**Table S1.** Comparison of the median percentage of PD-1+ cells in T cell subsets of the subjects that were not exposed to nivolumab for at least 200 days before enrollment on the trial, Cycle 1, Day 1.

| Immune markers | Responder median (n=7) | Nonresponder median (n=3) | Wilcoxon p-value | T-test p-value |
|---|---|---|---|---|
| % PD1 in CD3 | 23.24 | 19.38 | 0.49412 | 0.63 |
| % PD-1 in CD4 | 22.26 | 18.34 | 1 | 0.68 |
| % PD-1 in CD8 | 30.354 | 22.913 | 0.49412 | 0.55 |
| % PD-1 in CD4+/CD8+ | 39.4 | 32.09 | 0.81971 | 0.84 |
| %PD-1 in Perf+/GrB+/CD4+ | 38.41 | 37.5 | 1 | 0.69 |
| % PD-1 in Perf+/GrB-/CD4+ | 46.711 | 27.27 | 0.17146 | 0.26 |
| % PD-1 in Perf+/GrB-/CD8+ | 39.479 | 30 | 0.36193 | 0.29 |
| % PD-1 in Perf+/Grb+/CD8+ | 40.706 | 32.56 | 0.36193 | 0.38 |
| % PD-1 in CD4+/HLA-DR+ | 53.34 | 26.67 | 0.36193 | 0.18 |
| % PD-1 in CD8+/HLADR+ | 35.69 | 36.39 | 1 | 0.7 |
| % PD-1 in CD28+/CD4+ | 23.024 | 18.85 | 0.81971 | 0.65 |
| % PD-1 in CD28+/CD8+ | 19.712 | 17.72 | 0.49412 | 0.87 |
| % PD-1 in CD25+/CD4+ | 27.521 | 17.56 | 0.49412 | 0.57 |
| % PD-1 in CD25+/CD8+ | 19.601 | 15.84 | 0.6485 | 0.57 |
| % PD-1 in CD4+/FoxP3+ | 32.325 | 29.962 | 0.81971 | 0.68 |
| % PD-1 in CD8+/FoxP3+ | 45.455 | 25 | 0.49412 | 0.46 |
| % PD-1 in CD4+/Tbet+ | 39.631 | 28.57 | 0.49281 | 0.7 |
| % PD-1 in CD8+/Tbet+ | 36.684 | 33.93 | 0.49412 | 0.49 |
| % PD-1 in CD4+/GATA3+ | 38.89 | 43.93 | 0.65 | 0.55 |
| % PD-1 in CD8+/GATA3+ | 28.767 | 40.26 | 0.36193 | 0.27 |
| % PD-1 in CD4+/Fas+ | 35.881 | 25.81 | 0.81971 | 0.66 |
| % PD-1 in CD8+/Fas+ | 34.003 | 36.45 | 1 | 0.76 |
| % PD-1 in NKG2D+/CD4+ | 45.61 | 53.623 | 0.49412 | 0.28 |
| % PD-1 in NKG2D+/CD8+ | 38.673 | 29.6 | 0.49412 | 0.38 |
| % PD-1 in CD45RA+/CD4+ | 10.227 | 13.71 | 0.73166 | 0.44 |
| % PD-1 in CD45RA+/CD8+ | 19.53 | 10.98 | 0.49412 | 0.53 |
| % PD-1 in CD45RO+/CD4+ | 35.728 | 20.18 | 0.6485 | 0.43 |
| % PD-1 in CD45RO+/CD8+ | 34.791 | 35.97 | 1 | 0.54 |
| % PD-1 in CD62L+/CD4+ | 19.718 | 19.46 | 0.81971 | 0.9 |
| % PD-1 in CD62L+/CD8+ | 21.839 | 20.6 | 0.6485 | 0.69 |
| % PD-1 in PD-L1+/CD4+ | 31.974 | 19.05 | 0.81971 | 0.7 |
| % PD-1 in PD-L1+/CD8+ | 37.412 | 24.24 | 0.49412 | 0.31 |
| % PD-1 in LAG3+/CD4+ | 40.738 | 25.89 | 1 | 0.89 |
| % PD-1 in LAG3+/CD8+ | 43.966 | 33.33 | 0.49412 | 0.75 |
| % PD-1 in CTLA4+/CD4+ | 40.367 | 50 | 0.81971 | 0.83 |
| % PD-1 in CTLA4+/CD8+ | 43.651 | 12.7 | 0.36193 | 0.38 |
| % PD-1 in CD57+/CD4+ | 45.455 | 74.157 | 0.49412 | 0.25 |
| % PD-1 in CD57+/CD8+ | 44.702 | 24.26 | 0.36193 | 0.4 |
| % PD-1 in Tim3+/CD4+ | 44.186 | 52.63 | 0.25445 | 0.38 |

