Supplementary Materials

C-Reactive Protein and Lymphocyte-to-Monocyte Ratio Predict Recurrence in Stage III Melanoma Patients with Microscopic Sentinel Lymph Node Metastasis

Table S1a: Baseline tumor characteristics (primary; n=138).

	All patients
	n=138 (100%)
Site of primary	
Head and neck	4 (2.9)
Trunk	51 (37.0)
Upper extremity	20 (14.5)
Lower extremity	63 (45.7)
Type of primary	
SSM	39 (28.3)
NMM	63 (45.7)
ALM	6 (4.3)
LMM	0 (0.0)
other	24 (17.4)
unknown	6 (4.3)
Tumor thickness (Breslow)	
<1mm	3 (2.2)
≥1<2mm	36 (26.1)
≥2<4mm	53 (38.4)
≥4mm	46 (33.3)
Median in mm (IQR)*	3.1 (1.6-4.5)
Presence of ulceration	
No	74 (53.6)
Yes	64 (46.4)
Mitosis rate > 1/mm^2	
No	5 (3.6)
Yes	95 (68.8)
Unknown	38 (27.5)
*IQR = Interquartile range (Q1 – Q3).	

Table S1b: Baseline tumor characteristics (lymph; node metastasis; n=138).

	All patients
	n=138 (100%)
Average resected number of SLN	
1	69 (50.0)
2	38 (27.5)
3	15 (10.9)
≥4	16 (11.5)
Number of affected SLN	
1	108 (78.3)
2	22 (15.9)
3	8 (5.8)
Largest diameter of SLN metastasis (in mm)	
<1 (0.09-0.99)	10 (7.2)
≥1 <2 (1.00-1.99)	41 (29.7)
≥2 <3 (2.00-2.99)	28 (20.3)
≥3 <4 (3.00-3.99)	19 (13.8)
≥4	14 (10.1)
Unknown	26 (18.8)
Median in mm (IQR)*	1.3 (0.5-3.0)
Capsule invasion of SLN metastasis	
No	122 (88.4)
Yes	15 (10.9)
Unknown	1 (0.7)
Presence of satellite metastasis	
No	130 (94.2)
Yes	8 (5.8)
CLND	
No	66 (47.8)
Yes	72 (52.2)
-no additional metastasis in CLND	58 (61.7)
-additional metastasis in CLND	36 (38.3)
*IQR = Interquartile range (Q1 – Q3).	

Table S2a: Overview of results of all blood variables in univariate and multivariate Cox regression analysis for recurrence-free survival (RFS) with applied exclusion criteria.

Blood values (continuous parameters)	Univariate Cox regression analysis		Multivariate Cox regression analysis*		Exclusion criteria
	n p-value	HR (95% CI)	n p-value	HR (95% CI)	
NLR	125 **0.006**	**1.236** **(1.064-1.437)**	114 **0.019**	**1.340** **(1.050-1.711)**	Not excluded
dNLR	125 0.077	1.254 (0.975-1.612)	114 0.107	1.223 (0.957-1.563)	1)
LMR	125 **<0.001**	**0.689** **(0.564-0.841)**	114 **0.008**	**0.608** **(0.422-0.877)**	Not excluded
PLR	125 **0.008**	**1.005** **(1.001-1.008)**	114 **0.049**	**1.282** **(1.001-1.643)**	4)**
Platelet count	138 0.634	1.001 (0.998-1.003)	126 0.548	1.069 (0.860-1.327)	1)
Absolute leucocyte count	138 0.933	1.004 (0.923-1.092)	126 0.434	1.093 (0.865-1.380)	1)
Absolute lymphocyte count	125 **0.025**	**0.659** **(0.458-0.948)**	114 0.150	0.089 (0.606-1.080)	1) and 4)**
Relative lymphocyte count	125 **0.011**	**0.959** **(0.928-0.990)**	114 **0.038**	**0.758** **(0.583-0.985)**	3) and 4)
Absolute neutrophile count	125 0.492	1.032 (0.943-1.130)	114 0.248	1.141 (0.912-1.426)	1)
Relative neutrophile count	124 0.137	1.023 (0.993-1.054)	113 0.229	1.175 (0.904-1.527)	1)
Absolute eosinophile count	124 0.650	0.602 (0.067-5.394)	113 0.779	0.959 (0.713.1.288)	1)
Relative eosinophile count	124 0.467	0.929 (0.761-1.133)	113 0.376	0.875 (0.650-1.177)	1)
Absolute monocyte count	125 0.074	2.637 (0.911-7.632)	114 0.145	1.200 (0.939-1.534)	1)
Relative monocyte count	125 **<0.001**	**1.178 (1.069-1.299)**	114 **0.019**	**1.332** **(1.049-1.692)**	3) and 4)***
LDH	53 **0.010**	**1.008** **(1.002-1.013)**	50 0.358	1.277 (0.759-2.148)	1) and 2)
CRP	138 **<0.001**	**1.065 (1.026-1.105)**	126 **<0.001**	**1.457** **(1.214-1.747)**	Not excluded

