

Supplementary material

Investigating Serum and Tissue Expression Identified a Cytokine/Chemokine Signature as a Highly Effective Melanoma Marker

Marco Cesati, Francesca Scatozza, Daniela D'Arcangelo, Gian Carlo Antonini-Cappellini, Stefania Rossi, Claudio Tabolacci, Maurizio Nudo, Enzo Palese, Luigi Lembo, Giovanni Di Lella, Francesco Facchiano and Antonio Facchiano

Table S1. Serum expression general data in all controls (male + female).

Cytokine	N. of values	Min	25% Percent	Median	75% Percent	Max	Average	Std. Dev.	Normal distrib.
IL-1b	85	0.11	0.38	0.53	0.8	13.05	0.86	1.69	No
IL-1RA	121	1.08	12.92	26.77	41.43	495.76	38.10	60.69	No
IL-2	19	0.19	2.38	3.45	10.12	31.77	7.69	8.83	No
IL-4	135	0.23	2.4	2.95	3.67	7.5	3.03	1.07	No
IL-5	30	0.34	1.7	2.77	4.69	67.9	7.54	14.73	No
IL-6	21	1.63	3.97	5.37	6.63	43.29	7.96	9.35	No
IL-7	81	0.26	1.32	2.24	3.96	126.91	4.74	14.08	No
IL-8	128	1	4.84	6.63	8.67	47.92	8.11	5.93	No
IL-9	135	2.64	34.72	45.58	55.5	102.58	46.14	18.65	Yes
IL-10	39	0.18	3.26	7.08	9.98	1218.15	71.50	237.54	No
IL-12(p70)	110	0.63	8.75	15.74	30.61	1723.01	45.14	172.48	No
IL-13	27	0.34	1.08	2.43	5.3	85.16	7.05	16.46	No
IL-15	2	1.67	26.95	52.22	77.5	102.77	52.22	71.49	No
IL-17	108	0.06	7.45	16.72	27.34	74.21	17.89	12.71	No
Eotaxin	132	7.29	65.7	95.28	134.38	471.47	108.95	67.72	No
FGF-2	129	4.22	24.53	32.68	39.64	107.83	33.23	14.31	No
G-CSF	92	0.42	3.47	4.73	6.86	82.39	5.86	8.41	No
GM-CSF	48	0.17	4.95	10.21	20.92	79.5	14.90	15.03	No
IFN- γ	136	3.52	14.21	19.1	43.12	533.04	51.19	74.66	No
IP-10	136	30.06	303.72	438.69	659.71	1697.24	518.32	332.07	No
MCP-1(MCAF)	47	1.39	9.94	18.59	26.72	172.11	22.37	25.41	No
MIP-1a (CCL3)	133	0.09	1.33	1.78	2.26	10.31	1.92	1.06	No
MIP-1b (CCL4)	136	6.29	37.17	54.36	66.32	109.96	54.74	21.68	Yes
PDGF-BB	135	83.69	879.78	1603.74	2117.68	5973.83	1662.24	1065.25	No
RANTES (CCL5)	136	581.91	7639.38	11353.34	15467.61	40521.71	12123.35	7349.35	No
TNF- α	120	1.79	11.94	16.4	27.16	190.28	21.75	21.46	No
VEGF	135	1.32	31.27	59.75	99.52	383.94	74.67	61.44	No

Table S2. General data on serum expression in all melanoma patients (male+female)

