PNAS
www.pnas.org

Supplementary Information for

**Reversible suppression of T cell function in the bone marrow microenvironment of acute myeloid leukemia.**

Adam J. Lamble, Yoko Kosaka, Ted Laderas, Allie Maffit, Andy Kaempf, Lauren K. Brady, Weiwei Wang, Nicola Long, Jennifer N. Saultz, Motomi Mori, David Soong, Clare V. LeFave, Fei Huang, Homer Adams III, Marc Loriaux, Cristina E. Tognon, Pierrette Lo, Jeffrey W. Tyner, Guang Fan, Shannon K. McWeeney, Brian J. Druker and Evan F. Lind

Email: drukerb@ohsu.edu

**This PDF file includes:**

Supplemental Materials and Methods
Figures S1 to S3
Tables S1 to S4



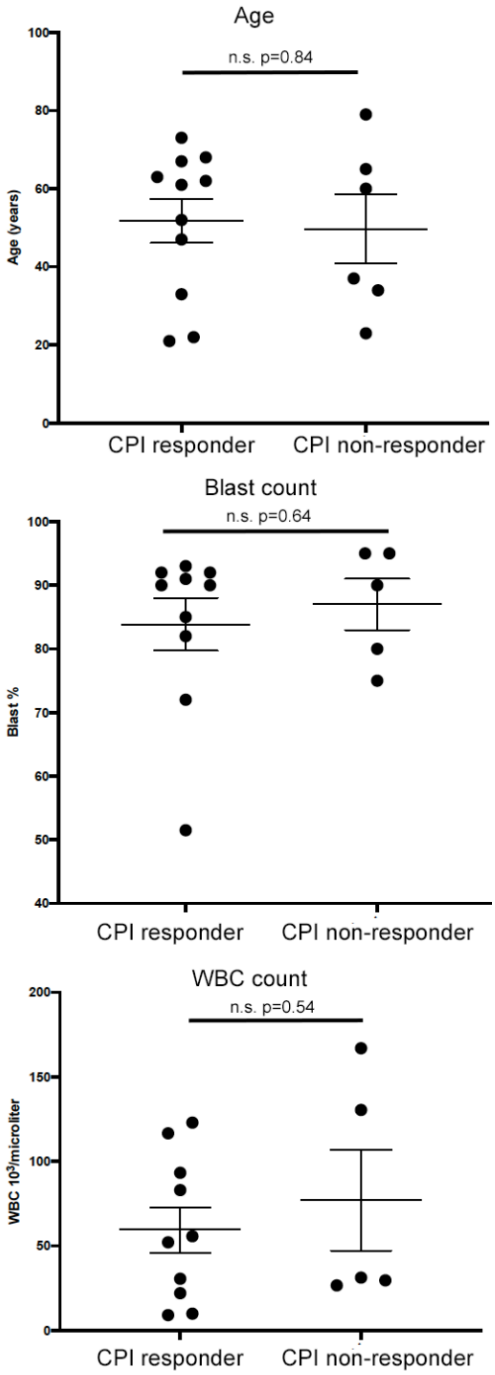
www.pnas.org/cgi/doi/10.1073/pnas.1916206117