Exclusion criteria:

1) Not significant in univariate or multivariate Cox regression analysis.
2) Patient number n<100.
3) Relative blood values, if there are absolute values provided.

4) High pairwise Pearson correlation (r ≥0.7) between blood values. In this case, the weaker parameter regarding exclusion criteria 1-3 was dropped.

* Note: Single blood value in multivariate Cox regression analysis with following co-variates: Age, AJCC-stage, capsule invasion, adjuvant interferon-α, size of biggest SLN metastasis.

** High correlation with NLR.

*** High correlation with LMR.

Note: Significant results are **bold.**

Table S2b: Overview of results of all blood variables in univariate and multivariate Cox regression analysis for overall survival (OS) with applied exclusion criteria.

Blood values (continuous parameter)	Univariate Cox regression analysis		Multivariate Cox regression analysis*		Exclusion criteria
	n	HR (95% CI)	n	HR (95% CI)	
	p-value		p-value		
NLR	124	1.174 (0967-1.426)	114	**1.396** **(1.004-1.941)**	4)**
	0.105		**0.047**		
dNLR	124	1.287 (0.945-1.753)	114	**1.410** **(1.024-1.942)**	Not excluded
	0.109		**0.035**		
LMR	124	0.818 (0.642-1.042)	114	0.769 (0.504-1.256)	1)
	0.103		0.326		
PLR	125	1.003 (0.998-1.007)	114	1.148 (0.804-1.639)	1)
	0.306		0.446		
Platelet count	136	1.000 (0.997-1.003)	126	1.103 (0.818-1.488)	1)
	0.977		0.518		
Absolute leucocyte count	136	1.044(0.943-1.155)	126	**1.334** **(1.022-1.742)**	Not excluded
	0.407		**0.034**		
Absolute lymphocyte count	124	0.777 (0.482-1.251)	114	0.935 (0.639-1.370)	1)
	0.299		0.732		
Relative lymphocyte count	124	0.966 (0.926-1.008)	114	0.710 (0.492-1.023)	1)
	0.109		0.066		
Absolute neutrophile count	124	1.081 (0.975-1.200)	114	**1.404** **(1.086-1.815)**	Not excluded
	0.140		**0.010**		
Relative neutrophile count	123	1.027 (0.987-1.068)	113	1.399 (0.951-2.057)	1)
	0.187		0.088		
Absolute eosinophile count	123	0.060 (0.002-1.757)	113	9.864 (0.564-1.323)	1)
	0.103		0.500		
Relative eosinophile count	123	0.745 (0.548-1.012)	113	0.764 (0.501-1.165)	1)
	0.059		0.211		
Absolute monocyte count	124	1.538 (0.358-6.620)	114	1.125 (0.808-1.569)	1)
	0.563		0.485		