Cytokine	N. of values	Min	25% Percent.	Median	75% Percent.	Max	Average	Std. Dev.	Normal distrib.
IL-1b	44	0.07	0.51	0.65	0.87	10.09	1.00	1.50	No
IL-1RA	75	0.74	10.58	17.83	35.39	340.17	32.28	46.41	No
IL-2	13	0.32	1.9	2.14	4.51	10.35	3.38	2.87	No
IL-4	95	0.07	2.31	2.88	3.53	5.43	2.89	1.03	Yes
IL-5	25	0.58	1.7	2.34	3.16	10.43	2.73	2.21	No
IL-6	16	0.14	0.83	3.17	5.84	31.83	5.49	8.03	No
IL-7	48	0.35	1.32	2.24	3.85	16.09	3.08	2.93	No
IL-8	83	2.02	5.05	6.4	9.83	27.81	8.08	4.79	No
IL-9	93	1.86	29.76	42.05	55.61	234.35	45.98	27.47	No
IL-10	22	0.34	2.33	4.56	6.93	16.26	5.11	3.71	No
IL-12(p70)	62	1.96	8.41	16.07	34.03	63.5	21.80	16.29	No
IL-13	19	0.06	1.47	2.99	4.16	13.98	3.49	3.36	No
IL-15	4	8.12	15.46	30.11	45.14	53.64	30.50	21.09	-
IL-17	73	0.31	7.29	13.1	22.7	45.8	15.95	11.16	No
Eotaxin	95	1.3	70.84	106.28	153.95	378.78	116.35	73.18	No
FGF-2	88	1.73	23.08	30.01	38.18	162.23	31.89	18.65	No
G-CSF	40	0.79	3.45	5.41	7.37	16.64	5.93	3.52	No
GM-CSF	46	0.54	7.15	10.63	16.21	121.29	14.99	18.08	No
IFN-γ	86	7.45	15.68	23.2	41.53	241.76	45.05	52.79	No
IP-10	94	55.76	341.37	501.41	768.25	9755	783.51	1147.09	No
MCP-1(MCAF)	30	2.1	5.24	12.49	26.72	123.1	18.58	22.35	No
MIP-1a (CCL3)	95	0.09	1.34	1.74	2.29	16.42	1.92	1.67	No
MIP-1b (CCL4)	94	4.69	34.22	56.26	76.59	385.12	61.41	44.75	No
PDGF-BB	94	33.55	713.58	1033.41	1742.26	5470.53	1325.42	957.25	No
RANTES (CCL5)	94	179.27	5143.95	8735.27	13839.75	29388.47	9751.18	6845.14	No
TNF-α	65	2.98	13.15	18.06	27.44	76.09	22.11	14.20	No
VEGF	92	2	32.82	57.98	114.34	330.16	76.52	62.07	No

Table S3. Correlation of the serum expression with Breslow thickness in male melanoma and in female melanoma.

Cytokines	Melanoma female			Melanoma male		
	N. of pairs	Spearman R	P value (2-tailed)	N. of pairs	Spearman R	P value (2-tailed)
IL-1b	20	0.07	0.78	22	0.05	0.83
IL-1Ra	36	0.09	0.62	35	-0.16	0.37
IL-2	8	-0.55	ns	4	0.95	ns
IL-4	44	0.24	0.12	45	-0.24	0.12
IL-5	17	0.02	0.95	7	-0.17	ns
IL-6	10	0.12	ns	5	0.36	ns
IL-7	25	0.23	0.28	21	0.40	0.07
IL-8	36	-0.23	0.17	41	-0.17	0.28
IL-9	43	0.18	0.24	44	-0.34	0.02
IL-10	12	-0.33	0.29	9	0.05	ns
IL-12(p70)	27	0.04	0.83	33	-0.06	0.75
IL-13	11	0.20	0.55	8	-0.48	ns
IL-15	3	1.00	n.s	0	0	n.s
IL-17	36	0.25	0.14	32	-0.15	0.41
Eotaxin	44	0.14	0.36	45	-0.03	0.84
FGF-2	41	0.21	0.18	42	-0.12	0.46
G-CSF	17	-0.01	0.96	20	0.14	0.57
GM-CSF	24	-0.47	0.02	18	-0.32	0.19
IFN- γ	40	0.17	0.28	42	-0.09	0.59
IP-10 (CXCL10)	44	0.22	0.15	44	-0.14	0.38
MCP-1(MCAF)	15	0.30	0.27	14	0.40	0.16
MIP-1a (CCL3)	44	0.16	0.30	45	-0.29	0.05
MIP-1b (CCL4)	44	0.22	0.16	44	-0.27	0.07
PDGF-BB	44	0.08	0.59	44	-0.28	0.07
RANTES (CCL5)	44	-0.21	0.17	44	-0.16	0.29
TNF- α	29	0.39	0.04	33	0.18	0.32
VEGF	43	0.01	0.97	43	-0.03	0.83

Table S4. Correlation of the serum expression with age, in female controls compared to female melanoma.