**Supplemental Materials and Methods**

*Immunophenotyping by time-of-flight mass cytometry (CyTOF)*

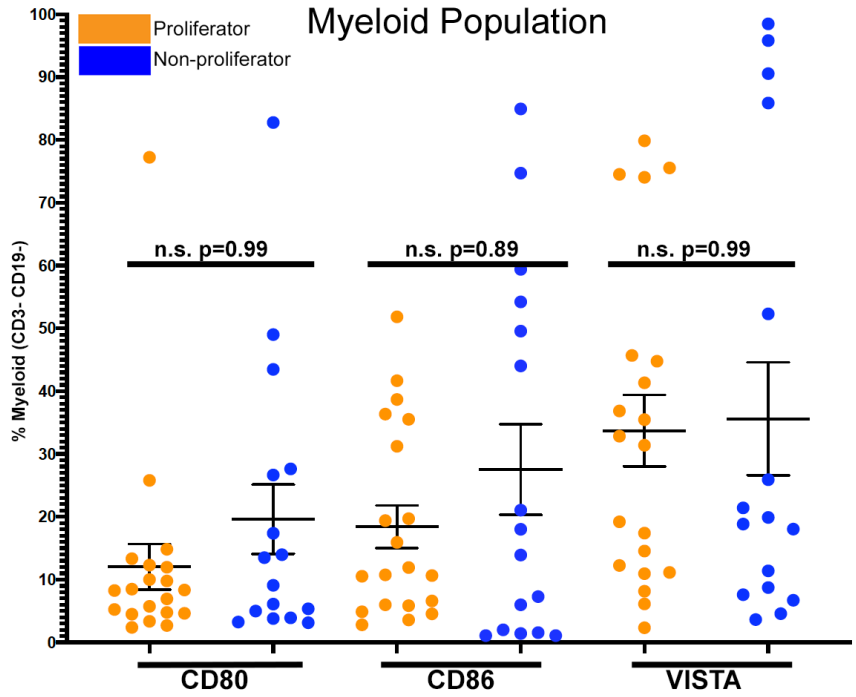
All antibodies and reagents were purchased from Fluidigm unless noted otherwise. Fresh samples were incubated with Cell-ID cisplatin reagent for 5 minutes at 5 μM final concentration in PBS. This was followed by quenching with Maxpar Cell Staining Buffer (CSB). Cells were then resuspended in CSB containing Human TruStain FcX (BioLegend) for 10 minutes followed by addition of the mix of metal-conjugated antibodies specific for surface molecules. After 30 minutes, cells were washed, fixed and permeabilized using Maxpar Fix I buffer for 5 minutes followed by washing and resuspending in Maxpar Perm-S buffer following manufacturer's instructions. Cells were then resuspended in Perm-S buffer containing intracellular antibodies for 30 minutes. Staining was followed by washing 3 times with CSB and resuspension in Maxpar Fix & Perm buffer containing Cell-ID Intercalator-Ir iridium solution. Immediately prior to analysis, samples were washed 3 times with CSB followed by 2 washes with Maxpar water. Samples were mixed with EQ four element calibration beads before acquisition on a CyTOF 1 instrument.

