**Supplementary Table 1: Number of plasma samples included in the study**

|  |  |  |
| --- | --- | --- |
| Timepoint | Anti-PD-1 monotherapy(Cohort 1) | Anti-CTLA-4 and anti-PD-1 (Cohort 2) |
| **Responders** | **Non-responders** | **Responders** | **Non-responders** |
| PRE | 16 | 24 | 47 | 11 |
| EDT | 16 | 22 | 46 | 11 |
| MID | 8 | 14 | 32 | 8 |
| LATE | 11 | 8 | 28 | 7 |

Abbreviations: PRE, baseline plasma taken 0-29 days before treatment initiation; EDT, early during treatment plasma taken at week 1-6 after therapy initiation; MID, mid timepoint plasma taken at week 7-11 after therapy; LATE, late timepoint plasma taken at week 12-18 after therapy. Patients undergoing complete or partial response were classified as responders, whereas non-responding patients had stable or progressive disease.

**Supplementary Table 2: Association of toxicity with treatment response**

|  |  |  |  |
| --- | --- | --- | --- |
| **Cohort 2** | **Responders****(RECIST CR or PR)** | **Non-responders****(RECIST SD or PD)** | **Fisher’s exact test****Odds ratio, p value** |
| Patients with severe irAEs | 19 | 2 | 0.25, p=0.098 |
| Patients with no-severe irAEs | 21 | 9 |
| **Cohort 3** | **Responders****(RECIST CR or PR)** | **Non-responders****(RECIST SD or PD)** | **Fisher’s exact test****Odds ratio, p value** |
| Patients with severe irAEs | 17 | 5 | 0.97, p>0.999 |
| Patients with no-severe irAEs | 21 | 6 |

Abbreviations: irAEs, immune-related adverse events; RECIST, response evaluation criteria in solid tumor; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease

**Supplementary Table 3: Differentially expressed cytokines in patients with severe irAEs compared to those with no-severe irAEs at PRE in Cohort 2**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Cytokine** | **T statistic** | **p value** | **FDR** | **Severe irAEs (log2 mean)** | **No-severe irAEs (log2 mean)** |
| Eotaxin-1 | 2.56 | 0.01 | 0.05 | 9.27 | 8.37 |
| FGF-2 | 2.98 | 0 | 0.02 | 6.81 | 5.76 |
| Fractalkine | 2.74 | 0 | 0.02 | 5.73 | 4.86 |
| G-CSF | 2.56 | 0.01 | 0.05 | 6.38 | 5.59 |
| GM-CSF | 2.65 | 0.01 | 0.04 | 6.56 | 5.45 |
| GROpan | 2.79 | 0.01 | 0.05 | 10.62 | 9.17 |
| IFN2 | 2.88 | 0 | 0.02 | 6.03 | 5.06 |
| IL-12P70 | 2.39 | 0.02 | 0.05 | 6.07 | 5.17 |
| IL-13 | 2.53 | 0.01 | 0.05 | 6.3 | 5.26 |
| IL-1a | 3.17 | 0 | 0.02 | 6.94 | 5.82 |
| IL-1B | 2.5 | 0.01 | 0.05 | 6.17 | 5.32 |
| IL-1RA | 3.16 | 0 | 0.02 | 7.48 | 6.26 |
| IL-2 | 2.65 | 0 | 0.03 | 6.63 | 5.74 |
| IL-3 | 2.47 | 0 | 0.03 | 5.78 | 5.05 |
| IL-9 | 2.13 | 0.02 | 0.05 | 6.12 | 5.28 |
| PDGF-AA | 3.07 | 0.01 | 0.05 | 13.66 | 12.93 |
| TARC | 3.84 | 0 | 0.03 | 11.33 | 10.24 |

Differentially expressed cytokines were selected using the *t*-test with an FDR-adjusted p value ≤ 0.05 and log2 fold change > 0.7 as cut-off criterion.

Abbreviations: irAEs, immune related adverse events; FDR, false discovery rate.