Cytokines	N. of Pairs	Female controls		N. of Pairs	Female melanoma	
		Spearman R	P value (2 tails)		Spearman R	P value (2 tails)
IL-1b	32	0.43	0.02	22	0.05	0.82
IL-1Ra	50	0.25	0.07	38	-0.04	0.80
IL-2	7	-0.11	ns	9	0.15	ns
IL-4	53	0.00	0.99	47	-0.13	0.38
IL-5	11	0.22	0.50	18	-0.04	0.88
IL-6	9	-0.01	ns	11	0.06	0.88
IL-7	29	0.72	< 0.0001	27	-0.04	0.82
IL-8	51	-0.03	0.83	39	-0.37	0.02
IL-9	53	-0.04	0.78	46	0.00	1.00
IL-10	14	0.41	0.15	13	0.02	0.95
IL-12(p70)	45	0.39	0.01	29	0.02	0.93
IL-13	5	0.10	ns	11	0.31	0.34
IL-15	-	-	-	4	-0.95	ns
IL-17	44	0.25	0.10	38	-0.08	0.61
Eotaxin	51	0.04	0.77	47	-0.12	0.43
FGF-2	51	-0.15	0.29	43	0.01	0.93
G-CSF	35	-0.18	0.29	19	-0.32	0.18
GM-CSF	19	-0.43	0.06	26	-0.07	0.74
IFN- γ	53	0.26	0.06	42	-0.12	0.43
IP-10 (CXCL10)	53	0.02	0.91	47	0.17	0.25
MCP-1(MCAF)	18	0.20	0.42	16	0.44	0.09
MIP-1a (CCL3)	52	0.23	0.10	47	-0.28	0.06
MIP-1b (CCL4)	53	0.15	0.29	47	-0.10	0.51
PDGF-BB	53	-0.15	0.29	47	-0.28	0.05
RANTES (CCL5) ((CCL5)(CCL5))	53	-0.13	0.34	47	-0.23	0.11
TNF- α	46	0.34	0.02	31	-0.14	0.47
VEGF	53	0.30	0.03	46	0.08	0.58

Table S5. Correlation of the serum expression with age, in male controls compared to male melanoma.

Cytokines	Male controls			Male melanoma		
	N. of Pairs	Spearman R	P value	N. of Pairs	Spearman R	P value (2-tailed)
IL-1b	40	0.08	0.62	22	-0.11	0.63
IL-1Ra	58	0.31	0.02	37	-0.09	0.59
IL-2	8	0.02	ns	4	0.32	ns
IL-4	64	0.26	0.04	48	0.01	0.96
IL-5	12	0.58	0.05	7	-0.58	ns
IL-6	8	0.61	ns	5	0.05	ns
IL-7	41	0.15	0.35	21	-0.22	0.35
IL-8	60	0.08	0.55	44	-0.07	0.65
IL-9	64	0.24	0.06	47	0.08	0.60
IL-10	19	0.12	0.62	9	-0.13	ns
IL-12(p70)	50	0.36	0.01	33	-0.15	0.40
IL-13	15	0.40	0.14	8	0	ns
IL-15	0	0	0	0	0	ns
IL-17	51	0.08	0.57	35	0.04	0.84
Eotaxin	63	0.23	0.06	48	0.02	0.87
FGF-2	60	0.12	0.35	45	-0.03	0.85
G-CSF	43	-0.08	0.62	21	-0.36	0.10
GM-CSF	23	0.31	0.15	20	-0.01	0.97
IFN- γ	64	0.16	0.22	44	-0.10	0.53
IP-10 (CXCL10)	64	0.34	0.01	47	0.20	0.17
MCP-1(MCAF)	23	0.14	0.53	14	-0.59	0.03
MIP-1a (CCL3)	63	0.18	0.16	48	-0.09	0.53
MIP-1b (CCL4)	64	0.45	0.0002	47	-0.22	0.13
PDGF-BB	64	0.15	0.25	47	0.03	0.84
RANTES (CCL5) UCCL5(CCL5)	64	0.08	0.53	47	0.10	0.49
TNF- α	56	0.11	0.41	34	-0.01	0.96
VEGF	64	0.28	0.02	46	0.07	0.65

Table S6. Anova analysis of tissue expression data.