**Fig. S1**. Clinical features according to checkpoint inhibitor (CPI) response group (among non-proliferators).



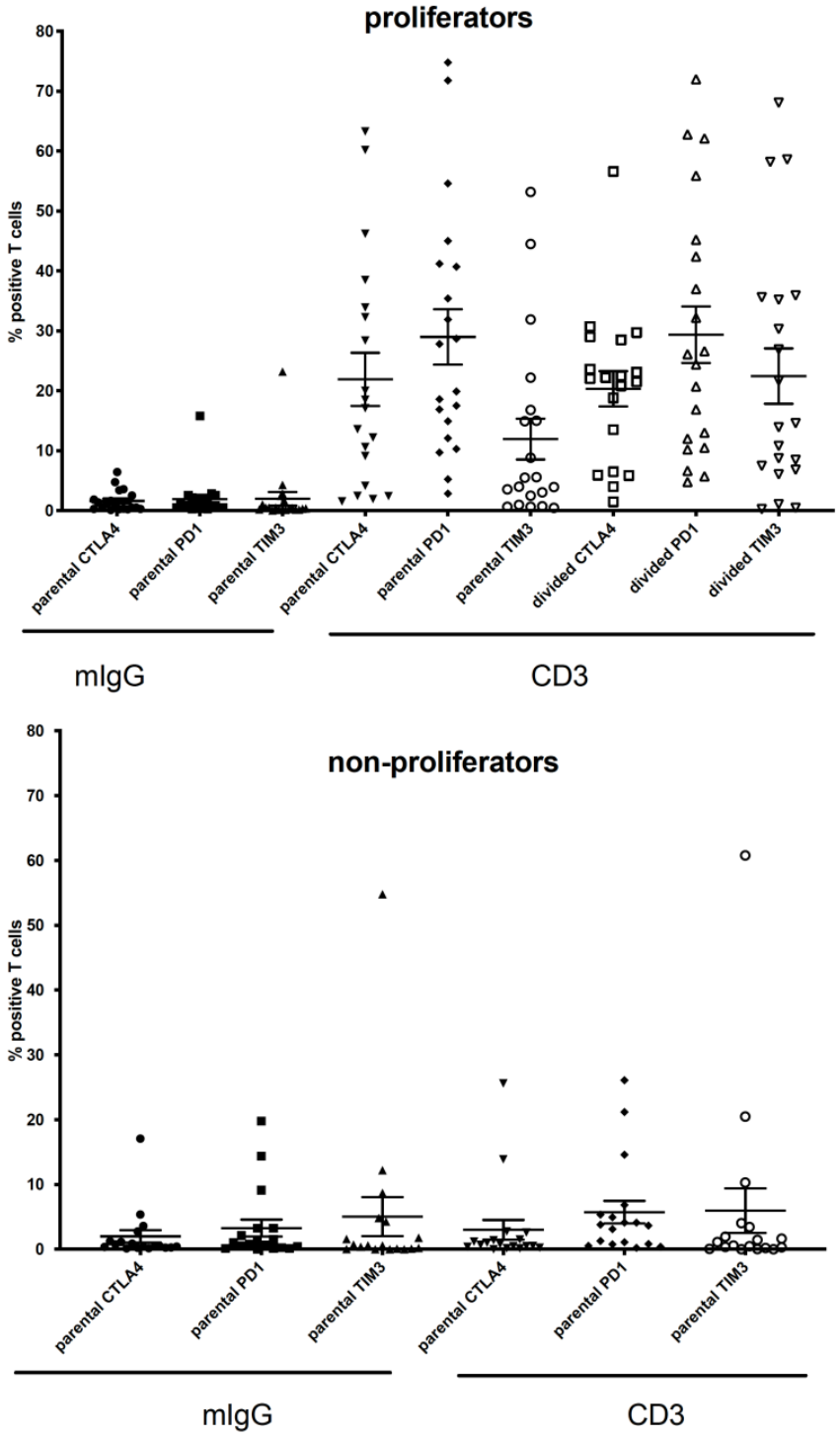

Patient age at sample collection, blast percentage in bone marrow, and white blood cell count in peripheral blood in responders vs. non-responders to CPI. Horizontal bars depict means ± 1 standard error and p-values were computed from equal-variance Student's t-tests.

**Fig. S2.** Expression of costimulatory molecules on myeloid cells.




Comparison of CD80, CD86 and VISTA expression between proliferator (orange) and non-proliferator (blue) groups displayed as % positive on the myeloid population. Bars represent mean +/- standard error of the mean. Statistics are one-way ANOVA with multiple comparisons using Bonferroni adjustment for multiple testing.

**Fig. S3.** Expression of checkpoint markers (CTLA-4, PD-1 and TIM3) on T cells in proliferation assay.




Cell Trace Violet (CTV) loaded samples were incubated in vitro with agonist anti-CD3 or control (mIgG) for 5 days. T cell-gated populations were analyzed for expression of indicated checkpoint molecule on the CTV high (parental) or cells that had undergone at least one round of division (divided).

**Table S1**. AML functional cohort characteristics.

| Patient or sample feature | All patients N = 49 | Proliferators n = 20 | Non-proliferators n = 18 | p-value |
|---|---|---|---|---|
| AML type | *de novo*: 44 (89.8%)<br>2°: 5 (10.2%) | *de novo*: 18 (90.0%)<br>2°: 2 (10.0%) | *de novo*: 17 (94.4%)<br>2°: 1 (5.6%) | 1.0 |