| | | | | |
|---|---|---|---|---|
| % PD-1 in Tim3+/CD8+ | 42.739 | 15.79 | 0.17146 | 0.17 |
| % PD-1 in Perf Hi/Granzyme B+/CD4+ | 53.571 | 33.333 | 1 | 0.91 |
| % PD-1 in Perf Hi/Granzyme B+/CD8+ | 41.387 | 39.604 | 0.49412 | 0.45 |

**Table S2.** Comparison of the median percentages of cells expressing the designated immune markers among CD8+/PD-1+ T cells in the subjects that were not exposed to nivolumab for at least 200 days before enrollment on the trial, Cycle 1, Day 1.

| Immune marker | Responder median (n=7) | Nonresponder median (n=3) | Wilcoxon p-value | T-test p-value |
|---|---|---|---|---|
| FoxP3+ | 12.1 | 7.69 | 0.25445 | 0.3 |
| Tbet+ | 15.962 | 7.22 | 0.36193 | 0.32 |
| GATA3+ | 1.48 | 1.03 | 1 | 0.94 |
| Perf+/GrB+ | 17.91 | 29.87 | 0.56764 | 0.58 |
| Perf+ Hi/GrB+ | 1.097 | 0.323 | 0.81752 | 0.81 |
| Perf+/GrB- | 5.105 | 3.09 | 0.6485 | 0.47 |
| HLA-DR+ | 28.36 | 32.99 | 1 | 0.94 |
| CD28+ | 94.72 | 92.78 | 0.6485 | 0.72 |
| CD25+ | 43.279 | 51.13 | 0.17146 | 0.14 |
| CD95+/Fas+ | 96.244 | 78.96 | 1 | 0.79 |
| NKG2D+ | 3.767 | 1.6 | 0.25445 | 0.69 |
| CD45RA+ | 7.069 | 2.606 | 0.49412 | 0.58 |
| CD45RO+ | 96.358 | 97.988 | 0.6485 | 0.9 |
| CD62L+ | 51.6 | 38.363 | 0.49412 | 0.72 |
| PD-L1+ | 5.778 | 2.378 | 0.49412 | 0.54 |
| PD-L2+ | 6.52 | 10.105 | 0.17146 | 0.29 |
| LAG3+ | 0.288 | 2.26 | 0.6485 | 0.97 |
| CTLA4+ | 0.274 | 1.13 | 0.06825 | 0.09 |
| CD57+ | 3.727 | 2.972 | 1 | 0.55 |
| Tim-3+ | 2.122 | 2.04 | 1 | 0.61 |

**Table S3.** Comparison of the median percentages of cells expressing the designated immune markers among CD4+/PD-1+ T cells in the subjects that were not exposed to nivolumab for at least 200 days before enrollment on the trial, Cycle 1, Day 1.

| Immune marker | Responder median (n=7) | Nonresponder median (n=3) | Wilcoxon p-value | T-test p-value |
|---|---|---|---|---|
| FoxP3+ | 12.1 | 7.69 | 0.25445 | 0.3 |
| Tbet+ | 15.962 | 7.22 | 0.36193 | 0.32 |
| GATA3+ | 1.48 | 1.03 | 1 | 0.94 |
| Perf+ GrB+ | 17.91 | 29.87 | 0.56764 | 0.58 |
| Perf+ HI GrB+ | 1.097 | 0.323 | 0.81752 | 0.81 |
| Perf+ GrB- | 5.105 | 3.09 | 0.6485 | 0.47 |
| HLA-DR+ | 28.36 | 32.99 | 1 | 0.94 |
| CD28+ | 94.72 | 92.78 | 0.6485 | 0.72 |
| CD25+ | 43.279 | 51.13 | 0.17146 | 0.14 |
| CD95 Fas | 96.244 | 78.96 | 1 | 0.79 |
| NKG2D | 3.767 | 1.6 | 0.25445 | 0.69 |
| CD45RA | 7.069 | 2.606 | 0.49412 | 0.58 |
| CD45RO | 96.358 | 97.988 | 0.6485 | 0.9 |
| CD62L | 51.6 | 38.363 | 0.49412 | 0.72 |
| PD-L1 | 5.778 | 2.378 | 0.49412 | 0.54 |
| PD-L2 | 6.52 | 10.105 | 0.17146 | 0.29 |
| LAG3 | 0.288 | 2.26 | 0.6485 | 0.97 |
| CTLA4 | 0.274 | 1.13 | 0.06825 | 0.09 |
| CD57 | 3.727 | 2.972 | 1 | 0.55 |
| Tim-3 | 2.122 | 2.04 | 1 | 0.61 |

