Supplementary Tables:

Supplementary Table 1: published evidence about serum biomarkers of immunotherapy in non-small-cell lung cancer.

Published evidence about the potential association of 100 serum markers with the clinical outcome of non-small-cell lung cancer patients receiving PD-(L)1 inhibitors was collected from 624 original publications identified in Pubmed as described in the Methods section (search terms ((predictive biomarker[Title/Abstract]) AND ((NSCLC[Title/Abstract]) OR (lung cancer[Title/Abstract]))).

For each of the serum markers listed below, each character of the string in the "evidence" column represents one study, while the study results are coded according to the following rule: 1 = association of the marker with PD-(L)1 inhibitor efficacy; 3 = association of the marker with the development of immune-related adverse events (toxicity); 5 = association of the marker with both efficacy and toxicity; 0 = no associated of the marker with efficacy or toxicity. Markers analyzed in the current study are highlighted in green, and their selection was based on both the previously published evidence and technical feasibility of parallel measurement (n=16). In addition, four promising markers with different published evidence were explored: two with data under (chemo-)immunotherapy for melanoma, but not NSCLC: angiogenin (PMID 17762972) and granzyme A (PMID 33746582); one with data for chemotherapy, but not immunotherapy in NSCLC: ICAM-1 (PMID 21550560, 23884579, 32853940, 25202042); and one with preclinical only data about relationship with anti-tumor immunity: IL-17F (PMID 32227296, 20635888).

| Serum marker       | evidence | Serum marker       | evidence |
|--------------------|----------|--------------------|----------|
| IL-8 (CXCL8)       | 111111000 | IL-13              | 100      |
| IP-10 (CXCL10)     | 11130    | IL-1RA             | 100      |
| RANTES (CCL5)      | 11300    | BMP-9              | 30       |
| IFN-γ              | 130000000 | CXCL11 (I-TAC)    | 30       |
| G-CSF              | 3010     | Leptin             | 30       |
| IL-10              | 100000000 | IL-12P40           | 30       |
| IL-2               | 113000000 | IL-16              | 30       |
| TNF-α              | 11300000 | CD137 (4-1-BB, TNFRSF9) | 10 |
| IL-6               | 11100000 | FGF                | 10       |
| IL-4               | 1100000  | Follistatin        | 10       |
| IL-5               | 100      | Granzyme B        | 10       |
| VEGF               | 100      | IL-18              | 10       |
| IL-1β              | 100000   | sGITR              | 10       |
| sTNFR1             | 1        | CXCL19             | 3        |
| IL-12P70           | 0        | sPD-L2             | 3        |
| sCD40L             | 0        | Perforin           | 1        |
| MCP-1 (CCL2)       | 1100000  | sIDO               | 1        |
| sPD-L1             | 110      | Angiotropoietin-2  | 0        |
| Eotaxin (CCL11)    | 100000   | b-NF               | 0        |
| MIP-1α (CCL3)      | 100000   | CCL21 (6kine)      | 0        |
| GRO (CXCL1)        | 50000    | CTACK (CCL27)      | 0        |
| MIP-1β (CCL4)      | 100000   | CXCL5 (ENA-78)     | 0        |
| IL-7               | 1000     | CXCL13 (BCA-1)     | 0        |
| CXCL9 (MIG)        | 300      | EGF                | 0        |
| MCP-3 (CCL7)       | 300      | Endoglin           | 0        |
| Cytokine       | Vendor | Sensitivity | Range standard curve | Limit of detection |
|---------------|--------|-------------|----------------------|--------------------|
| IL-1β         | BD     | E           | 274-200000 fg/ml     | 48.4 fg/ml         |
| IL-2          | BD     | E           | 274-200000 fg/ml     | 88.9 fg/ml         |
| IL-4          | BD     | E           | 274-200000 fg/ml     | 144.4 fg/ml        |
| IL-5          | BD     | E           | 274-200000 fg/ml     | 67.8 fg/ml         |
| IL-6          | BD     | E           | 274-200000 fg/ml     | 68.4 fg/ml         |
| IL-8          | BD     | E           | 274-200000 fg/ml     | 69.9 fg/ml         |
| IL-10         | BD     | E           | 274-200000 fg/ml     | 13.7 fg/ml         |
| IL-12p70      | BD     | E           | 274-200000 fg/ml     | 12.6 fg/ml         |
| IFN-γ         | BD     | E           | 274-200000 fg/ml     | 14.8 fg/ml         |
| TNF           | BD     | E           | 274-200000 fg/ml     | 67.3 fg/ml         |
| ICAM-1        | BD     | S           | 40-100000 pg/ml      | 25.7 pg/ml         |
| IP-10         | BD     | S           | 10-2500 pg/ml        | 0.5 pg/ml          |
| VEGF          | BD     | S           | 10-2500 pg/ml        | 4.5 pg/ml          |
| angiogenin    | BD     | S           | 10-2500 pg/ml        | 4.6 pg/ml          |
| sCD40L        | BD     | S           | 10-2500 pg/ml        | 2.3 pg/ml          |
| G-CSF         | BD     | S           | 10-2500 pg/ml        | 1.6 pg/ml          |
| CCL5          | BD     | S           | 10-2500 pg/ml        | 0.002 pg/ml        |
| Granzyme a    | BD     | S           | 40-100000 pg/ml      | 3.7 pg/ml          |
| TNF-RI        | BD     | S           | 40-100000 pg/ml      | 5.2 pg/ml          |

