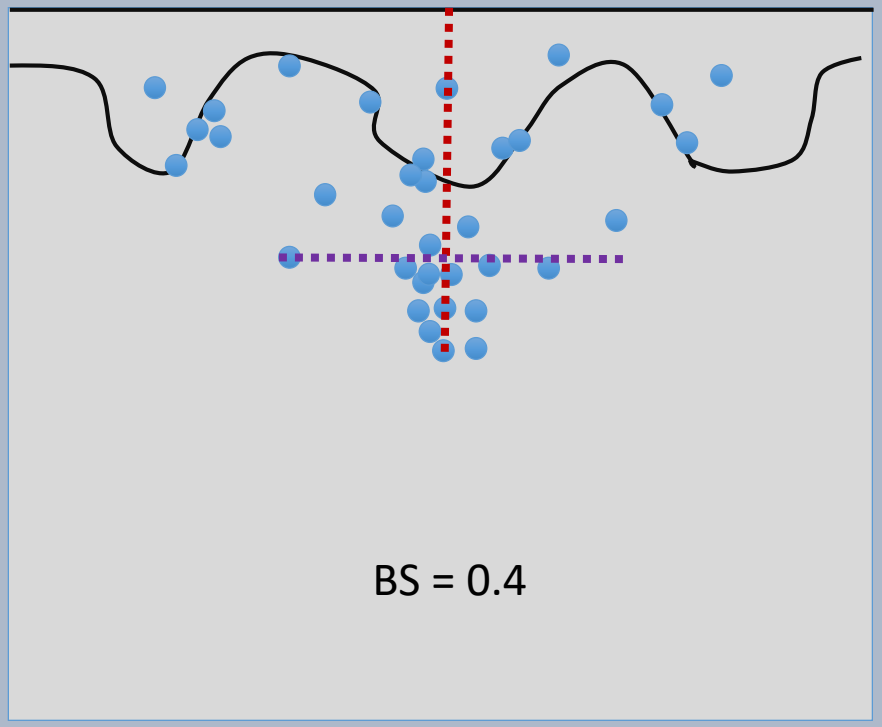
Breslow score: a combination of BT and BD