**Table S4.** Comparison of the median percentages of T cells expressing the designated immune markers between all responders and all non-responders, Cycle 1, Day 1.

| Immune marker | Responder median (n=9) | Nonresponder median (n=5) | Wilcoxon p-value | T-test p-value |
|---|---|---|---|---|
| FoxP3+/CD4+ | 3.124 | 1.776 | 0.0455 | 0.17 |
| Tbet+/CD4+ | 3.33 | 2.073 | 0.50499 | 0.44 |
| GATA3+/CD4+ | 0.494 | 0.074 | 0.1096 | 0.27 |
| Perf+/GrB+/CD4+ | 4.741 | 1.049 | 0.50499 | 0.27 |
| Perf+ HI/GrB+/CD4+ | 0.188 | 0.0323 | 0.28077 | 0.15 |
| Perf+/GrB-/CD4+ | 1.111 | 0.814 | 0.42371 | 0.41 |
| HLA-DR+/CD4+ | 4.572 | 7.934 | 0.14247 | 0.15 |
| CD28+/CD4+ | 21.746 | 37.327 | 0.28612 | 0.47 |
| CD25+/CD4+ | 12.778 | 26.971 | 0.35065 | 0.31 |
| CD95+/CD4+ | 15.958 | 20.057 | 0.23014 | 0.31 |
| NKG2D+/CD4+ | 1.55 | 0.974 | 0.5938 | 0.52 |
| CD45RA+/CD4+ | 5.774 | 0.817 | 0.14247 | 0.15 |
| CD45RO+/CD4+ | 17.619 | 41.877 | 0.23014 | 0.3 |
| CD62L+/CD4+ | 7.111 | 8.542 | 1 | 0.73 |
| PD-L1+/CD4+ | 1.885 | 1.42 | 0.78973 | 0.34 |
| PD-L2+/CD4+ | 1.03 | 2.414 | 0.35065 | 0.17 |
| LAG3+/CD4+ | 0.125 | 0.212 | 0.68883 | 0.68 |
| CTLA4+/CD4+ | 0.233 | 0.378 | 0.23014 | 0.18 |
| CD57+/CD4+ | 0.749 | 0.516 | 0.42371 | 0.38 |
| Tim-3+/CD4+ | 2.308 | 0.72 | 0.02341 | 0.055 |
| FoxP3+/CD8+ | 3.492 | 4.957 | 0.50499 | 0.26 |
| Tbet+/CD8+ | 11.291 | 3.466 | 0.28612 | 0.25 |
| GATA3+/CD8+ | 1.579 | 0.465 | 0.5938 | 0.53 |
| Perf+/GrB+/CD8+ | 20.961 | 18.595 | 0.78973 | 0.8 |
| Perf+ HI/GrB+/CD8+ | 6.977 | 6.603 | 0.78973 | 0.69 |
| Perf+/GrB-/CD8+ | 14.808 | 5.209 | 0.35065 | 0.38 |
| HLA-DR+/CD8+ | 8.693 | 7.7 | 0.89393 | 0.99 |
| CD28+/CD8+ | 16.032 | 11.975 | 0.78973 | 0.44 |
| CD25+/CD8+ | 9.302 | 6.725 | 0.78973 | 0.44 |
| CD95+/CD8+ | 29.133 | 17.765 | 0.50499 | 0.33 |
| NKG2D+/CD8+ | 9.302 | 5.329 | 0.5938 | 0.94 |
| CD45RA+/CD8+ | 16.849 | 12.876 | 0.18242 | 0.2 |
| CD45RO+/CD8+ | 18.413 | 24.056 | 0.06195 | 0.07 |
| CD62L+/CD8+ | 18.605 | 15.909 | 0.18242 | 0.14 |
| PD-L1+/CD8+ | 1.684 | 0.446 | 0.06195 | 0.15 |
| PD-L2+/CD8+ | 1.056 | 1.102 | 0.89393 | 0.61 |
| LAG3+/CD8+ | 0.448 | 0.243 | 1 | 0.56 |
| CTLA4+/CD8+ | 0.68 | 1.149 | 0.50499 | 0.51 |

