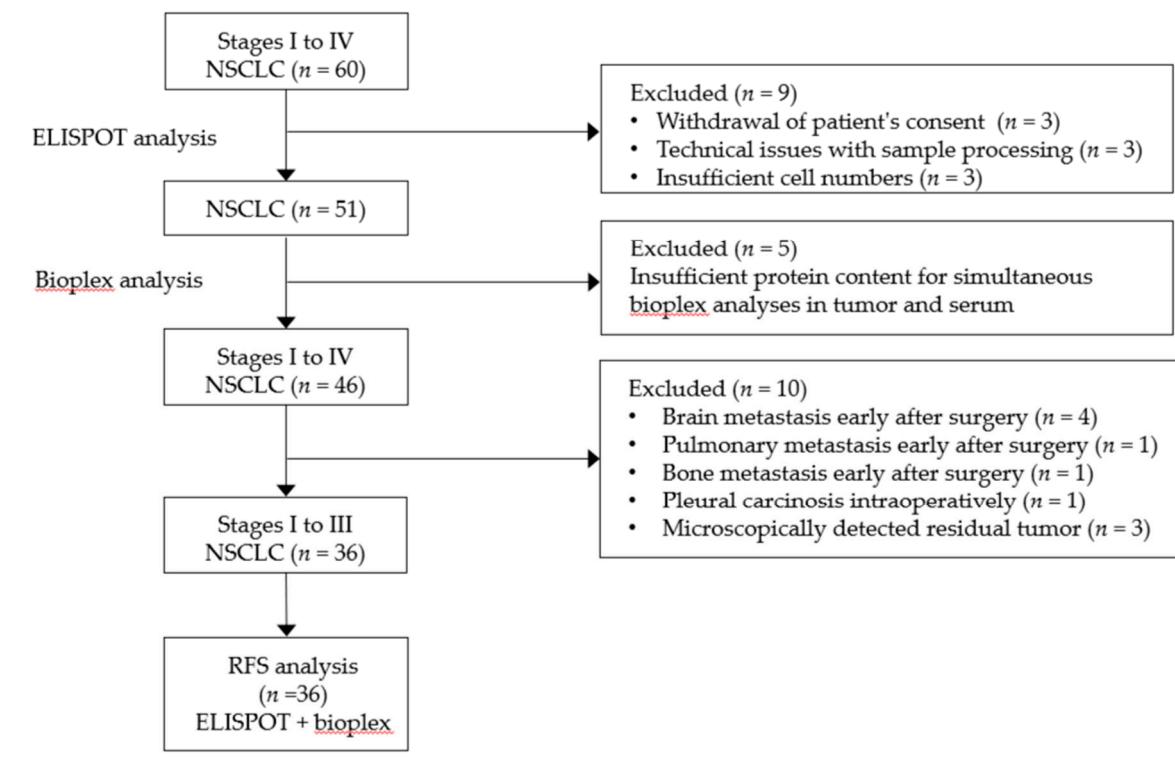
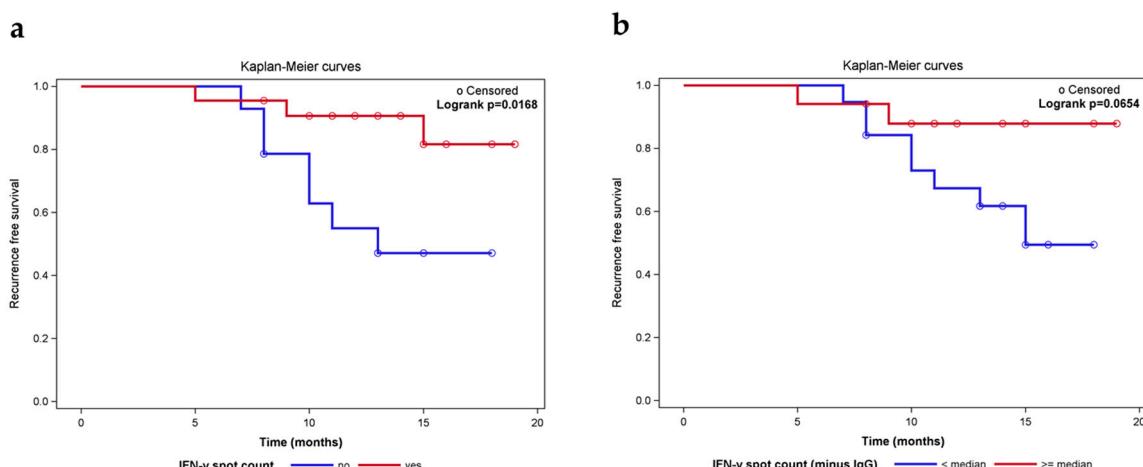