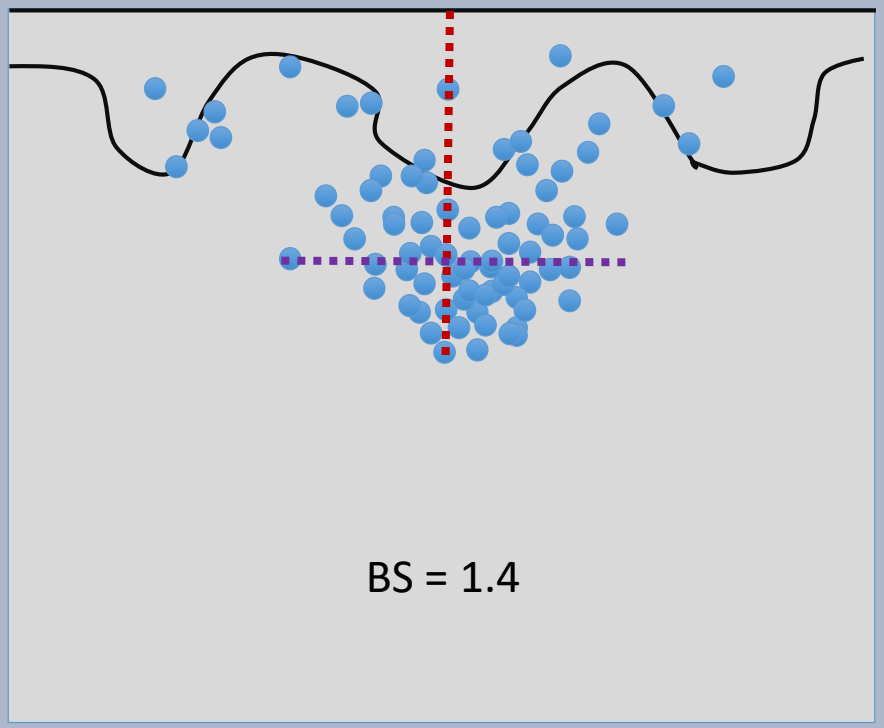


Thickness x Density ≈ number of invasive cells

in the BD window

Breslow score: a combination of BT and BD





Breslow score

BT x BD = BS

2.0 x 20/100 = 0.4

0 < BD < 1

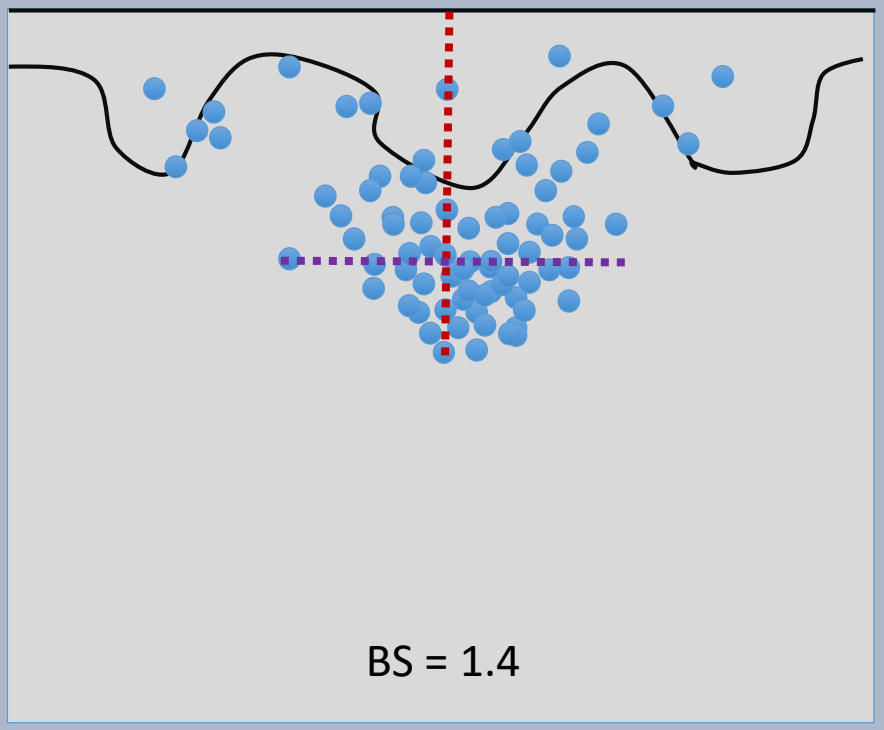
Breslow score

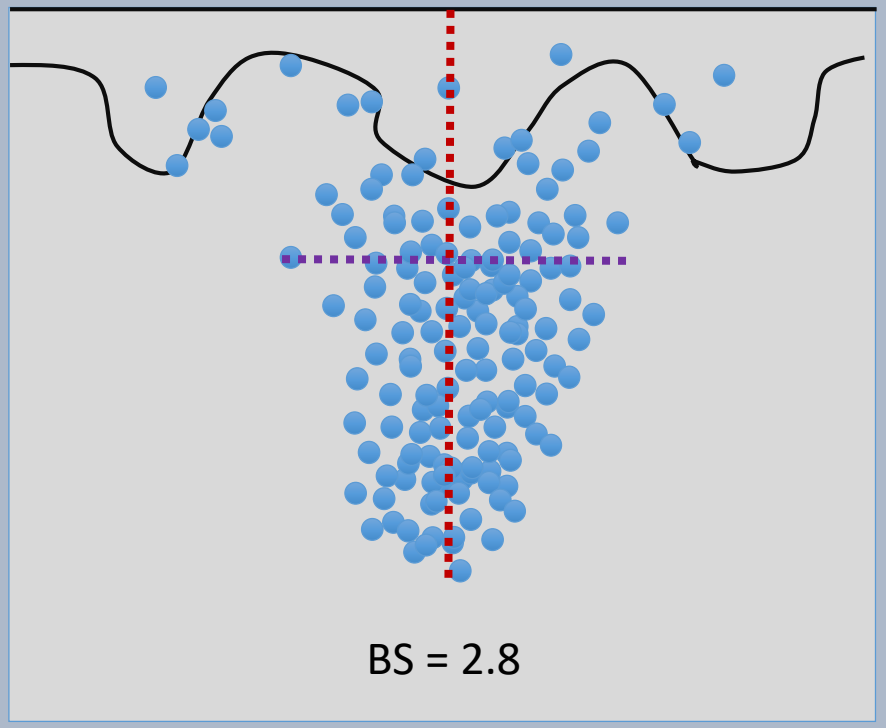
BT x BD = BS

2.0 x 70/100 = 1.4

0 < BD < 1

Breslow score: a combination of BT and BD