Cytokines	Normality assesment			Homogen. variances	Anova P value	Post hoc tests		
	Ctrls	Primary	Metast.			Ctrls vs. Primary	Ctrls vs. Metast.	Primary vs. Metast.
<i>IL-1b</i>	N	N	N	N	0.02	0.15	<u><0.0001</u>	0.71
<i>IL-1Ra</i>	N	Y	N	N	<u><0.0001</u>	<u><0.0001</u>	<u><0.0001</u>	1.31
<i>IL-2</i>	N	N	N	Y	0.61	-	-	-
<i>IL-4</i>	N	N	N	Y	0.14	-	-	-
<i>IL-5</i>	N	Y	N	Y	0.74	-	-	-
<i>IL-6</i>	N	Y	Y	N	<u><0.0001</u>	0.06	<u><0.0001</u>	0.06
<i>IL-7</i>	N	Y	N	N	<u><0.0001</u>	<u><0.0001</u>	<u><0.0001</u>	<u>0.01</u>
<i>IL-8</i>	N	N	N	N	0.92	-	-	-
<i>IL-9</i>	N	Y	N	Y	0.33	-	-	-
<i>IL-10</i>	Y	Y	N	Y	<u><0.0001</u>	<u>0.0004</u>	<u><0.0001</u>	0.43
<i>IL-12(p70)</i>	N	N	N	N	<u><0.0001</u>	<u><0.0001</u>	<u><0.0001</u>	0.12
<i>IL-13</i>	N	N	N	N	0.56	-	-	-
<i>IL-15</i>	N	Y	Y	N	<u><0.0001</u>	<u>0.001</u>	1.00	<u>0.01</u>
<i>IL-17</i>	N	N	N	N	<u><0.0001</u>	0.08	<u><0.0001</u>	<u>0.05</u>
<i>Eotaxin</i>	N	Y	N	Y	<u><0.0001</u>	0.18	<u><0.0001</u>	0.06
<i>FGF-2</i>	N	Y	Y	N	<u>0.005</u>	<u><0.0001</u>	0.53	<u><0.0001</u>
<i>G-CSF</i>	N	N	N	N	<u><0.0001</u>	<u>0.001</u>	0.30	<u>0.006</u>
<i>GM-CSF</i>	N	Y	N	Y	0.61	-	-	-
<i>IFN-γ</i>	N	N	N	N	<u><0.0001</u>	<u>0.03</u>	<u><0.0001</u>	<u>0.01</u>
<i>IP-10 (CXCL10)</i>	N	N	N	N	<u><0.0001</u>	0.36	<u><0.0001</u>	<u>0.01</u>
<i>MCP-1(MCAF)</i>	N	Y	N	Y	<u>0.0005</u>	0.63	<u>0.003</u>	<u>0.003</u>
<i>MIP-1a (CCL3)</i>	N	Y	N	Y	<u><0.0001</u>	<u><0.0001</u>	<u><0.0001</u>	0.07
<i>MIP-1b (CCL4)</i>	N	Y	N	N	<u><0.0001</u>	<u><0.0001</u>	<u><0.0001</u>	0.12
<i>PDGF-BB</i>	N	N	N	N	<u>0.007</u>	0.60	<u>0.001</u>	<u>0.003</u>
<i>RANTES (CCL5)</i>	N	Y	N	N	<u><0.0001</u>	<u><0.0001</u>	<u><0.0001</u>	1.00
<i>TNF-α</i>	N	Y	N	N	<u><0.0001</u>	<u><0.0001</u>	<u><0.0001</u>	<u>0.003</u>
<i>VEGF</i>	N	N	N	N	0.08	-	-	-

Table S7. Results of the SVM method applied to the serum expression dataset after age-matching. Results are similar to the analysis performed on unmatched data (see Table 7).

Missing values	Num. melanoma	Num. controls	Training set size	Testing set size	Predictors: Sex or Age	AUC (ROC)	Accuracy	No Info Rate	P- value
Removed*	54	63	83	34	Sex, Age	0.635	0.529	0.53	0.80
					Sex	0.542	0.529	0.53	0.57
					Age	0.538	0.588	0.53	0.30
					None	0.528	0.588	0.53	0.94
Set to 0 **	65	65	92	38	Sex, Age	0.609	0.632	0.50	0.07
					Sex	0.706	0.605	0.50	0.13
					Age	0.543	0.50	0.50	0.56
					None	0.579	0.632	0.50	0.07

*when the missing values were removed, IL4, IL8, IL9, Eotaxin, FGF2, IFN- γ , IP10, MIP-1a, MIP-1b, PDGF-BB, RANTES, and VEGF were simultaneously analyzed by the SVM.

** when the missing values were set to 0, all 27 cytokines/chemokines were simultaneously analyzed by SVM.