

Supplementary Table 1-5

Multifunctional T Cell Analyses to Study Response and Progression in Adoptive Cell Transfer Immunotherapy

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Supplementary Table 1. Antibody panel.

| Antibody | Manufacturer |
|----------------|--------------|
| IFN- γ | R&D |
| IL-10 | R&D |
| TNF- β | R&D |
| IL-4 | eBioscience |
| IL-5 | R&D |
| IL-1 β | eBioscience |
| TGF- β 1 | R&D |
| GM-CSF | eBioscience |
| TNF- α | eBioscience |
| IL-17A | eBioscience |
| Eotaxin | R&D |
| Granzyme B | R&D |
| IL-13 | R&D |
| IL-22 | R&D |
| MIP-1 α | R&D |
| IL-2 | R&D |
| MIP-1 β | R&D |
| IL-6 | R&D |
| TGF- β 2 | R&D |

Supplementary Table 2. Single cell cytokine panel and groupings of the functional protein identified by clustering.

| | CD8+ MART-1+ | CD8+ MART-1-negative | CD4+ MART-1-negative | CD4+ MART-1+ |
|--------------------|--------------|----------------------|----------------------|--------------|
| Proliferative | IL-2 | IL-2 | IL-2 | IL-2 |
| | GM-CSF | GM-CSF | GM-CSF | GM-CSF |
| | | IL-17A | TNF-a | IL-17A |
| | | | IL-4 | TNF-a |
| Th2 | IL-4 | | | IL-4 |
| | IL-5 | | | IL-5 |
| Regulatory | IL-10 | IL-10 | IL-10 | IL-10 |
| | TGF-b2 | TGF-b2 | TGF-b2 | TGF-b2 |
| | IL-17A | IL-22 | | |
| Inflammatory | IL-6 | IL-6 | IL-6 | IL-6 |
| | IL-1b | IL-1b | IL-1b | IL-1b |
| | IL-22 | CCL-11 | IL-17A | IL-22 |
| | | IL-5 | IL-5 | |
| Non-specific | IL-13 | IL-13 | IL-13 | IL-13 |
| | TGF-b1 | TGF-b1 | TGF-b1 | TGF-b1 |
| | TNF-b | TNF-b | TNF-b | TNF-b |
| | CCL-11 | | CCL-11 | CCL-11 |
| | | | | Granzyme B |
| Antitumor Effector | IFN-g | IFN-g | IFN-g | IFN-g |
| | MIP-1a | MIP-1a | MIP-1a | MIP-1a |
| | MIP-1b | MIP-1b | MIP-1b | MIP-1b |
| | Granzyme B | Granzyme B | Granzyme B | |
| | TNF-a | TNF-a | | |
| | | IL-4 | IL-22 | |

Supplementary Table 3. Melanoma antigen specific T cell populations studied.

| | | |
|---------------------------|------------------------|----------------------|
| Tyrosinase ₁ | NY-Eso1 | MELOE-1 |
| Tyrosinase ₃₆₈ | MAGE-A1 | PRAME ₁₀₀ |
| MART-1 ₂₆ | MAGE-C2 ₁₉₁ | PRAME ₃₀₀ |
| MART-1 ₃₂ | MAGE-C2 ₃₃₆ | MMP-2 |
| gp100 ₁₅₄ | MAGE-3 | Adipophilin |
| gp100 ₁₇₇ | MAGE-A4 | Survivin |
| gp100 ₂₀₉ | MAGE-A10 | |
| gp100 ₂₈₀ | CAMEL | |
| gp100 ₄₅₇ | HERV | |
| gp100 ₄₇₆ | SSX-2 | |
| gp100 ₆₁₉ | TAG-1 | |
| gp100 ₆₃₉ | NA-17 | |
| TRP-2 ₁₈₀ | CDK-4 | |
| TRP-2 ₃₆₀ | Prdx-5 | |
| RAB38 | | |

Supplementary Table 4. Functional differences of polyfunctional T cells and the rest T cells.