S: standard sensitivity; E: enhanced sensitivity
## Supplementary Table 3: Patient characteristics according to IO efficacy

|                  | 1L-IO | ICT | 2L-IO | >2L-IO |
|------------------|-------|-----|-------|--------|
| **Samples (n)**  | RP (n = 19) | LR (n = 27) | RP (n = 34) | LR (n = 74) | RP (n = 19) | LR (n = 15) | RP (n = 12) | LR (n = 4) |
| 1C               | 2     | 9   | 8     | 19    | 0     | 0     | 0     | 0      |
| 4C               | 5     | 23  | 4     | 63    | 7     | 15    | 0     | 4      |
| PD               | 3     | 5   | 16    | 11    | 5     | 6     | 1     | 1      |
| **Age (mean, range)**       | 72 (58-87) | 66 (48-83) | 65 (49-87) | 63 (37-81) | 64 (51-78) | 64 (48-78) | 61 (50-71) | 65 (60-71) |
| **Sex (n, %)**       | RP | LR |
| Female            | 5 (26%) | 7 (26%) |
| Male              | 14 (74%) | 20 (74%) |
| **Smoker s(n, %)** | RP | LR |
| never             | 2 (11%) | 2 (7%) |
| former            | 13 (68%) | 11 (41%) |
| current           | 4 (21%) | 14 (52%) |
| **ECOG (n, %)**    | RP | LR |
| 0                 | 8 (42%) | 11 (41%) |
| 1                 | 10 (53%) | 16 (59%) |
| ≥2                | 1 (5%) | 0 |
| **PD-L1 TPS (n, %)** | RP | LR |
| <1                | 1 (5%) | 0 |
| 1-49              | 6 (32%) | 4 (15%) |
| ≥50               | 12 (63%) | 23 (85%) |
| **Histology (n, %)** | RP | LR |
| ADC               | 10 (53%) | 16 (59%) |
| SCC               | 5 (26%) | 9 (33%) |
| Other NSCLC       | 4 (21%) | 2 (7%) |
| **Immunotherapy** | RP | LR |
| anti-PD-1         | 17 (89%) | 27 (100%) |
| anti-PD-L1        | 2 (11%) | 0 |
| **irAE (n, %)**    | RP | LR |
| Yes               | 3 (16%) | 11 (41%) |
| No                | 16 (84%) | 16 (59%) |

NSCLC: non-small-cell lung cancer; ICT: immunochemotherapy; 1L-IO: patients receiving PD-(L)1 inhibitors as monotherapy in the first line; 2L+IO: patients receiving PD-(L)1 inhibitors as monotherapy in the second-or-subsequent lines; RP: rapid progression (progression-free survival (PFS) < 120 days); LR: long-time response (PFS > 200 days); BL: baseline; C1: sample after 1 cycle of treatment; C4: sample after 4 cycles of treatment; PD: progressive disease; ECOG: Eastern Cooperative Oncology Group; PD-L1: programmed cell death protein ligand 1; TPS: tumor proportion score; ADC: adenocarcinoma; SCC: squamous-cell carcinoma, NSCLC: non-small-cell lung cancer; irAE: immune-related adverse events; 1L: first line.