| | | | | |
|---|---|---|---|---|
| CD57+/CD8+ | 6.732 | 6.816 | 0.68916 | 0.74 |
| Tim-3+/CD8+ | 2.326 | 1.406 | 0.08304 | 0.19 |

**Table S5.** Comparison of the median percentages of T cells expressing the designated immune markers between all responders and all non-responders, Cycle 4, Day 1.

| Immune marker | Responder median (n=8) | Nonresponder median (n=4) | Wilcoxon p-value | T-test p-value |
|---|---|---|---|---|
| FoxP3+/CD4+ | 3.384 | 3.11 | 0.93232 | 0.65 |
| Tbet+/CD4+ | 3.4745 | 1.755 | 0.4447 | 0.2 |
| GATA3+/CD4+ | 0.296 | 0.225 | 0.73365 | 0.4 |
| Perf+/GrB+/CD4+ | 5.8775 | 3.2455 | 0.26961 | 0.21 |
| Perf+ HI/GrB+ CD4+ | 2.6165 | 0.0709 | 0.0508 | 0.12 |
| Perf+/GrB-/CD4+ | 0.64 | 0.729 | 0.67113 | 0.17 |
| HLA-DR+/CD4+ | 4.772 | 9.445 | 0.10664 | 0.051 |
| CD28+/CD4+ | 14.0645 | 40.119 | 0.35024 | 0.4 |
| CD25+/CD4+ | 8.2555 | 22.2365 | 0.26961 | 0.48 |
| CD95+/CD4+ | 12.95 | 24.461 | 0.35024 | 0.37 |
| NKG2D+/CD4+ | 2.054 | 0.872 | 0.26961 | 0.18 |
| CD45RA+/CD4+ | 7.8805 | 4.764 | 0.20273 | 0.21 |
| CD45RO+/CD4+ | 11.7965 | 41.9895 | 0.26961 | 0.2 |
| CD62L+/CD4+ | 8.6185 | 12.1615 | 0.67113 | 0.77 |
| PD-L1+/CD4+ | 1.5115 | 1.2265 | 0.93232 | 0.87 |
| PD-L2+/CD4+ | 1.7025 | 3.0295 | 0.10664 | 0.07 |
| LAG3+/CD4+ | 0.126 | 0.2491 | 0.79752 | 0.56 |
| CTLA4+/CD4+ | 0.27 | 0.367 | 0.67113 | 0.55 |
| CD57+/CD4+ | 0.963 | 0.7 | 0.26961 | 0.34 |
| Tim-3+/CD4+ | 1.329 | 0.7605 | 0.26961 | 0.2 |
| FoxP3+/CD8+ | 4.075 | 4.6645 | 0.79891 | 0.22 |
| Tbet+/CD8+ | 28.649 | 3.068 | 0.14884 | 0.08 |
| GATA3+/CD8+ | 0.9285 | 0.6905 | 0.4447 | 0.36 |
| Perf+/GrB+/CD8+ | 29.4905 | 23.538 | 0.67113 | 0.7 |
| Perf+ HI/GrB+/CD8+ | 21.912 | 10.2415 | 0.20273 | 0.31 |
| Perf+/GrB-/CD8+ | 10.306 | 9.6285 | 0.93232 | 0.6 |
| HLA-DR+/CD8+ | 9.082 | 11.4115 | 0.55221 | 0.41 |
| CD28+/CD8+ | 17.696 | 15.6425 | 0.79891 | 0.55 |
| CD25+/CD8+ | 6.5735 | 10.9625 | 0.4447 | 0.23 |
| CD95+/CD8+ | 26.8285 | 26.9235 | 0.79891 | 0.67 |
| NKG2D+/CD8+ | 15.669 | 9.384 | 0.93232 | 0.83 |
| CD45RA+/CD8+ | 20.1525 | 11.568 | 0.26961 | 0.19 |
| CD45RO+/CD8+ | 17.424 | 26.5885 | 0.02186 | 0.03 |
| CD62L+/CD8+ | 25.059 | 18.1145 | 0.4447 | 0.52 |
| PD-L1+/CD8+ | 1.479 | 0.835 | 0.93232 | 0.33 |
| PD-L2+/CD8+ | 1.2 | 1.4485 | 0.55221 | 0.71 |
| LAG3+/CD8+ | 0.293 | 0.637 | 0.10664 | 0.06 |
| CTLA4+/CD8+ | 0.5805 | 1.3945 | 0.4447 | 0.14 |
| CD57+/CD8+ | 8.5715 | 9.394 | 1 | 0.9 |