# Supplementary Materials: Circulating Interleukin-4 is Associated with a Systemic T Cell Response against Tumor-Associated Antigens in Treatment-Naïve Patients with Resectable Non-Small-Cell Lung Cancer

Seyer Safi, Yoshikane Yamauchi, Hans Hoffmann, Wilko Weichert, Philipp J. Jost, Hauke Winter, Thomas Muley and Philipp Beckhove

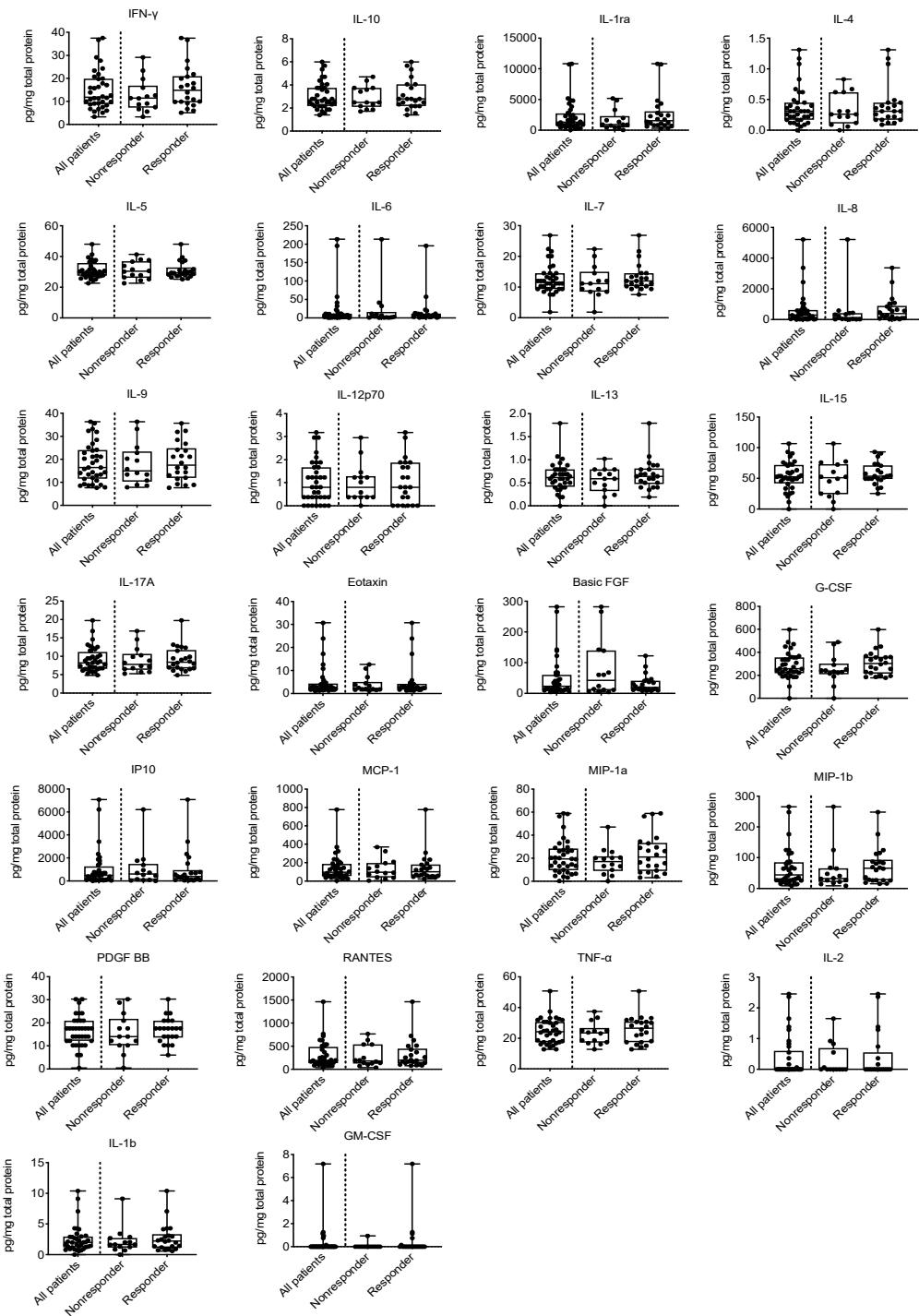


**Figure S1.** Consort diagram of the study.

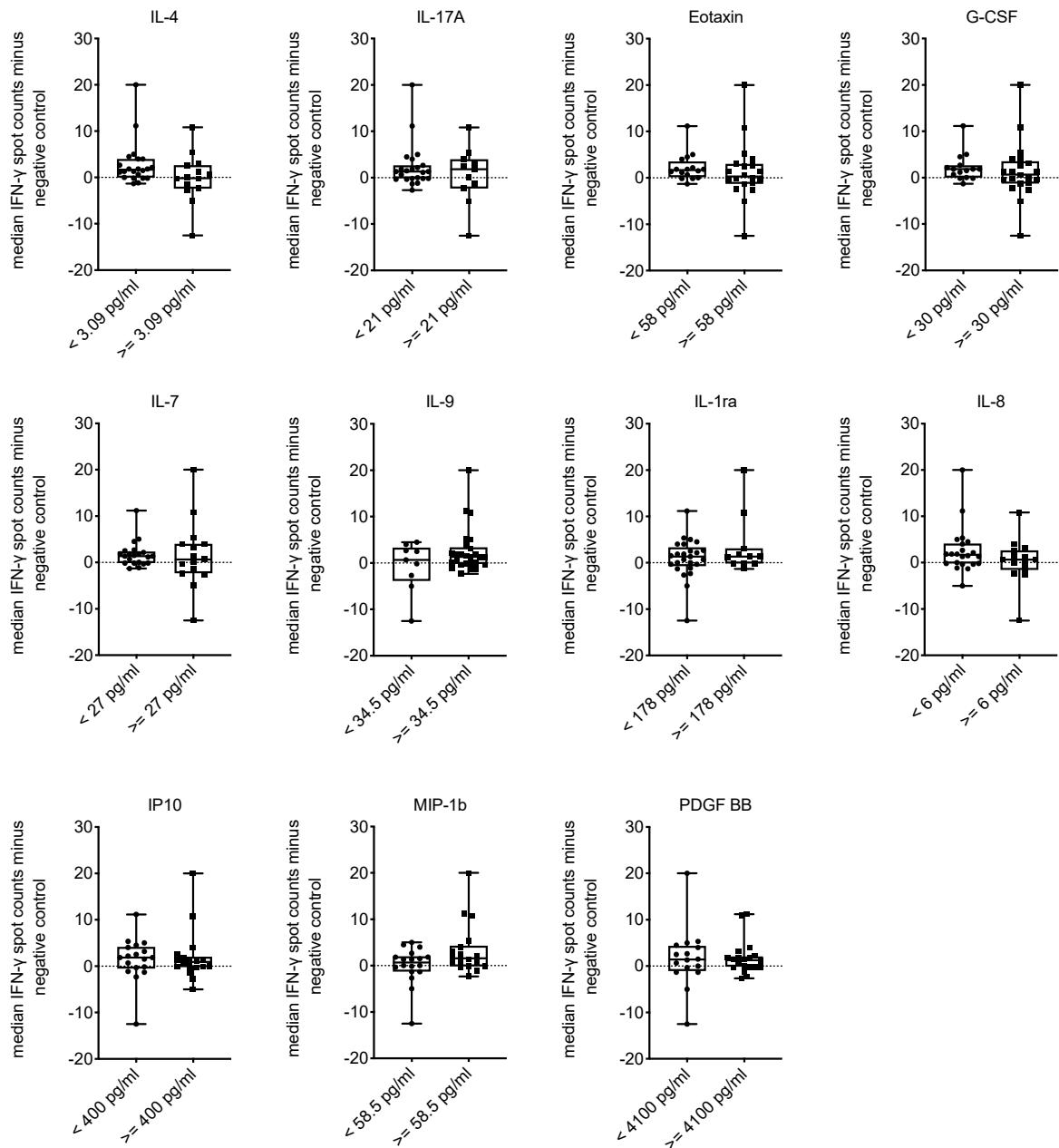


**Figure S2.** The number of IFN- $\gamma$  spot counts significantly correlates with RFS. The median value of IFN- $\gamma$  spot counts was calculated from the 36 patients. Survival based on IFN- $\gamma$  spot