SUPPLEMENTARY APPENDIX

Supplement to: 'Interferon-gamma signature as prognostic and predictive marker in macroscopic stage III melanoma'

Content   Page

Table S1. Characteristics of patients who received adjuvant therapy in discovery cohort 2

	N	%
Type of adjuvant therapy		
Anti-PD-1[a]	39	100
Time to start adjuvant therapy (in weeks)		
Median	9.0	
IQR	7.9-11.7	
Range	4.0-23.1[b]	
Treatment ongoing		
No	39	100
Time on adjuvant treatment		
Median	10	
IQR	3-10	
Range	0-12	
Reason stop end of adjuvant therapy		
End of treatment	22	56
Toxicity[c]	6	15
Recurrence	10	26
Other[d]	1	3
COVID break		
No	24	62
Yes[e]	15	39

Characteristics of adjuvant therapy, reported for the 39 patients who received at least 1 dose of adjuvant therapy.

Reported as number (%), percentages may not total 100 due to rounding.

[a] One patient participated in a trial and was randomized between either anti-PD-1 monotherapy, or combination anti-PD-1 and anti-CTLA-4. [b] One patient started with targeted therapy, due to COVID, but switched to anti-PD-1 due to toxicity. [c] One patient was first treated with targeted therapy due to logistic challenges following the COVID-19 pandemic, then proceeded with adjuvant anti-PD-1. [d] One patient skipped two cycles due to COVID break, did not receive last cycle due to abscess in appendectomy scar. [e] COVID break: eight patients completed 10 cycles instead of 12, 1 patient received 11 cycles and 1 patient had a delayed start of treatment.

Table S2. Cox regression analyses

	Crude HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value
Age	1.01 (0.99-1.03)	0.513		
Sex	1.46 (0.88-2.44)	0.143		
Mutation status				
BRAF	1.62 (0.39-6.75)	0.505		
NRAS	1.52 (0.35-6.65)	0.578		
cKIT	2.82 (0.25-31.37)	0.398		
No driver mutations	2.16 (0.46-10.19)	0.330		
Unknown	ref			
Type of surgery				
LND	**0.31 (0.21-0.81)**	**0.010**	0.69 (0.34-1.43)	0.325
ITM	0.61 (0.28-1.33)	0.212	0.98 (0.44-2.23)	0.975
LND + ITM	0.59 (0.23-1.50)	0.266	0.81 (0.31-2.09)	0.661
ILP ± LND	ref		ref	
S100b	**2.39 (1.23-4.63)**	**0.010**	**2.64 (1.29-5.38)**	**0.008**
LDH	1.85 (0.66-5.19)	0.243		
Adjuvant therapy	**0.25 (0.14-0.45)**	**<0.001**	**0.25 (0.13-0.48)**	**<0.001**

HR: hazard ratio; CI: confidence interval; LND: lymph node dissection; ITM: in-transit metastasis; ILP: isolated limb perfusion.

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Table S3. Baseline characteristics of patients of whom RNA sequencing data is available