| | | | | |
|---|---|---|---|---|
| Tim-3+/CD8+ | 4.285 | 3.1505 | 0.55221 | 0.41 |

**Table S6.** Comparison of the median percentages of T cells expressing the designated immune markers between all responders and all non-responders, Cycle 4, Day 1.

| Immune marker | Responder median (n=3) | Nonresponder median (n=1) |
|---|---|---|
| FoxP3+/CD4+ | 2.182 | 3.256 |
| Tbet+/CD4+ | 0.919 | 1.598 |
| GATA3+/CD4+ | 0.364 | 0.109 |
| Perf+/GrB+/CD4+ | 0.727 | 0.287 |
| Perf+ HI/GrB+/CD4+ | 0.0249 | 0.029 |
| Perf+/GrB-/CD4+ | 0.647 | 1.125 |
| HLA-DR+/CD4+ | 3.05 | 4.506 |
| CD28+/CD4+ | 13.893 | 31.723 |
| CD25+/CD4+ | 8.389 | 19.805 |
| CD95+/CD4+ | 13.064 | 29.543 |
| NKG2D+/CD4+ | 2.545 | 0.35 |
| CD45RA+/CD4+ | 10.146 | 15.214 |
| CD45RO+/CD4+ | 12.997 | 30.488 |
| CD62L+/CD4+ | 8.123 | 18.586 |
| PD-L1+/CD4+ | 0.368 | 0.339 |
| PD-L2+ CD4+ | 1.257 | 0.876 |
| LAG3+/CD4+ | 0 | 0.178 |
| CTLA4+/CD4+ | 0.0332 | 0.441 |
| CD57+/CD4+ | 0.239 | 0.223 |
| Tim-3+/CD4+ | 0.488 | 0.501 |
| FoxP3+/CD8+ | 1.558 | 3.091 |
| Tbet+/CD8+ | 14.545 | 1.578 |
| GATA3+/CD8+ | 1.091 | 0.194 |
| Perf+/GrB+/CD8+ | 9.818 | 1.433 |
| Perf+ HI/GrB+/CD8+ | 6.182 | 0.201 |
| Perf+/GrB-/CD8+ | 24.35 | 16.455 |
| HLA-DR+/CD8+ | 5.902 | 10.931 |
| CD28+/CD8+ | 11.353 | 11.66 |
| CD25+/CD8+ | 2.182 | 3.236 |
| CD95+/CD8+ | 9.326 | 12.611 |
| NKG2D+/CD8+ | 9.9 | 8.081 |
| CD45RA+/CD8+ | 9.383 | 14.046 |
| CD45RO+/CD8+ | 12.378 | 21.454 |
| CD62L+/CD8+ | 16.86 | 15.76 |
| PD-L1+/CD8+ | 0.147 | 0.152 |
| PD-L2+/CD8+ | 0.177 | 0.457 |
| LAG3+/CD8+ | 0.192 | 0.131 |
| CTLA4+/CD8+ | 0.365 | 0.421 |