| Age | median: 58 years<br>range: 21 – 81<br>[1 missing] | median: 54 years<br>range: 41 - 76 | median: 60 years<br>range: 21 – 79<br>[1 missing] | 0.385 |
| Gender | female: 28 (57.1%)<br>male: 21 (42.9%) | female: 14 (70.0%)<br>male: 6 (30.0%) | female: 10 (55.6%)<br>male: 8 (44.4%) | 0.503 |
| ELN risk group | favorable: 19 (38.8%)<br>intermed: 16 (32.7%)<br>adverse: 9 (18.4%)<br>NA: 5 (10.2%) | favorable: 9 (45.0%)<br>intermed: 8 (40.0%)<br>adverse: 2 (10.0%)<br>NA: 1 (5.0%) | favorable: 8 (44.4%)<br>intermed: 5 (27.8%)<br>adverse: 3 (16.7%)<br>NA: 2 (11.1%) | 0.733 |
| WBC ($10^3$ / µL) in blood | median: 56.7<br>range: 9.0 – 166.9<br>[7 missing] | median: 68.3<br>range: 24.9 – 137.5<br>[2 missing] | median: 52.1<br>range: 9.0 – 166.9<br>[3 missing] | 0.294 |
| Myeloblast % in bone marrow | median: 82.5<br>range: 18.0 – 97.0<br>[7 missing] | median: 74.4<br>range: 18.0 – 97.0<br>[2 missing] | median: 90.0<br>range: 51.5 – 95.0<br>[3 missing] | 0.050 |
| Normal karyotype | no: 15 (30.6%)<br>yes: 29 (59.2%)<br>NA: 5 (10.2%) | no: 5 (25.0%)<br>yes: 13 (65.0%)<br>NA: 2 (10.0%) | no: 5 (27.8%)<br>yes: 11 (61.1%)<br>NA: 2 (11.1%) | 1.0 |
| FLT3-ITD | no: 35 (71.4%)<br>yes: 13 (26.5%)<br>NA: 1 (2.0%) | no: 13 (65.0%)<br>yes: 7 (35.0%) | no: 14 (77.8%)<br>yes: 3 (16.7%)<br>NA: 1 (5.6%) | 0.288 |
| NPM1 mutant | no: 26 (53.1%)<br>yes: 22 (44.9%)<br>NA: 1 (2.0%) | no: 7 (35.0%)<br>yes: 13 (65.0%) | no: 9 (50.0%)<br>yes: 8 (44.4%)<br>NA: 1 (5.6%) | 0.331 |