		Observation cohort 1A		Adjuvant treatment cohort 2A	
		Recurrence N=18	No recurrence N=6	Recurrence N=9	No recurrence N=15
Age					
	Median	71	73	55	61
	Range	55-77	57-82	45-63	54-73
Sex					
	Male	11 (61)	3 (50)	4 (44)	7 (47)
	Female	7 (39)	3 (50)	5 (56)	8 (53)
Site primary					
	Extremities	13 (72)	3 (50)	4 (44)	8 (53)
	Trunk	4 (22)	3 (50)	5 (56)	5 (33)
	Head&neck	0	0	0	1 (7)
	Acral	0	0	0	1 (7)
	Mucosal	1 (6)	0	0	0
	MUP	0	0	0	0
Breslow thickness					
	≤1.0mm	1 (6)	0	0	3 (20)
	1.01-2.0	4 (22)	1 (17)	2 (22)	2 (13)
	2.01-4.0	6 (33)	4 (80)	5 (56)	4 (27)
	>4.0	5 (28)	0	1 (11)	6 (40)
	Unknown	2 (11)	1 (17)	1 (11))	0
Ulceration					
	No	6 (33)	3 (50)	6 (67)	11 (73)
	Yes	7 (39)	2 (33)	1 (11)	3 (20)
	Unknown	5 (2)	1 (17)	2 (22)	1 (7)
Stage (AJCC 8th edition)					
	IIIB	2 (11)	1 (17)	2 (22)	4 (27)
	IIIC	16 (89)	4 (67)	6 (67)	10 (67)
	IIID	0	0	0	1 (7)
	Unknown	0	1 (17)	1 (11)	0
Mutation status					
	BRAF	9 (50)	4 (67)	6 (67)	9 (60)
	NRAS	6 (33)	1 (17)	1 (11)	6 (40)
	cKIT	1 (6)	0	0	0
	No driver mutations	1 (6)	0	2 (22)	0
	Unknown	1 (6)	1 (17)	0	0
Type surgery					
	LND	8 (44)	2 (33)	7 (78)	11 (73)
	ITM	2 (11)	2 (33)	0	3 (20)
	LND + ITM	2 (11)	1 (17)	2 (22)	1 (7)
	ILP ± LND	6 (33)	1 (17)	0	0
Type sample					
	LND	12 (67)	3 (50)	9 (100)	12 (80)
	ITM	6 (33)	3 (50)	0	3 (20)
S100b					
	≤ULN	14 (78)	6 (100)	7 (78)	14 (93)
	>ULN	2 (11)	0	1 (11)	1 (7)
	Unknown	2 (11)	0	1 (11)	0
LDH					
	≤ULN	16 (89)	4 (67)	8 (89)	15 (100)
	>ULN	1 (6)	2 (33)	0	0
	Unknown	1 (6)	0	1 (14)	0

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Baseline characteristics of patients of whom RNA sequencing data is available, reported per cohort for patient with and without recurrence.

Reported as number (%), percentages may not total 100 due to rounding.

IQR: interquartile range; MUP: melanoma of unknown primary; LND: lymph node dissection; ITM: in-transit metastasis; ILP: isolated limb perfusion; ULN: upper limit of normal; LDH: lactate dehydrogenase.

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Table S4. Correlation IFNγ with clinical parameters in all patients of whom RNA sequencing data is available

	Pearson coefficient	P value	Spearman coefficient	P value
T stage	0.140	0.295	0.154	0.248
Stage (AJCC 8th edition)	0.021	0.871	0.095	0.455
Breslow thickness	-0.182	0.151	-0.063	0.621
Ulceration	-0.042	0.744	-0.020	0.877
S100B surgery	0.169	0.181	-0.031	0.811
S100B adjuvant	0.223	0.307	0.234	0.282
LDH surgery	-0.020	0.875	-0.073	0.568
LDH adjuvant	0.141	0.522	0.176	0.423
Age	-0.021	0.868	-0.009	0.946
Mutation status	-0.035	0.783	0.003	0.979
Type of surgery	-0.111	0.382	-0.125	0.326

Correlation of interferon-gamma (IFNγ) with known prognostic clinical parameters.

LDH: lactate dehydrogenase.