Relative monocyte count	124	1.050 0.486	(0.915-1.206)	114	0.939 0.704	(0.679-1.299)	1)
LDH	51	**1.027** **<0.001**	**(1.014-1.041)**	48	**4.877** **0.007**	**(1.550-15.342)**	2)
CRP	136	1.019 0.548	(0.958-1.084)	126	1.216 0.157	(0.928-1.594)	1)

Exclusion criteria:

5) Not significant in univariate or multivariate Cox regression analysis.
6) Patient number n<100.
7) Relative blood values, if there are absolute values provided.
8) High pairwise Pearson correlation (r ≥0.7) between blood values. In this case, the weaker parameter regarding exclusion criteria 1-3 was dropped.

* Note: Single blood value in multivariate Cox regression analysis with following co-variates: Age, AJCC-stage, capsule invasion, adjuvant interferon-α, size of biggest SLN metastasis.

** High correlation with dNLR.

Note: Significant results are **bold.**

Table S3. Univariate Cox regression analysis with covariates for recurrence-free survival and overall survival.

	Univariate Cox analysis	
Variable (Reference bold)	**Recurrence-free survival** HR (95% CI) p-value	**Overall survival** HR (95% CI) p-value
Age (n=55; n=83) >65 years vs **≤65 years**	**1.985** **(1.261-3.125)** **0.003**	**3.060** **(1.657-5.650)** **<0.001**
AJCC-stage (n=36, n=32, n=70, n=0) IIIB vs **IIIA** IIIC vs **IIIA** IIID vs **IIIA**	2.247 (0.992-5.087) 0.052 **4.303 (2.114-8.759) <0.001** /	1.656 (0.589-4.658) 0.339 **2.685 (1.111-6.490)** **0.028** /
Capsule invasion (n=15; n=122) yes vs **no**	1.677 (0.858-3.277) 0.130	**4.394** **(2.120-9.109)** **<0.001**
Adjuvant interferon-α		

(n=45; n=92)		
yes vs **no**	0.692 (0.422-1.133) 0.143	**0.329** **(0.156-0.692)** **0.003**
Size of biggest SLN metastasis (n=66; n=62)		
> 1mm vs ≤ **1mm**	**1.849** **(1.139-3.004)** **0.013**	**3.444** **(1.674-7.086)** **<0.001**
Gender (n=67; n=71)		
female vs **male**	0.721 (0.458-1.136) 0.158	0.672 (0.364-1.239) 0.203
Ulceration (n=64, n=74)		
yes vs **no**	**2.390 (1.505-3.794)** **<0.001**	**2.044** **(1.108-3.770)** **0.022**
Mitotic rate (n=95; n=5)		
>1/mm² vs ≤ **1/mm²**	0.260 (0.036-1.881) 0.182	0,045 (0.000-64.245) 0.403
Number of affected SLN (n=108; n=22; n=8)		
2 vs **1**	0.784 (0.401-1.534) 0.477	0.493 (0.175-1.385) 0.179
3 vs **1**	1.921 (0.873-4.227) 0.104	0.621 (0.149-2.579) 0.512
Satellite metastases (n=8; n=130)		
yes vs **no**	0.683 (0.249-1.873) 0.459	0.968 (0.298-3.138) 0.956
CLND (n=72; n=66)		
yes vs **no**	**1.710** **(1.072-2.729)** **0.024**	1.356 (0.728-2.525) 0.337
Note: Significant results are **bold.**		

Table S4. Univariate and multivariate Cox regression analysis with multiple blood value cut-offs of NLR, LMR and CRP for recurrence-free survival.