1 PD-1-inhibitors: nivolumab, pembrolizumab; PD-L1-inhibitors: atezolizumab, durvalumab.
### Supplementary Table 4: Association of serum cytokines with IO efficacy and irAE in patient groups

| Marker | LR vs. RP ICT | ICT irAE | LR vs. RP IL-1O | 1L-IO irAE | LR vs. RP 2L-IO | 2L-IO irAE |
|--------|---------------|----------|-----------------|------------|-----------------|-----------|
|        | baseline under treatment | vs. ctrl (n=100) | baseline under treatment | vs. ctrl (n=50) | baseline under treatment | vs. ctrl (n=50) |
|        | (n=108) | | | | | |
| IL-1β  | FC=3 p=0.689 | FC=1 p=1 | FC=1 p=0.786 | FC=0.1 p=0.009 | FC=0.8 p=0.187 | FC=1 p=1 | FC=0.1 p=0.617 |
|         | | FC=0 | | FC=15 | | | FC=0.9 p=0.791 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.436 |
| IL-2   | FC=2 p=0.898 | FC=10 p=0.35 | FC=10 p=0.638 | FC=0.1 p=0.407 | FC=7 p=0.773 | FC=10 p=0.637 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| IL-4   | FC=2 p=0.891 | FC=10 p=0.516 | FC=10 p=0.506 | FC=0.7 p=0.344 | FC=8 p=0.906 | FC=10 p=0.476 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| IL-5   | FC=2 p=0.527 | FC=10 p=0.53 | FC=10 p=0.53 | FC=0.288 | FC=4 p=0.213 | FC=10 p=0.193 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| IL-6   | FC=0.7 p=0.005 | FC=1 p=0.116 | FC=0.16 p=0.53 | FC=0.111 | FC=4 p=0.084 | FC=0.1 p=1 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| IL-8   | FC=0.7 p=0.016 | FC=0.167 | FC=0.843 | FC=0.132 | FC=0.16 p=0.028 | FC=0.6 p=0.723 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| IL-10  | FC=1 p=0.694 | FC=0.499 | FC=0.263 | FC=0.029 | FC=0.4 p=0.473 | FC=0.8 p=0.927 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| IP-10  | FC=0.8 p=0.037 | FC=0.233 | FC=0.103 | FC=0.116 | FC=0.8 p=0.723 | FC=0.7 p=0.283 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| IL-12p70 | FC=5 p=0.857 | FC=15 p=0.884 | FC=10 p=0.536 | FC=0.588 | FC=10 p=0.257 | FC=10 p=0.392 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| IL-17F | FC=1 p=0.193 | FC=0.171 | FC=0.426 | FC=0.328 | FC=0.24 p=0.814 | FC=0.7 p=0.418 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| CCL5   | FC=1 p=0.11 | FC=0.9 | FC=0.426 | FC=0.328 | FC=0.24 p=0.814 | FC=0.7 p=0.418 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| CD34+L | FC=1 p=0.171 | FC=0.9 | FC=0.426 | FC=0.328 | FC=0.24 p=0.814 | FC=0.7 p=0.418 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| G-CSF  | FC=1 p=0.344 | FC=1.2 | FC=0.122 | FC=0.344 | FC=0.24 p=0.814 | FC=0.7 p=0.418 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| Granzyme A | FC=0.5 p=0.197 | FC=0.394 | FC=0.254 | FC=0.254 | FC=0.24 p=0.814 | FC=0.7 p=0.418 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| ICAM-1 | FC=2 p=0.491 | FC=0.491 | FC=0.491 | FC=0.491 | FC=0.24 p=0.814 | FC=0.7 p=0.418 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| IFN-γ  | FC=0.4 p=0.444 | FC=1 p=1 | FC=0.517 | FC=0.517 | FC=0.24 p=0.814 | FC=0.7 p=0.418 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| TNF    | FC=5 p=0.897 | FC=1.2 | FC=0.946 | FC=0.53 | FC=0.548 | FC=0.567 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| TNF-RI | FC=0.8 p=0.006 | FC=0.7 | FC=0.053 | FC=0.152 | FC=0.062 | FC=0.034 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| Angiogenin | FC=0.6 p=0.068 | FC=1 p=1 | FC=0.835 | FC=0.693 | FC=0.0002 | FC=0.031 | FC=1.5 p=0.018 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| VEGF   | FC=0.8 p=0.327 | FC=0.394 | FC=0.394 | FC=0.394 | FC=0.24 p=0.814 | FC=0.7 p=0.418 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |
|         | | | | | | | FC=0.1 p=0.127 |
| NLR    | FC=1 p=0.963 | FC=0.9 | FC=0.873 | FC=0.115 | FC=0.04 p=0.2 | FC=0.024 | FC=1 p=1 |
|         | | | | | | | FC=10 p=0.355 |