Footnotes

- "Proliferators" are patients whose bone marrow samples had >50% T cell proliferation (defined as the anti-CD3+mIgG proliferation % minus the mIgG+mIgG proliferation %, with proliferation requiring at least one division) whereas "Non-proliferators" are patients whose marrow samples had <5% T cell proliferation.
- p-values are from Fisher's exact test (for categorical variables) or Kruskal-Wallis test (for continuous variables)
- among the 5 secondary (2°) AML patients, 3 had prior MDS/MPN and 2 had therapy-related AML after breast cancer

Newly diagnosed AML patient demographic, hematologic, and genomic variables of the functional T cell assay cohort (N=49) and in subgroups of T cell proliferators (n=20) and non-proliferators (n=18).

**Table S2.** Percentage of T cells that divided in the functional assay described in Figures 1 and 3.

| mIgG+mIgG | CD3+mIgG | CD3+PD1 | CD3+CTLA4 | CD3+TIM3 |
|---|---|---|---|---|
| **Proliferators** | | | | |
| 5.2 | 83.2 | 85.4 | 83.0 | 82.4 |
| 5.1 | 96.0 | 97.7 | 96.1 | 95.4 |
| 20.8 | 91.6 | 91.7 | 91.7 | 88.2 |
| 1.5 | 75.1 | 96.6 | 96.3 | 97.5 |
| 23.4 | 93.8 | 96.6 | 97.5 | 98.1 |
| 2.2 | 94.3 | 96.0 | 94.2 | 94.6 |
| 27.0 | 80.2 | 90.7 | 90.8 | 92.2 |
| 0.7 | 84.5 | 87.8 | n.d. | n.d. |
| 0.7 | 63.0 | 89.6 | 83.4 | 91.3 |
| 1.9 | 67.6 | 92.1 | 89.3 | 88.1 |
| 5.5 | 71.9 | 75.2 | 75.2 | 81.9 |
| 0.4 | 82.9 | 84.4 | 84.3 | 85.3 |
| 1.1 | 71.4 | 75.1 | 72.2 | 71.9 |
| 9.5 | 92.2 | 95.1 | 93.3 | 93.3 |
| 1.0 | 89.8 | 93.4 | 93.9 | 92.2 |
| 3.0 | 86.5 | 92.7 | 90.7 | 90.2 |
| 0.5 | 72.6 | 80.1 | 80.1 | 80.4 |
| 1.0 | 65.2 | 85.6 | n.d. | 80.4 |
| 8.8 | 83.2 | 89.7 | 87.9 | 87.3 |
| 0.7 | 56.2 | 96.9 | 98.3 | 96.9 |
| **Non-proliferators** | | | | |
| 0.4 | 0.5 | 6.0 | n.d. | 5.1 |
| 0.2 | 0.4 | 2.6 | 0.4 | 0.3 |
| 0.2 | 0.3 | 22.5 | 5.5 | 4.2 |
| 0.2 | 0.4 | 2.2 | 0.5 | 1.2 |
| 0.2 | 3.8 | 46.9 | 39.5 | 48.8 |
| 0.3 | 2.0 | 15.1 | 8.2 | 15.3 |
| 0.6 | 5.1 | 70.8 | 58.8 | 32.6 |
| 0.1 | 1.7 | 20.1 | 6.1 | 7.2 |
| 0.7 | 1.3 | 6.5 | 3.5 | 2.2 |
| 1.1 | 2.2 | 38.2 | 39.9 | 37.2 |
| 0.7 | 0.7 | 2.3 | 2.5 | 15.1 |
| 0.3 | 0.2 | 1.9 | 0.8 | 1.0 |
| 23.8 | 26.6 | 22.4 | 27.2 | 27.0 |
| 9.3 | 9.6 | 20.1 | n.d. | 36.7 |
| 0.5 | 1.3 | 2.8 | 1.8 | 0.9 |
| 0.4 | 0.8 | 1.0 | 1.1 | 1.0 |
| 0.2 | 1.9 | 4.7 | 3.1 | 3.0 |
| 0.6 | 2.7 | 7.1 | 4.2 | 4.0 |
| **Intermediate Proliferators** | | | | |
| 0.9 | 35.0 | 61.0 | 55.3 | 70.3 |
| 0.5 | 41.2 | 84.8 | 82.3 | 87.4 |
| 2.0 | 8.1 | 25.4 | 11.8 | 17.1 |
| 0.5 | 23.4 | 37.4 | 45.9 | 20.9 |
| 0.3 | 39.8 | 40.3 | 39.4 | 36.8 |
| 1.3 | 49.8 | 60.5 | n.d. | 61.1 |
| 0.8 | 31.7 | 51.8 | 57.0 | 58.0 |
| 0.6 | 19.5 | 30.8 | 35.6 | 38.6 |
| 2.4 | 20.9 | 36.5 | 27.6 | 27.7 |
| 1.2 | 30.1 | 93.9 | 94.7 | 93.9 |
| 1.5 | 33.8 | 78.7 | 85.1 | 79.6 |