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Table S5. Baseline characteristics of patients of whom PD-L1 expression data is available
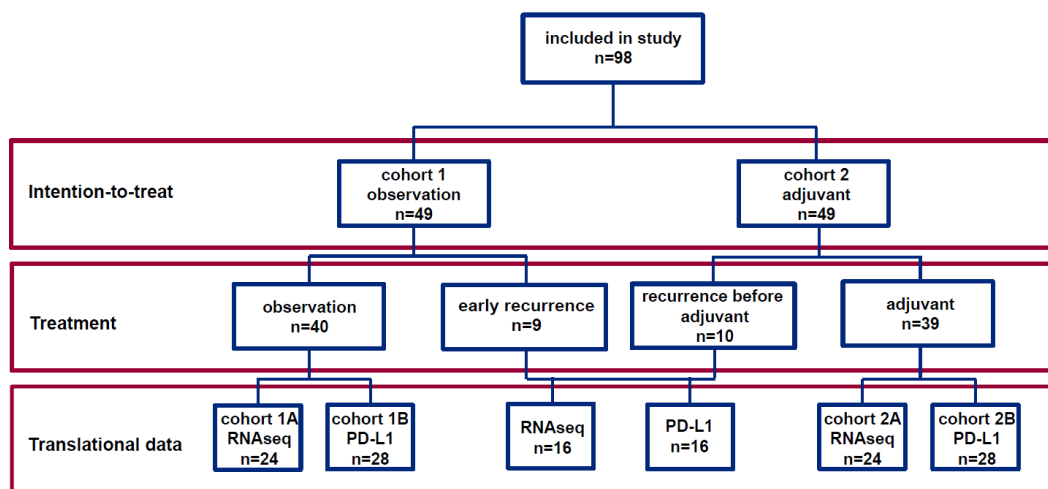
		Observation cohort 1B		Adjuvant treatment cohort 2B	
		Recurrence	No recurrence	Recurrence	No recurrence
		N=21	N=7	N=11	N=17
Age					
	Median	67	75	59	61
	Range	51-74	73-81	55-70	54-71
Sex					
	Male	9 (43)	4 (57)	5 (46)	7 (41)
	Female	12 (57)	3 (43)	6 (55)	10 (59)
Site primary					
	Extremities	14 (67)	3 (43)	4 (36)	7 (41)
	Trunk	5 (24)	4 (57)	7 (64)	9 (53)
	Head&neck	0	0	0	0
	Acral	0	0	0	1 (6)
	Mucosal	1 (5)	0	0	0
	MUP	1 (5)	0	0	0
Breslow thickness					
	≤1.0mm	1 (5)	0	0	3 (18)
	1.01-2.0	5 (24)	2 (29)	1 (9)	4 (24)
	2.01-4.0	10 (48)	3 (43)	5 (6)	4 (24)
	>4.0	3 (14)	1 (14)	3 (27)	6 (35)
	Unknown	2 (10)	1 (14)	2 (18)	0
Ulceration					
	No	7 (33)	2 (29)	5 (45)	10 (59)
	Yes	7 (33)	3 (43)	3 (27)	6 (35)
	Unknown	7 (33)	2 (29)	3 (27)	1 (6)
Stage (AJCC 8th edition)					
	IIIB	1 (5)	0	2 (18)	4 (24)
	IIIC	20 (95)	6 (86)	8 (73)	12 (71)
	IIID	0	0	0	1 (6)
	Unknown	0	1 (14)	1 (9)	0
Mutation status					
	BRAF	12 (57)	3 (43)	8 (73)	11 (65)
	NRAS	5 (24)	0	2 (18)	6 (35)
	cKIT	1 (5)	0	0	0
	No driver mutations	2 (10)	2 (29)	1 (9)	0
	Unknown	1 (5)	2 (29)	0	0
Type surgery					
	LND	8 (38)	2 (29)	8 (73)	12 (71)
	ITM	6 (29)	2 (29)	1 (9)	3 (18)
	LND + ITM	1 (5)	2 (29)	2 (18)	1 (6)
	ILP ± LND	6 (29)	1 (4)	0	1 (6)
Type sample					
	LND	12 (57)	4 (57)	10 (1)	14 (82)
	ITM	9 (43)	3 (43)	1 (9)	3 (18)
S100b					
	≤ULN	15 (71)	6 (86)	9 (82)	16 (94)
	>ULN	4 (19)	0	2 (18)	1 (6)
	Unknown	2 (10)	1 (14)	0	0
LDH					
	≤ULN	15 (71)	4 (57)	10 (91)	17 (100)
	>ULN	3 (14)	2 (29)	0	0
	Unknown	3 (14)	1 (14)	1 (9)	0