Breslow score

BT x BD = BS

2.0 x 70/100 = 1.4

0 < BD < 1

Breslow score

BT x BD = BS

4.0 x 70/100 = 2.8

0 < BD < 1

TRAINING

970 Leicester patients
Finding combination of
BD + AJCC8 // BS + AJCC8

↓

Ln(BS) + AJCC8

VALIDATION

359 Nottingham patients

FINAL MODEL

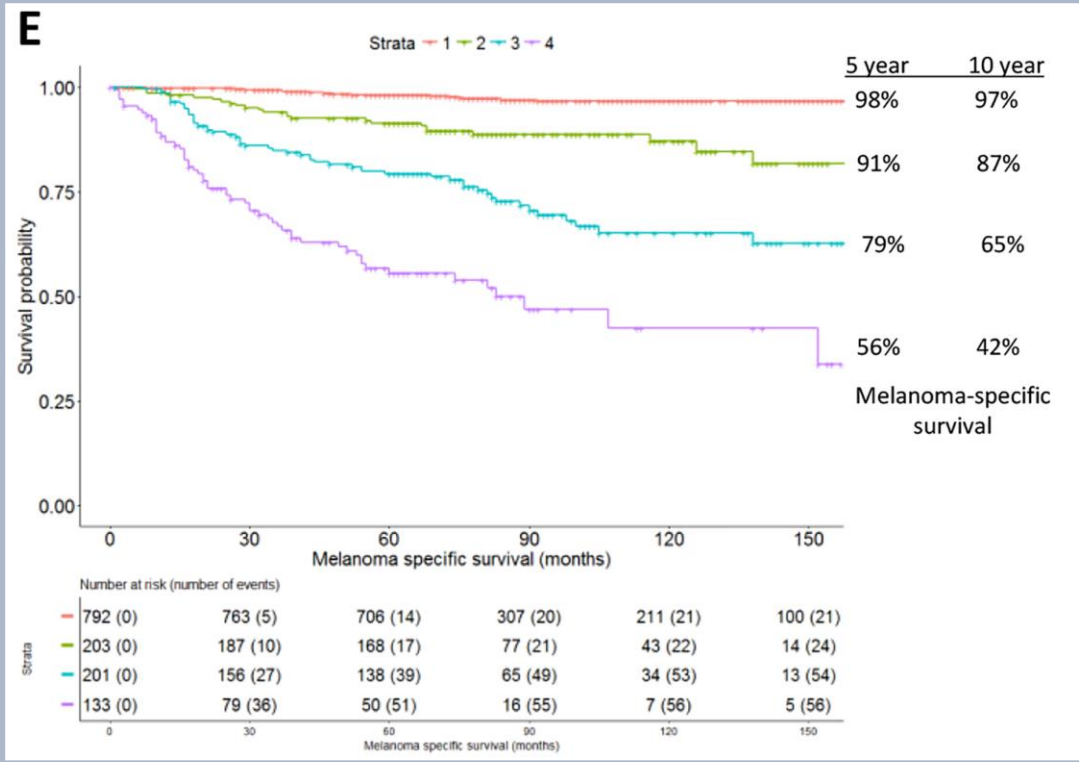
1329 Leics -Notts patients

↓

Prognostic index(PI)