counts above

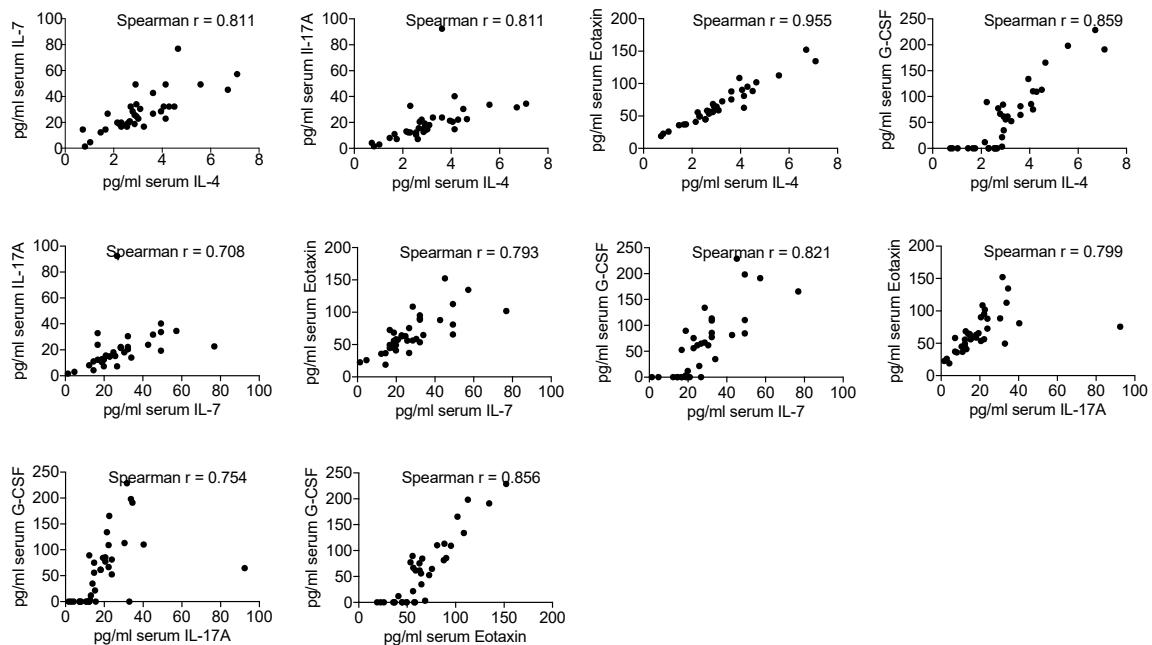
and below the median was correlated with response using two different spot count calculation methods: division of TA-specific spot counts by the corresponding IgG control counts (**a**) or subtraction of the IgG control counts from the corresponding TA-specific spot counts (**b**).