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Baseline characteristics of patients of whom PD-L1 expression data is available, reported per cohort for patients with and without recurrence.
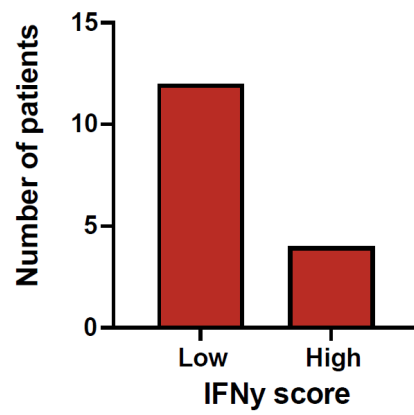
Reported as number (%), percentages may not total 100 due to rounding.

IQR: interquartile range; MUP: melanoma of unknown primary; LND: lymph node dissection; ITM: in-transit metastasis; ILP: isolated limb perfusion; ULN: upper limit of normal; LDH: lactate dehydrogenase.

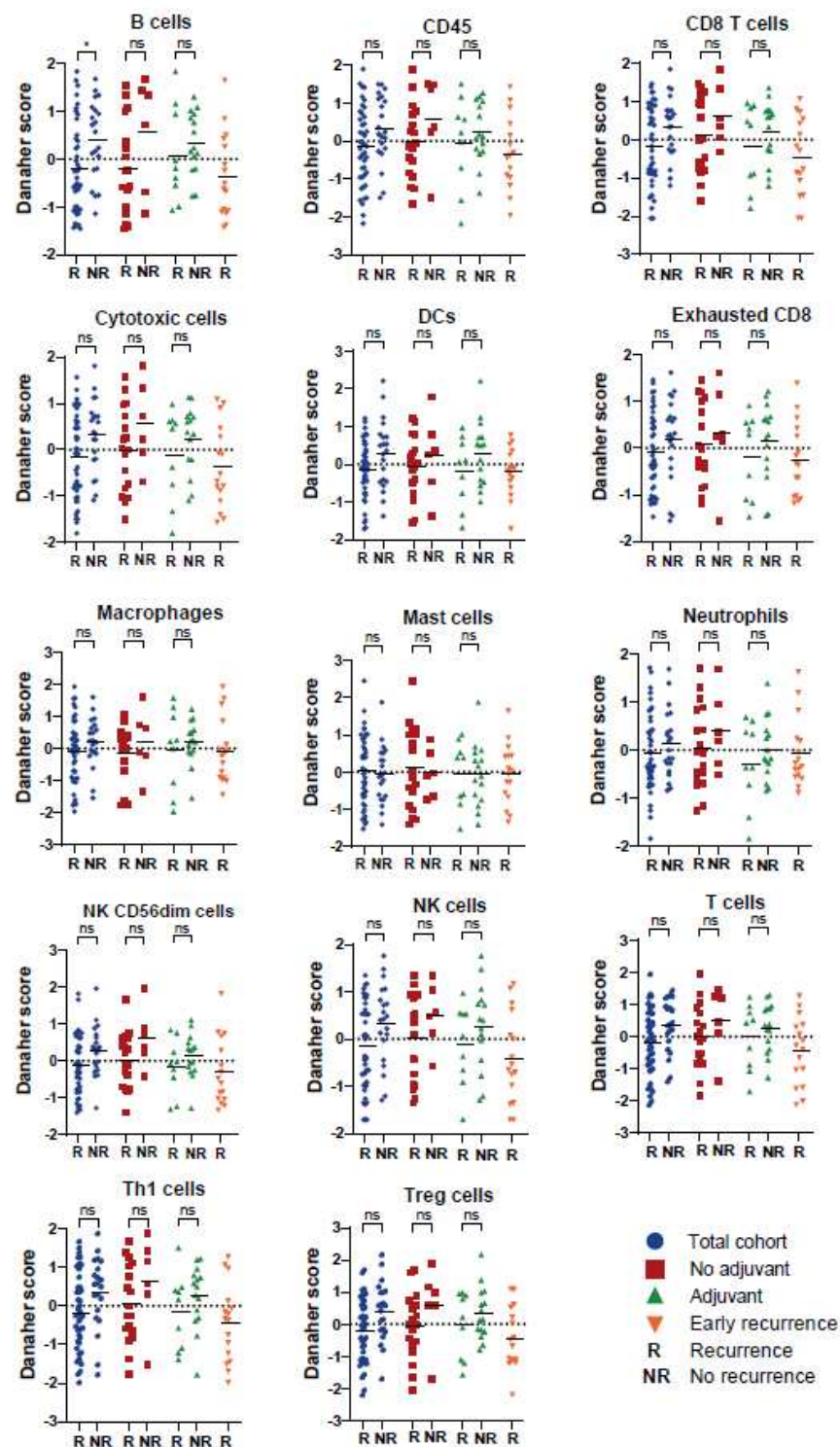**Figure S1.** Flow diagram of patient selection