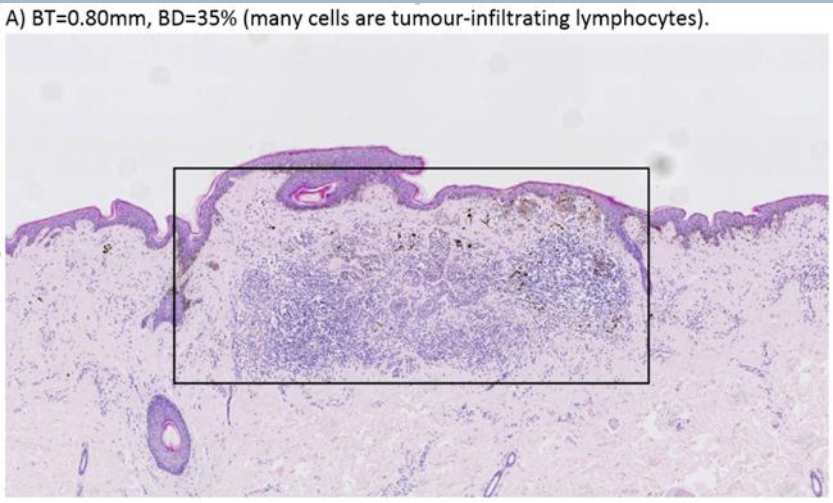
↓

Cut into 4 PI groups



	AJCC8 (%)					
PI Group	**IA**	**IB**	**IIA**	**IIB**	**IIC**	**III**
1	699 (100)	85 (33)	8 (6)	0 (0)	0 (0)	0 (0)
2	0 (0)	169 (67)	34 (23)	0 (0)	0 (0)	0 (0)
3	0 (0)	0 (0)	104 (71)	53 (87)	42 (29)	2 (8)
4	0 (0)	0 (0)	0 (0)	8 (13)	101 (71)	24 (92)