In mean fluorescence intensity

| | IL2 | IL17A | IFNg | TNFa | IL13 | TNFb | CCL11 | GB | TGFb1 | IL4 | IL5 | IL6 | IL10 | GMCSF | TGFb2 | MIP1a | MIP1b | IL22 | IL1b | Mean | Median |
|-----------------------|----------|-----------|-----------|-----------|----------|----------|----------|----------|----------|-----------|----------|-----------|-----------|------------|-----------|----------|----------|----------|-----------|-------------|------------|
| MFI of polyfunctional | 299 | 434 | 5220 | 6096 | 222 | 236 | 1546 | 4104 | 105 | 222 | 284 | 2742 | 189 | 1472 | 424 | 10922 | 9723 | 324 | 478 | 2371 | 434 |
| MFI of the rest | 36 | 39 | 152 | 227 | 35 | 47 | 195 | 789 | 36 | 23 | 68 | 38 | 15 | 7 | 36 | 1330 | 1358 | 63 | 48 | 239 | 47 |
| Ratio | 8 | 11 | 34 | 27 | 6 | 5 | 8 | 5 | 3 | 10 | 4 | 71 | 12 | 211 | 12 | 8 | 7 | 5 | 10 | 10 | 9 |

In mean molecular number

| | IL2 | IL17A | IFNg | TNFa | IL13 | TNFb | CCL11 | GB | TGFb1 | IL4 | IL5 | IL6 | IL10 | GMCSF | TGFb2 | MIP1a | MIP1b | IL22 | IL1b | Mean | Median |
|--|-----------|------------|-------------|------------|-----------|-----------|-----------|-----------|-----------|------------|-----------|-------------|------------|--------------|------------|-----------|-----------|-----------|------------|----------------|----------------|
| Mean molecule number of polyfunctional | 2.3E+05 | 4.8E+05 | 6.9E+07 | 9.4E+07 | 1.2E+05 | 1.4E+05 | 6.0E+06 | 4.3E+07 | 2.8E+04 | 1.2E+05 | 2.0E+05 | 1.9E+07 | 9.0E+04 | 5.5E+06 | 4.5E+05 | 3.0E+08 | 2.4E+08 | 2.7E+05 | 5.8E+05 | 4.1E+07 | 4.8E+05 |
| Mean molecule number of the rest | 2.4E+03 | 2.8E+03 | 4.3E+04 | 9.6E+04 | 2.2E+03 | 4.0E+03 | 7.1E+04 | 1.2E+06 | 2.4E+03 | 9.4E+02 | 8.5E+03 | 2.8E+03 | 4.3E+02 | 9.0E+01 | 2.4E+03 | 3.3E+06 | 3.4E+06 | 7.5E+03 | 4.2E+03 | 4.3E+05 | 4.0E+03 |
| Ratio | 93 | 168 | 1599 | 978 | 55 | 35 | 85 | 37 | 11 | 132 | 24 | 6909 | 209 | 60546 | 192 | 92 | 70 | 36 | 137 | 96 | 118 |

Supplementary Table 5. Antibody panel used for flow cytometry immunophenotyping analysis.

| Fluorochrome | Tube a | Tube b | Tube c |
|-------------------|----------------------|--------------------------|-------------------|
| Pacific Blue | CD8 (3B5)* | | |
| FITC | CD45RO (UCHL1)** | CD279 [PD1] (MHH4)** | CD28 (CD28.2)** |
| PE | Mart1 Tetramer*** | | |
| Dump Channel | 7AAD*** | | |
| ECD | HLA-DR (Immu-357)*** | CD45RA (2H4DHILDB9)*** | CD62L (DREG56)*** |
| APC/AlexaFluor647 | CD127 (hIL07R-M21)** | CCR7 (3D12)** | CD44 (G44-26)** |
| AlexaFluor700 | CD3 (UCHT-1)* | | |
| APC-Cy7 | CD25 (M-A251)** | CD195[CCR5] (2D7/CCR5)** | CD27 (M-T271)** |
| PE-Cy7 | CD4 (RPA-T4)\$ | | |

In parenthesis the clone used for detecting the CDs. * Life Science; ** BDbioscience;*** Beckman Coulter; \$ Biolegend.