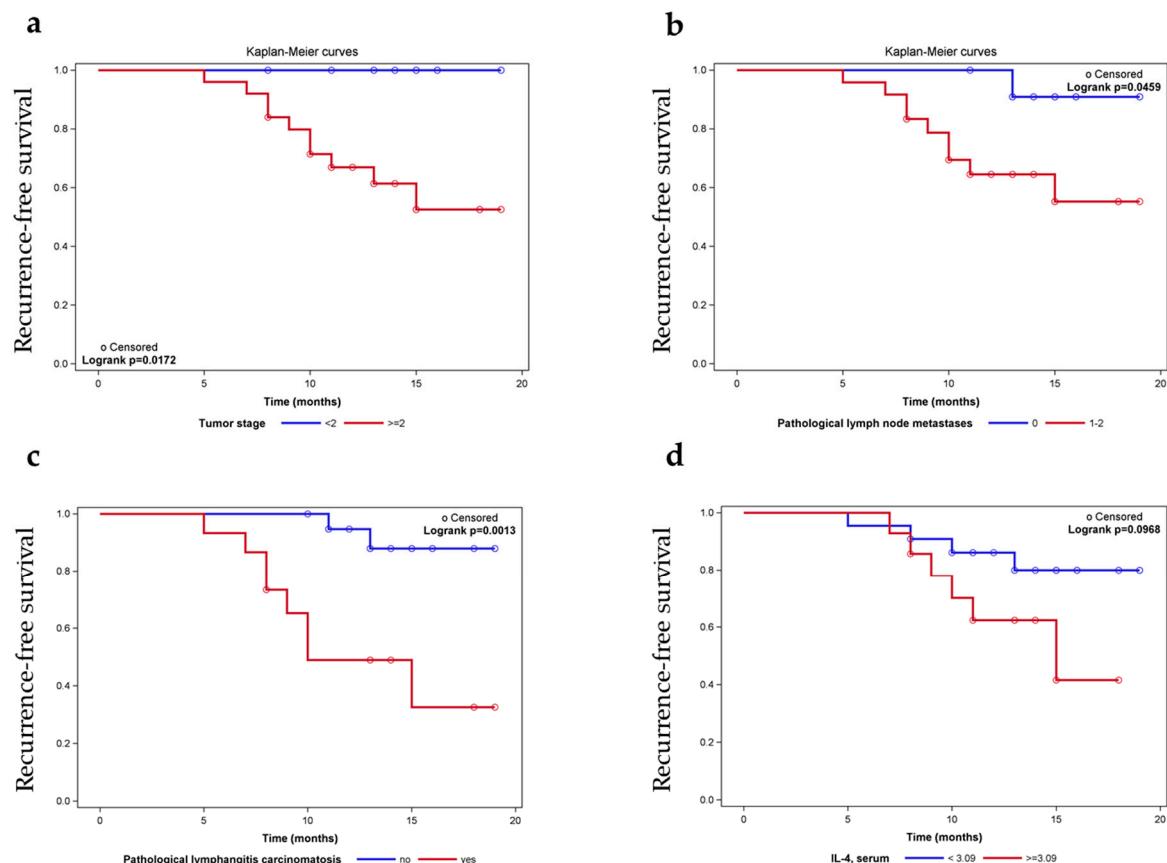
**Figure S3.** Box and whisker plots presenting the tumor cytokine levels in all patients (maximum  $n = 36$ ), responders only ( $n = 22$ ) and nonresponders only ( $n = 14$ ).



**Figure S4.** Associations between median IFN- $\gamma$  spot counts normalized to the values for the negative control and categorized serum cytokine levels.



**Figure S5.** Correlations between serum levels of specific cytokines.



**Figure S6.** Kaplan-Meier survival curves for postoperative tumor stage (a), lymph node status (b), lymphangiosis carcinomatosis (c) and preoperative dichotomized serum IL-4 levels (d). *p*-values represent the results of log-rank tests.

**Table S1.** Characteristics of patients with (responders) and without (nonresponders) circulating TA-specific T cells.

Variable	T cell nonresponder <i>n</i> = 14	T cell responder <i>n</i> = 22	<i>p</i> -value *
Age, mean	70.0	63.9	<b>0.059</b>
Age ≥ 68 years, %	71.4	40.9	<b>0.097</b>
Male, %	42.9	50.0	0.742
Tumor stages II and III, %	71.4	68.2	1.000
Adjuvant chemotherapy given, %	42.9	50.0	0.742
Lymph node metastases present (pN1/2), %	71.4	63.6	0.727
Lymphangiosis carcinomatosa present (pL1) *, %	57.1	33.3	0.187

\* Fisher's exact test/t-test; # *n* = 35; TA-specific = tumor antigen-specific. Bold values indicate strong effects with a significant difference at *p* < 0.15.