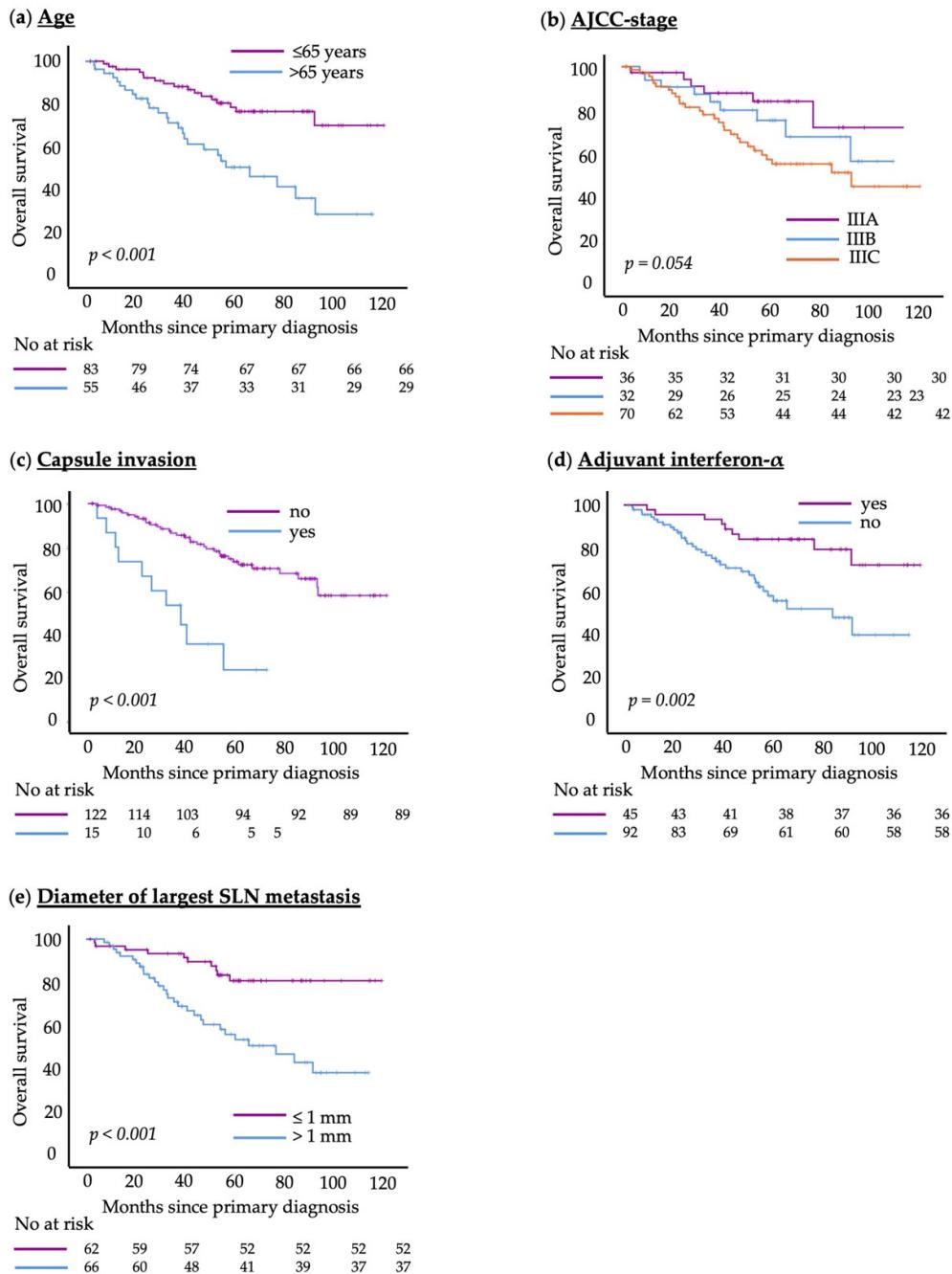| Blood value combinations					
Univariate Cox analysis			**Multivariate Cox analysis ***		
Event number p-value		HR (95% CI)	Event number p-value		HR (95% CI)
NLR (≥3.5) + CRP (>3.0)			NLR (≥3.5) + CRP (>3.0)		
1 (n=45) vs **0** (n=71) events	**2.673 (1.616-4.421)**	**<0.001**	1 (n=39) vs **0** (n=67) events	**2.370 (1.352-4.156)**	**0.003**
2 (n=9) vs **0** (n=71) events	**4.131 (1.872-9.117)**	**<0.001**	2 (n=8) vs **0** (n=67) events	**4.838 (2.009-11.652)**	**<0.001**
1 (n=45) vs **2** (n=9) events	0.647 (0.298-1.402)	0.270	1 (n=39) vs **2** (n=8) events	0.490 (0.200-1.203)	0.119
LMR (<3.5) + CRP (>3.0)			LMR (<3.5) + CRP (>3.0)		
1 (n=57) vs **0** (n=51) events	**3.365 (1.870-6.054)**	**<0.001**	1 (n=53) vs **0** (n=48) events	**3.239 (1.710-6.135)**	**<0.001**
2 (n=17) vs **0** (n=51) events	**6.386 (3.119-13.076)**	**<0.001**	2 (n=13) vs **0** (n=48) events	**7.700 (3.436-17.255)**	**<0.001**
1 (n=57) vs **2** (n=17) events	0.527 (0.289-9.60) 0.036		1 (n=53) vs **2** (n=13) events	**0.421 (0.212-0.835)**	**0.013**
NLR (≥3.5) + LMR (<3.5)			NLR (≥3.5) + LMR (<3.5)		
1 (n=36) vs **0** (n=63) events	**2.133 (1.217-3.738)**	**0.008**	1 (n=32) vs **0** (n=59) events	**2.216 (1.211-4.055)**	**0.010**
2 (n=26) vs **0** (n=63) events	**2.614 (1.463-4.671)**	**0.001**	2 (n=23) vs **0** (n=59) events	**2.160 (1.113-4.189)**	**0.023**