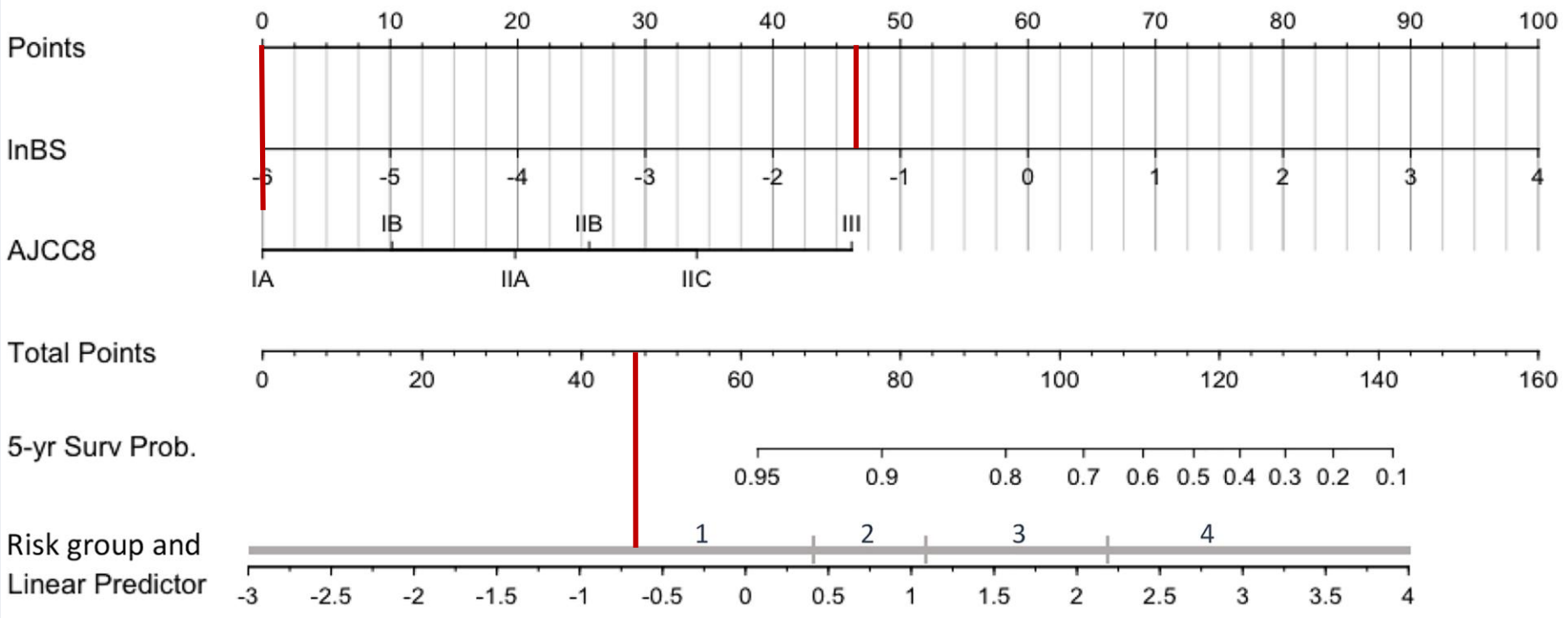
BT = 0.8 mm BD = 35%



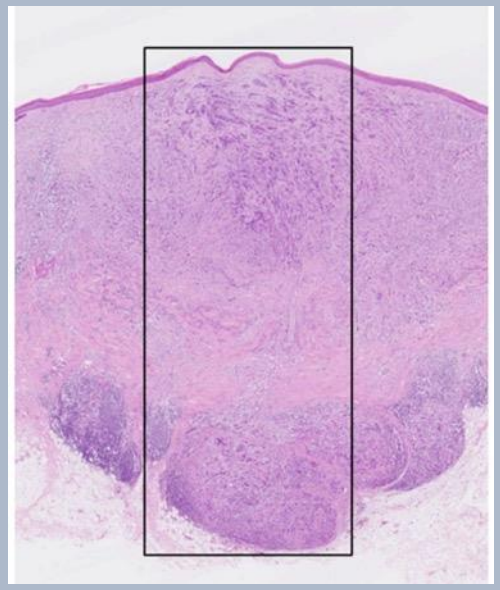
BS = 0.8 x 35/100 = 0.28
Ln(0.28) = -1.3 ≈ 47 points

BT 0.8, no ulcer = pT1b = AJCC8 IA
IA = 0 points

Total points = 47
> 95% 5 year MSS
Prognostic group 1 (98% 5 year MSS)