| | | |
|---|---|---|
| CD57+/CD8+ | 5.338 | 3.485 |
| Tim-3+/CD8+ | 4 | 1.219 |

**Table S7.** Antibodies used for mass cytometry.

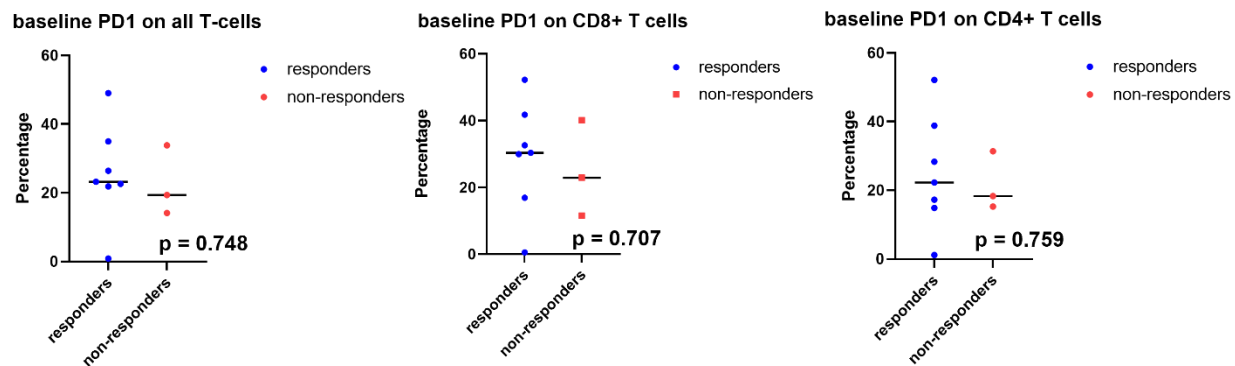| Metal | Mass | Target | Clone | Vendor |
|---|---|---|---|---|
| Y | 89 | CD235 | HIR2 | Biolegend |
| In | 113 | CD3 | UCHT1 | Biolegend |
| In | 115 | CD45 | HI30 | Biolegend |
| La | 139 | Granzyme B | QA16A02 | Biolegend |
| Ce | 140 | cPARP | F21-852 | BD Biosciences |
| Nd | 142 | CD7 | CD7-6B7 | Biolegend |
| Nd | 143 | T-Bet | eBio4B10 | eBioscience |
| Nd | 144 | CD95 (Fas) | DX2 | Biolegend |
| Nd | 145 | CD4 | RPA-T4 | Biolegend |
| Nd | 146 | CD8a | RPA-T8 | Biolegend |
| Sm | 147 | CD56 | HCD56 | Biolegend |
| Nd | 148 | CD62L | DREG-56 | Biolegend |
| Sm | 149 | CD127 | A019D5 | Fluidigm |
| Nd | 150 | CD45RO | UCHL1 | Biolegend |
| Eu | 151 | CD14 | M5E2 | Fluidigm |
| Sm | 152 | CD33 | WM53 | Biolegend |
| Eu | 153 | HLA-DR | L243 | Biolegend |
| Sm | 154 | TIM-3 | F382E2 | Biolegend |
| Gd | 155 | CD279 (PD-1) | EH12.2H7 | Biolegend |
| Gd | 157 | CD161 | HP-3G10 | Biolegend |
| Gd | 158 | CD45RA | HI100 | Biolegend |
| Tb | 159 | GATA3 | 16E10A23 | Biolegend |
| Gd | 160 | CD28 | CD28.2 | Fluidigm |
| Dy | 161 | CD16 | 3G8 | Biolegend |
| Dy | 162 | Foxp3 | PCH101 | Fluidigm |
| Dy | 163 | Perforin | B-D48 | Abcam |
| Dy | 164 | CD15 | W6D3 | Biolegend |
| Er | 166 | NKG2D | 1D11 | Biolegend |
| Tm | 169 | CD25 | 2A3 | Fluidigm |
| Er | 170 | CTLA-4 | 14D3 | Fluidigm |
| Yb | 171 | CD226 | DX11 | Fluidigm |
| Yb | 172 | CD273 (PD-L2) | 24F.10C12 | Fluidigm |
| Yb | 173 | CD19 | HIB19 | Biolegend |
| Yb | 174 | CD57 | HNK-1 | Biolegend |
| Lu | 175 | CD274 (PD-L1) | 29E.2A3 | Biolegend |
| Yb | 176 | LAG3 | 1009614 | R&D Systems |
| Bi | 209 | CD11b | ICRF44 | Biolegend |