1 (n=36) vs **2** (n=26) events	0.816 (0.448-1.488)	0.507	1 (n=32) vs **2** (n=23) events	1.026 (0.521-2.020)	0.941
NLR (≥3.5) + LMR (<3.5) + CRP (>3.0)			NLR (≥3.5) + LMR (<3.5) + CRP (>3.0)		
1 (n=41) vs **0** (n=48) events	**3.054 (1.612-5.788)**	**<0.001**	1 (n=39) vs **0** (n=45) events	**3.398 (1.709-6.755)**	**<0.001**
2 (n=28) vs **0** (n=48) events	**4.167 (2.148-8.083)**	**<0.001**	2 (n=23) vs **0** (n=45) events	**3.515 (1.647-7.500)**	**0.001**
3 (n=8) vs **0** (n=48) events	**6.059 (2.456-14.945)**	**<0.001**	3 (n=7) vs **0** (n=45) events	**7.690 (2.789-21.202)**	**<0.001**
1 (n=41) vs **3** (n=8) events	0.504 (0.218-1.165)	0.109	1 (n=39) vs **3** (n=7) events	0.442 (0.173-1.128)	0.087
2 (n=28) vs **3** (n=8) events	0.688 (0.293-1.617)	0.391	2 (n=23) vs **3** (n=7) events	0.457 (0.167-1.251)	0.128
*Single blood value in multivariate Cox regression analysis with following covariates: Age, AJCC-stage, capsule invasion, adjuvant Interferon-α, size of biggest SLN metastasis. Note: Significant results are **bold.**					

Supplementary figure

Figure S1. Kaplan-Meier survival curves showing overall survival for covariates of the multivariate Cox regression model: (**a**) patient age; (**b**) AJCC-stage; (**c**) capsule invasion of sentinel lymph node (SLN) metastasis; (**d**) adjuvant interferon-α therapy; (**e**) diameter of largest SLN metastasis. The log-rank test was used to compare between groups; p <0.05 was considered significant.