**Table S2.** Characteristics of older and younger patients.

Variable	All Patients <i>n</i> = 36	Patients <68 Years <i>n</i> = 17	Patients ≥68 Years <i>n</i> = 19	<i>p</i> -value *
Median age [years], (IQR)	68 (59 to 74)			
Male, %	47% (17)	53 (9)	42 (8)	0.739
Current or former smoker, %	83% (30)	94%	74%	0.182
Lobectomy for tumor resection, %	78% (28)	76% (13)	79% (15)	1.000
Median tumor size [mm], (IQR)	30 (21 to 42)	33 (22 to 42)	28.5 (21 to 39)	0.381
Pulmonary adenocarcinoma, %	64% (23)	76% (13)	53% (10)	0.177
Postoperative UICC tumor stage, 7 <sup>th</sup> edition				
I	30.5% (11)	35% (6)	26% (5)	0.913
II	27.8% (10)	24% (4)	32% (6)	
III	41.7% (15)	41% (7)	42% (8)	
IV	0			
Lymph node metastases present (pN1/2), %	66.7% (24)	59% (10)	74% (14)	0.483
Lymphangiosis carcinomatosa present (pL1) *, %	42.9% (15)	29% (5)	56% (10)	0.176

\* Fisher's exact test/Wilcoxon test; IQR = Interquartile range; # *n* = 35.

**Table S3.** Bioplex data for cytokine levels measured in tumor tissues from 36 patients with NSCLC.

Variable	<i>n</i>	Median	Mean	Min	Max	Comment
IFN-γ, tumor	36	11.850	14.8297222	3.33	37.56	
IL-2, tumor	34	0	0.3750000	0	2.45	
IL-10, tumor	36	2.710	3.0552778	1.4	6.00	
IL-1b, tumor	36	1.995	2.5180556	0	10.37	
IL-1ra, tumor	36	1287.56	2224.54	33.8	10800.78	
IL-4, tumor	36	0.310	0.3758333	0	1.31	
IL-5, tumor	36	29.625	31.0300000	22.55	47.95	
IL-6, tumor	36	5.445	19.9475000	0	213.28	
IL-7, tumor	36	11.560	12.6602778	1.84	26.86	
IL-8, tumor	36	298.570	617.796667	4.87	5217.00	
IL-9, tumor	36	16.430	18.5375000	7.63	36.31	
IL-12p70, tumor	36	0.800	1.0350000	0	3.18	
IL-13, tumor	36	0.615	0.6283333	0	1.79	
IL-15, tumor	36	52.600	53.7963889	0	106.71	

IL17A, tumor	36	8.210	9.1169444	4.87	19.74
Eotaxin, tumor	36	2.590	4.9183333	1.12	30.67
Basic_FGF, tumor	36	22.810	49.5975000	4.34	281.93
G-CSF, tumor	36	257.570	285.376111	0	598.25
GM_CSF, tumor	35	0	0.3257143	0	7.18
IP10, tumor	36	489.790	1018.18	4.41	7069.80
MCP-1, tumor	36	96.975	140.116111	4.33	777.20
MIP-1a, tumor	36	19.205	21.3177778	0.10	58.85
MIP-1b, tumor	36	43.905	66.1322222	8.29	265.64
PDGF_BB, tumor	36	17.580	16.3380556	0.46	30.26
RANTES, tumor	36	192.805	304.706667	35.36	1462.04
TNF-a, tumor	36	24.215	24.6386111	12.79	50.70
VEGF, tumor	35	0	0	0	No analysis *

\* Due to the large number of measurements out of range, this variable was excluded from analysis.