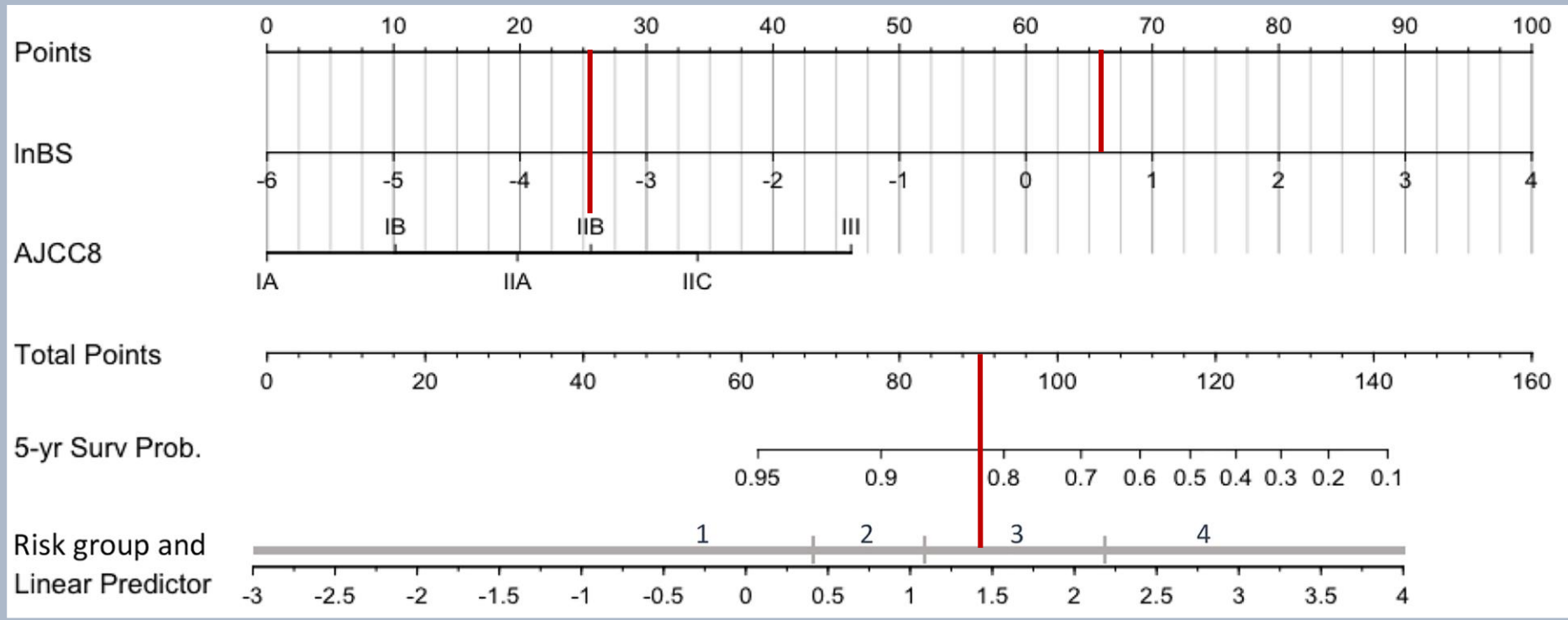
BT = 5.0 mm BD = 35%



BS = 5.0 x 35/100 = 1.75
Ln(1.75) = 0.6 ≈ 66 points

BT 5.0, no ulcer = pT4a = AJCC8 IIB
IIB ≈ 25 points

Total points = 91
5 year MSS between 80 and 90%
Prognostic group 3 (79% 5 year MSS)



BD can be used as an adjunct to AJCC8 staging

Breslow density – where next?