**Figure S1.** Quantification of the average PD1 positive cells between responders (n=7) and non-responders (n=3) at baseline in all T-cells (left), CD8+ T-cells (middle) and CD4+ T-cells (right). Each dot represents an individual patient.
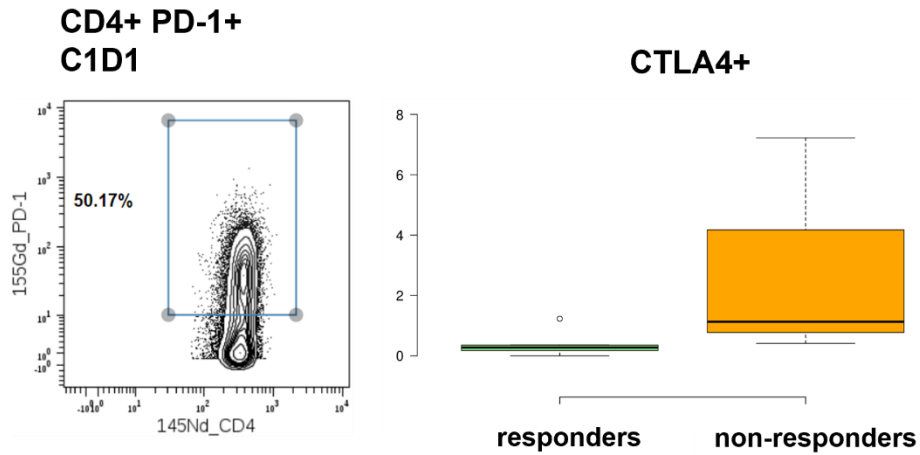
**Figure S2.** Representative percentage of CD4+/PD-1+ cells on C1D1 (**left**) and analysis of CTLA-4 expression in CD4+/PD-1+ T cells (**right**) showed a trend towards higher percentage of CTLA4+ in non-responders. p-value 0.068 (Wilcoxon) and 0.09 (t-test). Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots. n = 7 (responders) and 3 (non-responders).
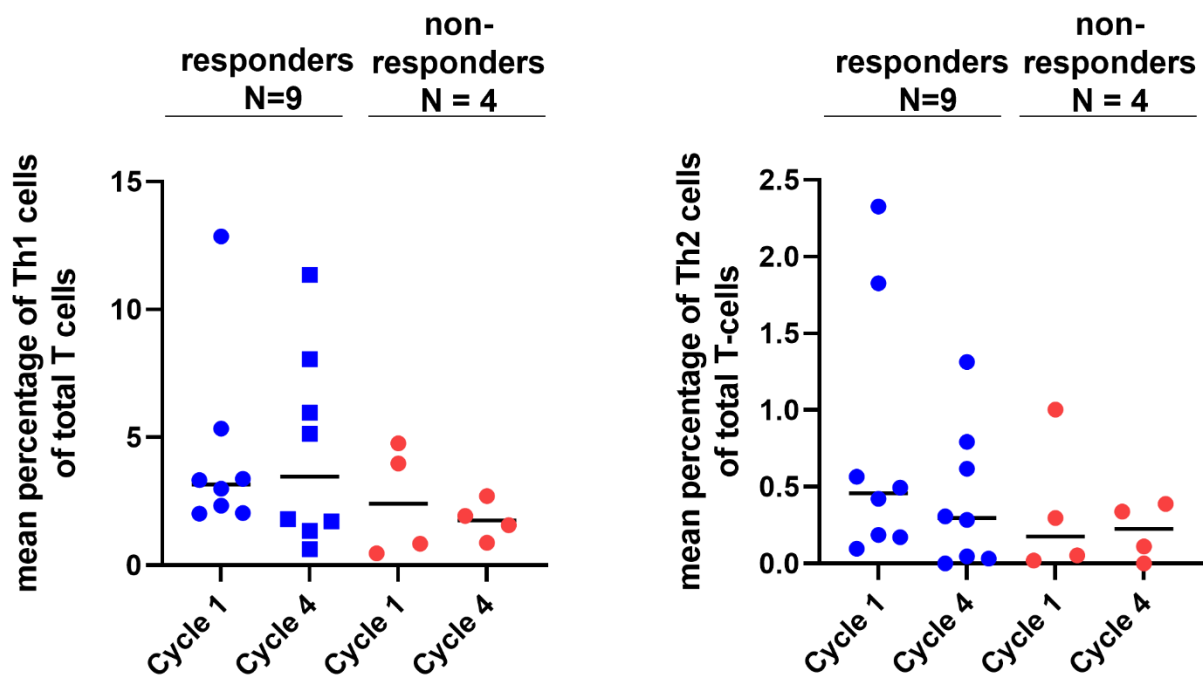
**Figure S3.** Quantification of the average Th1 (left, CD4+Tbet+) and Th2 (CD4+GATA3+) cells at the indicated time points in responders (blue, n=9) and non-responders (red, n=4). Each dot represents an individual patient.