RNAseq: RNA sequencing.

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Figure S2. Interferon-gamma score

Bar plots with number of patients with an early recurrence with low and high IFNγ score.
IFNγ: interferon-gamma.
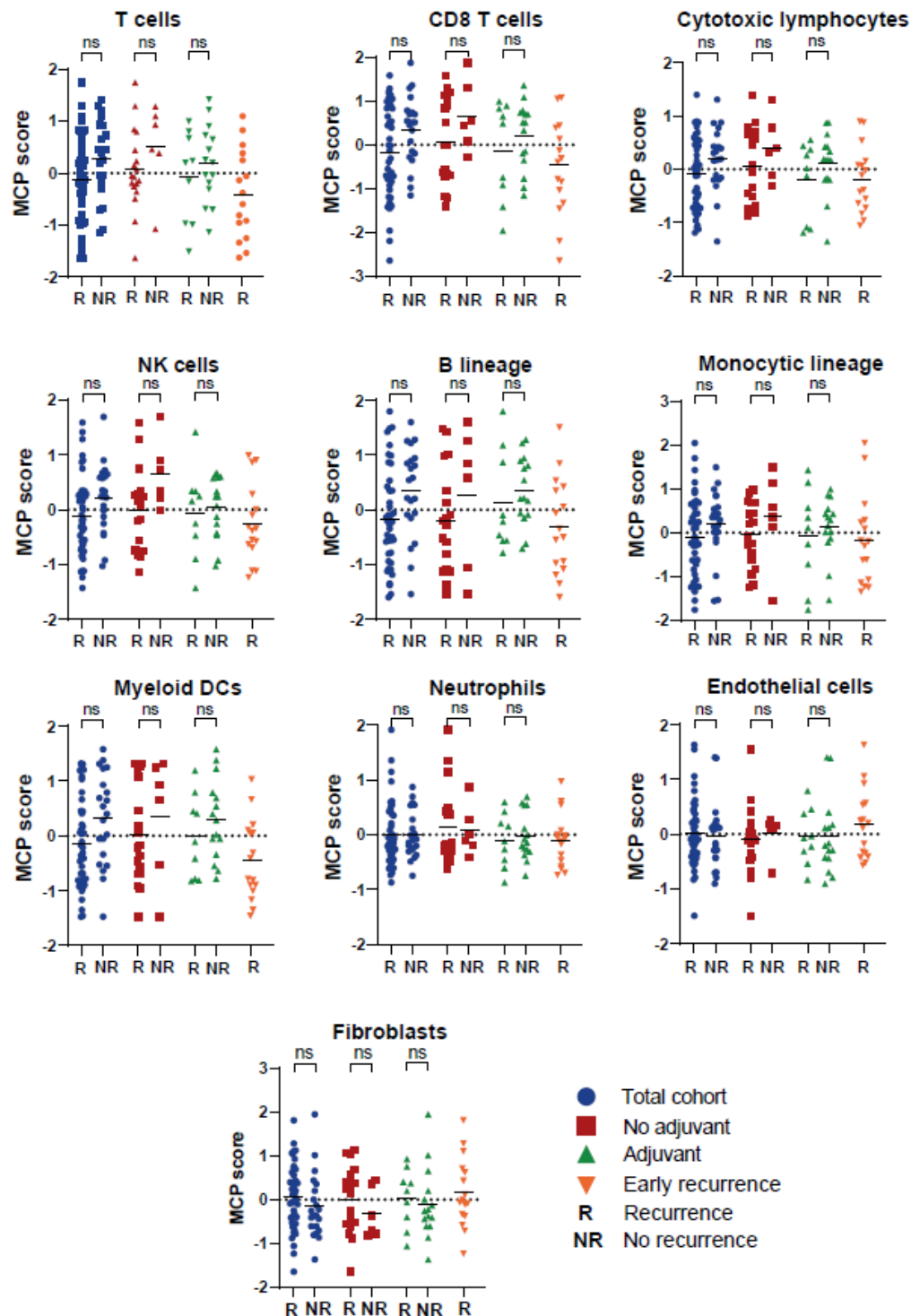
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Figure S3. Overview of immune subsets of Danaher
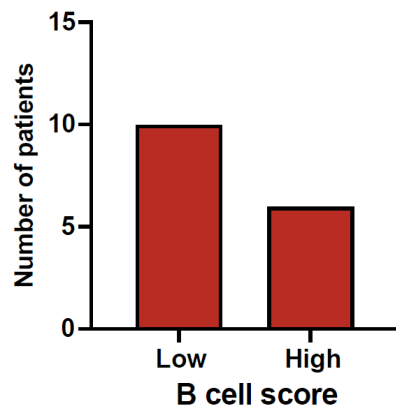