Clinical validity

External validation

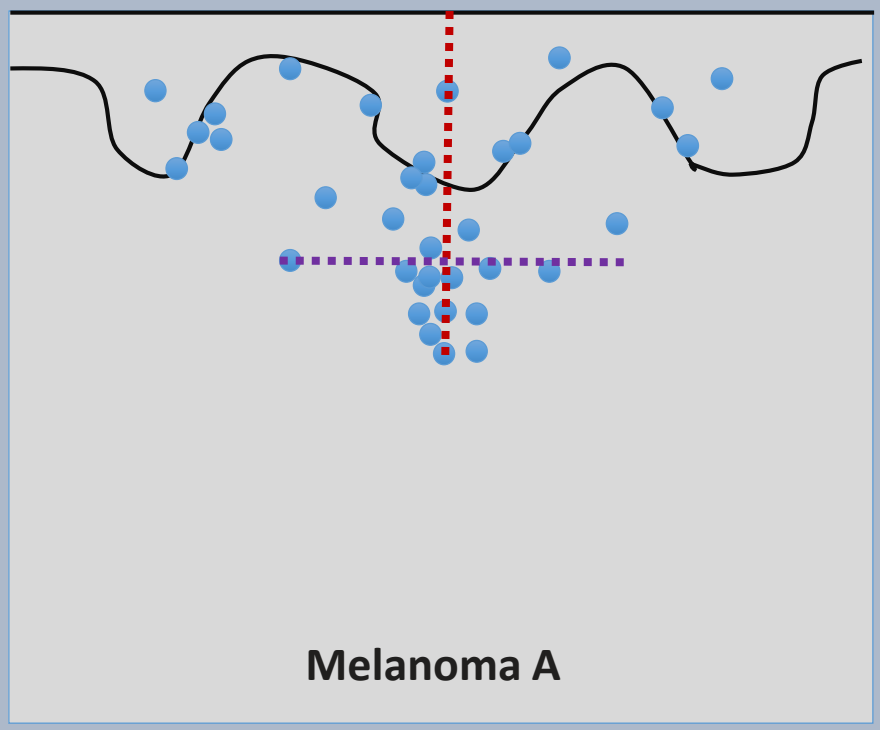
Prospective studies

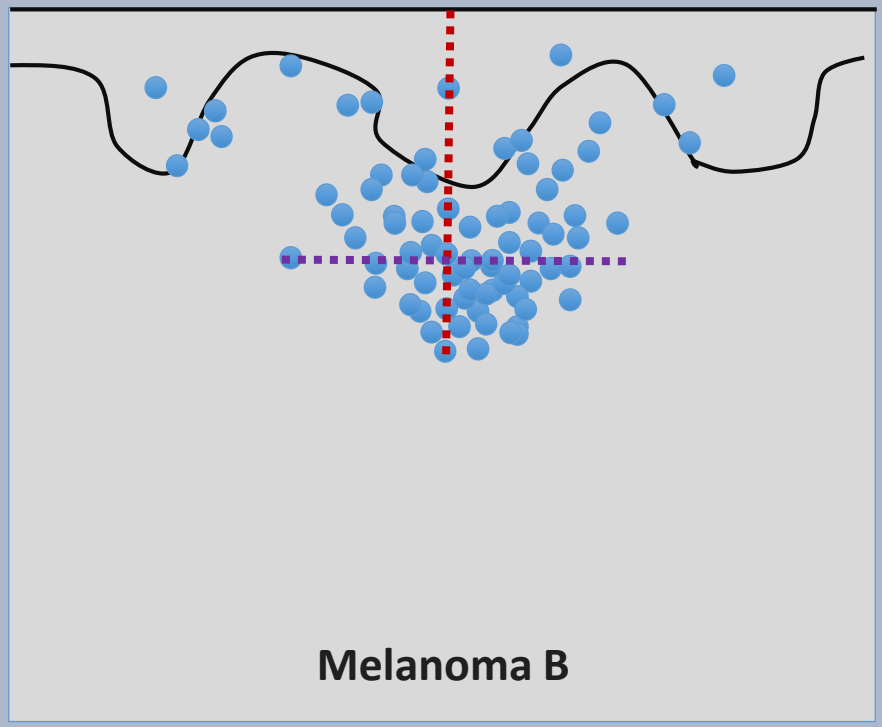
Clinical utility

Impact studies

Breslow density – is there anything better?

Is BD/BS the best way to capture the 2nd dimension?





BRESLOW DENSITY: SUMMARY

Summary

01

BD adds 2nd dimension to BT

02

BD is quick, easy and cheap to measure

05

Can be used with AJCC8

03

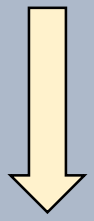
BD accuracy comparable to image analysis & precision is excellent

04

BD is a powerful prognostic feature

WILL YOU BE MEASURING BRESLOW DENSITY

ON YOUR NEXT MELANOMA?



Facilitate further research

Clinical validity

Clinical utility

Co-investigators

Mark Bamford
Jeremy Yarrow
Jay Pancholi
Katerina Flatman
Somaia El-Sheik
Kah Wee Teo
Marie O'Riordan
Rebecca Harrison

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Hope Foundation

THE END
THANK YOU