**Table S4.** Bioplex data for cytokine levels measured in serum samples from 36 patients with NSCLC.

Variable	n	Median	Mean	Min	Max	Comment
IFN-γ, serum	36	0.020	33.6964	0	1115.20	
IL-2, serum	35	0	0.2923	0	6.32	No analysis *
IL-10, serum	35	1.910	4.3320	0	35.51	
IL-1b, serum	35	0	0.2054	0	6.42	No analysis *
IL-1ra, serum	36	122.825	141.0461	2.65	439.88	
IL-4, serum	36	2.880	3.12361	0.72	7.09	
IL-5, serum	36	0	6.9158333	0	181.18	
IL-6, serum	35	0.130	2.3891429	0	26.44	
IL-7, serum	36	25.270	27.9028	1.36	76.87	
IL-8, serum	36	5.430	493.9936	0	17533.00	
IL-9, serum	36	41.255	60.67250	17.02	421.14	
IL-12p70, serum	36	0	5.17222	0	99.94	
IL-13, serum	36	0.690	1.9081	0	22.82	
IL-15, serum	35	0	19.8594	0	291.46	
IL17A, serum	36	16.825	19.9481	1.59	92.42	
Eotaxin, serum	36	60.660	66.4169	18.89	152.19	
Basic_FGF, serum	36	3.390	12.9931	0	105.17	
G-CSF, serum	36	58.715	60.5144	0	228.63	
GM_CSF, serum	35	0	0.5489	0	14.51	No analysis *
IP10, serum	36	391.320	440.3514	158.29	1819.70	
MCP-1, serum	36	38.805	40.7219	4.16	144.14	
MIP-1a, serum	36	1.790	2.1783	0.33	5.69	
MIP-1b, serum	36	58.475	61.7344	37.02	111.69	

PDGF_BB, serum	36	4176.47	4138.54	1315.26	8051.01
RANTES, serum	36	9162.47	9288.39	1175.35	15606.02
TNF-a, serum	36	13.805	30.2406	1.83	263.96
VEGF, serum	35	0	1.5963	0	55.87      No analysis *

\* Due to the large number of measurements out of range, this variable was excluded from analysis.

**Table S5.** Association between median IFN- $\gamma$  spot counts adjusted for the negative control values and the cytokine levels<sup>1</sup>. Results from crude and age-adjusted linear regression models are shown.

Variable	Cutoff <sup>1</sup> (pg/mL)	Crude Linear Regression		Age-adjusted Linear Regression	
		Effect <sup>2</sup>	p-value	Effect <sup>2</sup>	p-value
IL-4, serum	3.09	-2.87	<b>0.097</b>	-2.82	<b>0.108</b>
IL-17A, serum	21	-1.64	0.355	-1.66	0.357
Eotaxin, serum	58	-0.91	0.599	-0.84	0.631
G-CSF, serum	30	-1.06	0.539	-1.05	0.546
IL-7, serum	27	-0.04	0.980	-0.20	0.914
IL-9, serum	34.5	3.16	<b>0.104</b>	3.13	<b>0.112</b>
IL-1ra, serum	178	2.50	0.174	2.45	0.199
IL-8, serum	6	-2.16	0.214	-2.80	<b>0.138</b>
IP10, serum	400	0.54	0.755	0.70	0.692
MIP-1b, serum	58.5	3.33	<b>0.046</b>	3.32	<b>0.054</b>
PDGF BB, serum	4100	0.03	0.987	-0.29	0.881

<sup>1</sup> Optimal cutoff values calculated for logistic regression; <sup>2</sup> Difference in predicted means for the category “< cutoff” minus predicted means for the category “>= cutoff”. Bold values indicate strong effects with a significant difference at  $p < 0.15$ .

**Table S6.** Nonparametric comparisons of the intratumor cytokine distributions in responders and nonresponders and correlation coefficients between intratumor cytokine levels and the TA-specific response.