*: significant difference (P<0.005); ns: not significant difference. Immune subsets as defined by Danaher et al. Gene expression markers of tumor infiltrating leukocytes. J Immunother Cancer 2017.

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Figure S4. Overview of immune subsets of the MCP counter

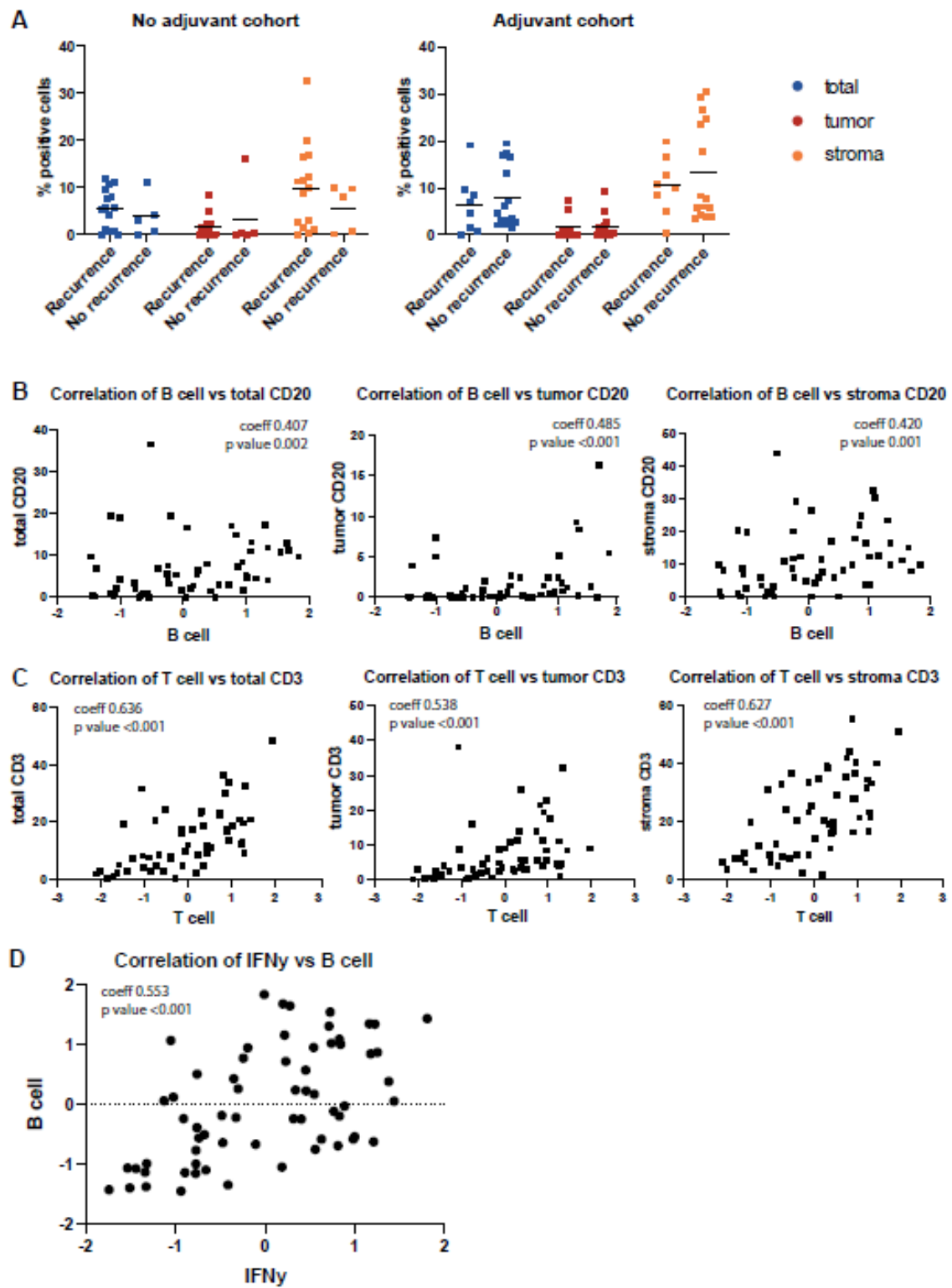

*: significant difference (P<0.005); ns: not significant difference. Immune subsets as defined by Becht et al. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. Genome Biol 2016.