Results with p<0.05 have been marked in green if passing and in gray if failing the false discovery rate <0.1 threshold. For abbreviations, please see Table 2.

### Supplementary Table 5: Characteristics of immune-related adverse events (irAE) in study patients
### irAE Grade (n, %)

| Grade | 1L-IO (n=14) | ICT (n=19) | 2+L-IO (n=10) |
|-------|--------------|------------|---------------|
| 1     | 0            | 5 (26%)    | 2 (20%)       |
| 2     | 3 (21%)      | 7 (37%)    | 5 (50%)       |
| 3     | 9 (64%)      | 7 (37%)    | 2 (20%)       |
| 4     | 2 (14%)      | 0          | 1 (10%)       |

### Affected organ system (n, %)

| Organ System | 1L-IO (n=14) | ICT (n=19) | 2+L-IO (n=10) |
|--------------|--------------|------------|---------------|
| Skin         | 1 (7%)       | 0          | 3 (30%)       |
| Liver        | 1 (7%)       | 1 (5%)     | 2 (20%)       |
| Colon        | 6 (43%)      | 1 (5%)     | 1 (10%)       |
| Musculoskeletal | 3 (21%)   | 4 (21%)    | 0             |
| Lung         | 1 (7%)       | 2 (11%)    | 2 (20%)       |
| Endocrine    | 2 (14%)      | 6 (32%)    | 2 (20%)       |
| Heart        | 0            | 2 (11%)    | 0             |
| Blood system | 0            | 1 (5%)     | 0             |
| Nervous system | 0          | 1 (5%)     | 0             |
| Kidney       | 0            | 1 (5%)     | 0             |

### Treatment with steroids (n, %)

|       | 1L-IO (n=14) | ICT (n=19) | 2+L-IO (n=10) |
|-------|--------------|------------|---------------|
| Yes   | 13 (93%)     | 14 (74%)   | 7 (70%)       |
| No    | 1 (7%)       | 5 (26%)    | 3 (30%)       |

irAE: immune-related adverse event; ICT: immunochemotherapy; 1L-IO: patients receiving PD-(L)1 inhibitors as monotherapy in the first line; ctrl: age-matched healthy controls; 2+L-IO: patients receiving PD-(L)1 inhibitors as monotherapy in the second-or-subsequent lines.
Supplementary Figures:

Supplementary Figure 1: Receiver operating characteristic curve for TNF-RI
Receiver operating characteristic (ROC) curve and Youden index analysis of the long-term responder (LR) vs. rapid progressor (RP) status of patients with metastatic NSCLC under first-line (chemo-)immunotherapy was used in order to derive an appropriate cut-off for the baseline serum TNF-RI concentration at the time of diagnosis. Area under the curve (AUC) was 0.703 (95% confidence interval 0.612-0.794), p=0.00003. The TNF-RI cut-off was 2139.7 pg/ml.