Cytokine	n	Coefficients of Correlation		Nonresponder	Responder	Wilcoxon Rank-sum Test
		Rang biserial	Spearman	Median	Median	p-value
IFN- $\gamma$ , tumor	36	0.2403	0.2029	11.41	14.85	0.244
IL-2, tumor	34	-0.0184	-0.0181	0.0	0.0	0.934
IL-10, tumor	36	0.0942	0.0798	2.48	2.79	0.651
IL-1b, tumor	36	0.1623	0.1371	1.66	2.21	0.432
IL-1ra, tumor	36	0.2078	0.1755	900.33	1537.01	0.314
IL-4, tumor	36	0.1526	0.1293	0.26	0.31	0.459
IL-5, tumor	36	-0.0065	-0.0055	30.33	29.28	0.987
IL-6, tumor	36	0.1916	0.1618	3.67	6.84	0.353
IL-7, tumor	36	0.2078	0.1762	11.13	11.99	0.312
IL-8, tumor	36	0.3507	0.2962	131.33	388.25	<b>0.091</b>
IL-9, tumor	36	0.1071	0.0905	14.90	17.44	0.607
IL-12p70, tumor	36	0.0130	0.0111	0.80	0.80	0.961
IL-13, tumor	36	0.1753	0.1487	0.59	0.64	0.394
IL-15, tumor	36	0.1916	0.1621	51.05	53.62	0.352
IL-17A, tumor	36	0.0812	0.0686	7.79	8.42	0.699
Eotaxin, tumor	36	0.0584	0.0494	2.18	2.79	0.784
Basic FGF, tumor	36	-0.1331	-0.1125	41.77	19.31	0.521
G-CSF, tumor	36	0.2662	0.2249	237.0	302.34	0.197
GM-CSF, tumor	36	0.1538	0.1961	0	0	0.272
IP10, tumor	35	-0.0584	-0.0494	609.75	384.50	0.784
MCP-1, tumor	36	-0.0065	-0.0055	96.98	102.32	0.987
MIP-1a, tumor	36	0.2273	0.1920	16.95	20.97	0.271
MIP-1b, tumor	36	0.2533	0.2139	31.93	65.93	0.220
PDGF BB, tumor	36	0.1331	0.1139	14.04	17.58	0.515
RANTES, tumor	36	0.0714	0.0603	180.53	196.02	0.735
TNF- $\alpha$ , tumor	36	0.1234	0.1043	23.43	26.57	0.552

Spearman rank correlation coefficients  $>|0.3288|$  are considered statistically significant for  $n = 36$ . A responder was defined as a patient with T cells in the peripheral blood that were reactive to any of the 14 tested tumor-associated antigens in ELISPOT analyses, and a nonresponder was defined as a patient without such T cells in the peripheral blood. Bold values indicate strong effects with a significant difference a  $p < 0.10$ .

**Table S7.** Association of dichotomized serum cytokine levels with potential risk factors or confounders. p-values are presented as the results of Fisher's exact test.

Variables							
Dichotomized at the Optimal Cutoff	Age, Years <68 vs. $\geq 68$	Stage I vs. $\geq II$	pL no vs. yes	Adj CTX no vs. yes	pN 0 vs. $\geq 1$	Sex Male vs. Female	
IL-4, serum	0.742	1.000	<b>0.080</b>	0.496	0.293	0.322	
IL-17A, serum	1.000	0.708	0.157	0.299	0.468	0.177	
Eotaxin, serum	1.000	0.718	<b>0.087</b>	0.749	0.730	0.749	
G-CSF, serum	1.000	0.729	<b>0.037</b>	1.000	0.499	1.000	
IL-7, serum	0.316	1.000	0.315	0.739	0.732	0.525	
IL-9, serum	0.451	1.000	1.000	0.706	1.000	0.706	
IL-1ra, serum	0.721	0.252	1.000	<b>0.003</b>	0.125	0.721	

IL-8, serum	0.171	0.467	0.511	0.496	0.727	<b>0.097</b>
IP10, serum	<b>0.044</b>	1.000	0.738	0.181	0.725	1.000
MIP-1b, serum	0.505	0.471	1.000	1.000	0.725	1.000
PDGF BB, serum	<b>0.107</b>	0.718	0.506	0.335	0.482	0.335

Adj CTX = adjuvant chemotherapy, pL = lymphangiosis carcinomatosa, pN = lymph node metastases.

Bold values indicate strong effects with significance <0.15.

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