**Supplementary Table 4: Differentially expressed cytokines in patients with severe irAEs compared to those with no-severe irAEs at EDT in Cohort 2**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Cytokine** | **T statistic** | **p value** | **FDR** | **Severe irAEs (log2 mean)** | **No-severe irAEs (log2 mean)** |
| EGF | 3.32 | 0 | 0.02 | 7.69 | 5.98 |
| FGF-2 | 2.8 | 0 | 0.03 | 6.75 | 5.77 |
| Fractalkine | 2.85 | 0 | 0.02 | 5.76 | 4.89 |
| G-CSF | 2.35 | 0.01 | 0.04 | 6.45 | 5.71 |
| GM-CSF | 2.48 | 0.01 | 0.03 | 6.5 | 5.45 |
| IFN2 | 2.56 | 0 | 0.03 | 5.93 | 5.08 |
| IL-12P70 | 2.5 | 0.01 | 0.04 | 6.02 | 5.18 |
| IL-13 | 2.51 | 0.01 | 0.04 | 6.25 | 5.24 |
| IL-1a | 2.52 | 0.01 | 0.04 | 6.68 | 5.94 |
| IL-1B | 2.59 | 0 | 0.02 | 6.12 | 5.26 |
| IL-1RA | 2.42 | 0.01 | 0.05 | 7.41 | 6.46 |
| IL-2 | 2.69 | 0 | 0.02 | 6.51 | 5.72 |
| IL-4 | 2.5 | 0 | 0.02 | 5.9 | 5.19 |
| MIP-1a | 2.77 | 0 | 0.03 | 6.8 | 5.64 |
| MIP-1B | 2.53 | 0.01 | 0.03 | 6.98 | 6.15 |
| VEGF-A | 3.31 | 0 | 0.02 | 6.92 | 5.6 |

Differentially expressed cytokines were selected using the *t*-test with an FDR-adjusted p value ≤ 0.05 and log2 fold change > 0.7 as cut-off criterion.

Abbreviations: irAEs, immune related adverse events; FDR, false discovery rate.

**Supplementary Table 5: Univariate analysis of cytokine expression and association with overall survival**

|  |  |  |
| --- | --- | --- |
| **Cytokine** | **Hazard ratio (95% CI)** | **Log-rank test****P value** |
| Discovery cohort 1 – anti-PD-1 monotherapy |
| TRAIL | 0.318 (0.141-0.717) | 0.0038 |
| MCP-1 | 2.696 (1.184-6.139) | 0.0141 |
| IL-2 | 0.447 (0.202-0.988) | 0.0410 |
| Discovery cohort 2 - anti-CTLA-4 and anti-PD-1 |
| TNFα | 5.193 (1.119-24.083) | 0.0189 |
| IL-8 | 5.143 (1.109-23.842) | 0.0197 |
| IP-10 | 4.693 (1.013-21.733) | 0.0293 |
| Validation cohort 3 - anti-CTLA-4 and anti-PD-1\* |
| SDF-1 | Undefined | 0.0189 |

Abbreviations: CI, confidence interval. \*Five patients in validation cohort 3 were treated with neoadjuvant combination immunotherapy for clinically detectable, resectable stage III disease and as such were not included in the survival analysis.

**Supplementary Table 6: Univariate analysis of cytokine expression and association with RECIST response**

|  |  |  |
| --- | --- | --- |
| **Cytokine** | **Odds ratio (95% CI)** | **Fisher’s exact test****P value** |
| Discovery cohort 1 – anti-PD-1 monotherapy |
| IL-2 | 0.175 (0.031-0.815) | 0.0225 |
| IP-10 | 5.708 (1.227-32.73) | 0.0225 |
| MCP-4 | 0.175 (0.031-0.815) | 0.0225 |
| TARC | 0.175 (0.031-0.815) | 0.0225 |
| ENA78 | 0.175 (0.031-0.815) | 0.0225 |

Abbreviations: CI, confidence interval



**Supplementary Figure 1: Distribution of relative fluorescence intensity units of 65 circulating cytokines in plasma collected from 98 melanoma patients (cohorts 1 and 2) prior to therapy initiation.** Box plots showing the relative fluorescence intensity units (RFU) of each cytokine. Each box represents the interquartile ranges, the central line indicates median, and the whiskers indicate the minimum and maximum values.



**Supplementary Figure 2: Hierarchical clustering of cytokine expression profiles in cohorts 1 and 2.**Unsupervised hierarchical clustering of circulating cytokines (Euclidean distance with average linkage of relative fluorescence units) derived from immune therapy treated patients at four timepoints. PRE, baseline plasma taken 0-29 days before treatment initiation; EDT, early during treatment plasma taken at week 1-6 after therapy initiation; MID, mid timepoint plasma taken at week 7-11 after therapy; LATE, late timepoint plasma taken at week 12-18 after therapy. Patients treated with PD-1 monotherapy have the prefix M and those treated with combination therapy are labelled with the prefix C.