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Figure S5. B cell score

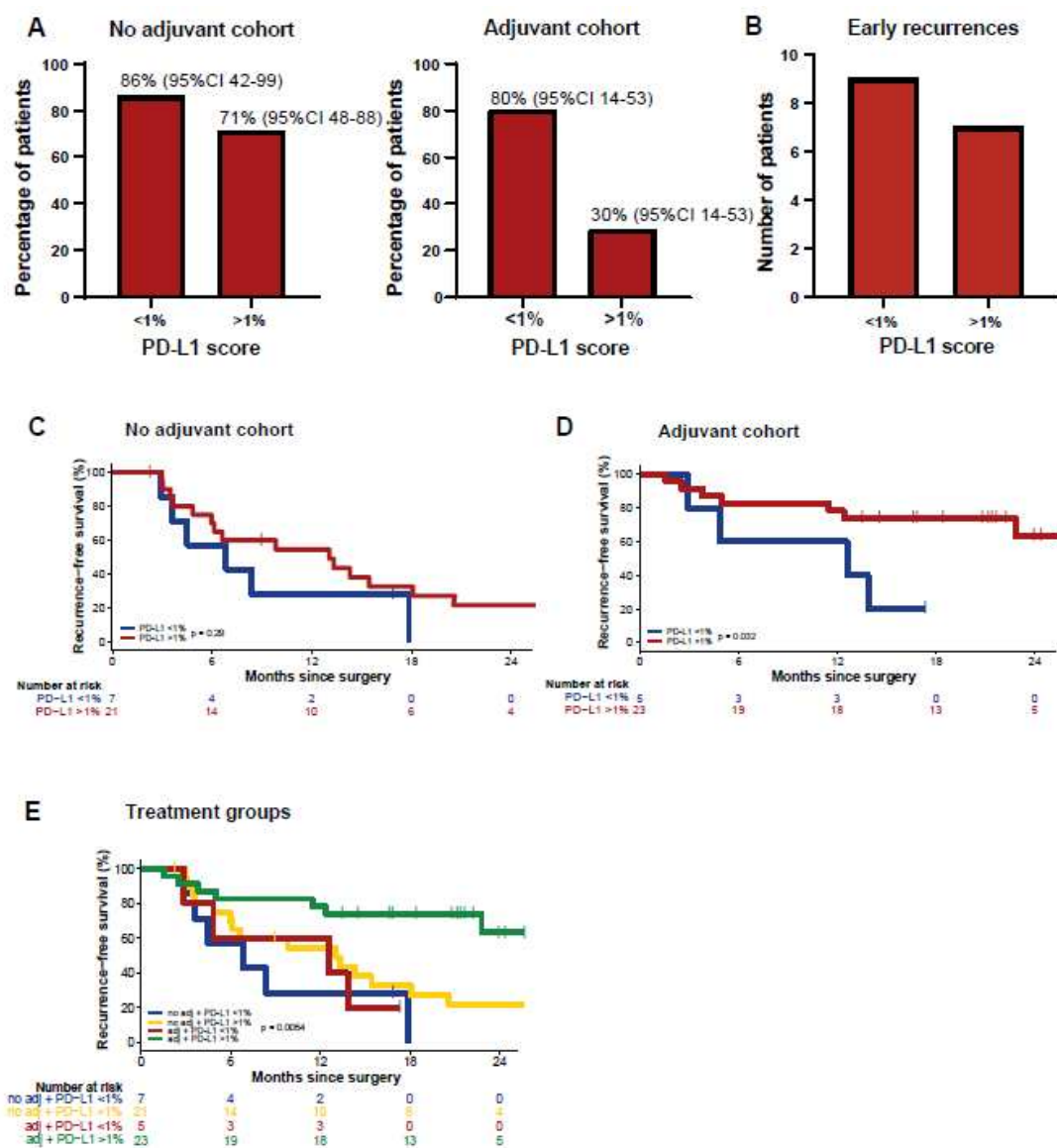Bar plots with number of patients with an early recurrence with low and high B cell score.

Figure S6. CD20 and CD3 staining

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A. Dot plots percentage CD20$^+$ cells of patients with and without recurrence reported for total sample, tumor and stroma area.

B. Correlation of B cell score based on RNAseq compared to percentage CD20$^+$ cells for the total samples, tumor and stroma area.

C. Correlation of T cell score based on RNAseq compared to percentage CD3$^+$ cells for the total samples, tumor and stroma area.

D. Correlation of B cell score compared to IFNγ score.

RNAseq: RNA sequencing.

Figure S7. PD-L1 staining

A. Bar plots with percentage of patients with recurrence, split for a low and high PD-L1 score.

B. Bar plots with number of patients with an early recurrence with low and high PD-L1 score.

C. Recurrence-free survival for patients with a low versus a high PD-L1 score in the no adjuvant cohort.

D. Recurrence-free survival for patients with a low versus a high PD-L1 score in the adjuvant cohort.

E. Recurrence-free survival for patients with a low versus a high PD-L1 score for both the no adjuvant and adjuvant cohort.