%T cell division was assessed by Cell Trace Violet dilution as described in the main text.

- Proliferator: difference in %T cell division between mIg+mIg and CD3+mIg >50% (n=20)
- Non-Proliferator: difference in %T cell division between mIg+mIg and CD3+mIg <5% (n=18)
- Intermediate Proliferator: difference in %T cell division between mIg+mIg and CD3+mIg of 5-49% (n=11)
- Checkpoint responder (blue cells): %T cell division with CPI treatment >5-fold over CD3+mIg (n=12)

**Table S3.** AML clinical cohort characteristics.

| Patient feature or outcome | All patients N = 80 | "low" T-cell % N = 40 | "high" T-cell % N = 40 | p-value |
|---|---|---|---|---|
| AML type | *de novo*: 76 (95.0%)<br>2°: 4 (5.0%) | *de novo*: 38 (95.0%)<br>2°: 2 (5.0%) | *de novo*: 38 (95.0%)<br>2°: 2 (5.0%) | 1.0 |
| Age | median: 61 years<br>range: 18 - 83 | median: 58.5 years<br>range: 25 - 73 | median: 61 years<br>range: 18 - 83 | 0.769 |
| Gender | female: 37 (46.3%)<br>male: 43 (53.8%) | female: 21 (52.5%)<br>male: 19 (47.5%) | female: 16 (40.0%)<br>male: 24 (60.0%) | 0.370 |
| ELN (2017) risk group | favorable: 33 (41.3%)<br>intermed: 20 (25.0%)<br>adverse: 27 (33.8%) | favorable: 13 (32.5%)<br>intermed: 13 (32.5%)<br>adverse: 14 (35.0%) | favorable: 20 (50.0%)<br>intermed: 7 (17.5%)<br>adverse: 13 (32.5%) | 0.185 |
| WBC ($10^3$ / µL) | median: 15.5<br>range: 0.5 – 250.0 | median: 28.5<br>range: 0.5 – 250.0 | median: 8.0<br>range: 1.0 – 198.0 | 0.001 |
| Myeloblast % (of WBC) | median: 56.0<br>range: 6.7 – 95.6 | median: 56.3<br>range: 14.3 – 95.6 | median: 55.5<br>range: 6.7 – 94.3 | 0.381 |
| Lymphocyte % (of WBC) | median: 8.4<br>range: 1.4 – 53.6 | median: 5.9<br>range: 1.4 – 52.6 | median: 12.1<br>range: 2.2 – 53.6 | 0.006 |
| T-cell % (of lymphocytes) | median: 72.5<br>range: 41.0 – 90.9 | median: 61.0<br>range: 41.0 – 72.3 | median: 79.1<br>range: 72.7 – 90.9 | |
| T-cell absolute numbers (cells / µL) | median: 1035<br>range: 57 - 7050 | median: 1029<br>range: 57 - 7050 | median: 1046<br>range: 109 - 3482 | |
| Treatment regimen | 7+3: 69 (86.2%)<br>ATRA: 3 (3.8%)<br>HMA: 7 (8.8%)<br>none: 1 (1.2%) | 7+3: 34 (85.0%)<br>ATRA: 1 (2.5%)<br>HMA: 4 (10.0%)<br>none: 1 (2.5%) | 7+3: 35 (87.5%)<br>ATRA: 2 (5.0%)<br>HMA: 3 (7.5%)<br>none: 0 (0.0%) | 1.0 |
| Response to induction therapy | CR/CRi: 59 (73.8%)<br>PR: 0 (0.0%)<br>none: 13 (16.3%)<br>NA: 8 (10.0%) | CR/CRi: 28 (70.0%)<br>PR: 0 (0.0%)<br>none: 7 (17.5%)<br>NA: 5 (12.5%) | CR/CRi: 31 (77.5%)<br>PR: 0 (0.0%)<br>none: 6 (15.0%)<br>NA: 3 (7.5%) | 0.764 |
| Bone Marrow Transplant | no: 47 (58.8%)<br>yes: 33 (41.3%) | no: 22 (55.0%)<br>yes: 18 (45.0%) | no: 25 (62.5%)<br>yes: 15 (37.5%) | 0.650 |
| Follow-up time from diagnosis | KM median: 48.1 mo<br>Alive at 2 yrs: 52.0% | KM median: 13.6 mo<br>Alive at 2 yrs: 40.0% | KM median: N/A<br>Alive at 2 yrs: 64.3% | 0.030 |
| Vital status | Alive: 40 (50.0%)<br>Dead: 40 (50.0%) | Alive: 15 (37.5%)<br>Dead: 25 (62.5%) | Alive: 25 (62.5%)<br>Dead: 15 (37.5%) | |

Footnotes

- "low" and "high" T-cell percentage groups were created by dichotomizing on the sample median of 72.5%
- p-values are from Fisher's exact test (for categorical variables), Kruskal-Wallis test (for continuous variables), or log-rank test (for follow-up time until death or last contact)
- among the 4 secondary (2°) AML patients, 2 had prior MDS, 1 had prior CMML (chronic myelomonocytic leukemia), and one had therapy-related AML after breast cancer
- reported cell percentages and absolute numbers are from multicolor flow analysis on bone marrow samples taken at the time of AML diagnosis
- For treatment regimen groups: (i) "7+3" includes similar regimens involving cytarabine and an anthracycline for numbers of days that differ from 7 and 3, respectively; (ii) the APL patients administered all-trans retinoic acid (ATRA) also received cytarabine and/or an anthracycline and thus have response to induction data; (iii) all patients in the hypomethylating agent (HMA) group received decitabine, with one patient also receiving azacytidine; and (iv) the lone untreated patient died 1 day after AML diagnosis
- response to induction was evaluated over 2 months for patients who received an initial course of standard induction therapy (i.e., 7+3 or similar) or ATRA-based therapy
- 'KM' refers to Kaplan-Meier estimation of survival (i.e., time to death or last contact)

Newly diagnosed AML patient demographic, bone marrow sample, and outcome variables of the clinical cohort (N=80) of patients with samples analyzed by flow cytometry, included to evaluate the relationship between marrow T cell percentage at diagnosis and overall survival

**Table S4.** Mass Cytometry staining panels.

| Panel 1 | | |
|---|---|---|
| **Antigen** | **Clone** | **Metal Tag** |
| CD45 | HI30 | 141Pr |
| CD19 | HIB19 | 142Nd |
| CD127(IL-7R) | A019D5 | 143Nd |
| CD38 | HIT2 | 144Nd |
| CD4 | RPA-T4 | 145Nd |
| CD8 | RPA-T8 | 146Nd |
| CD11c | Bu 15 | 147Sm |
| CD16(FcgRIII) | 3G8 | 148Nd |
| CD25(IL-2R) | 2A3 | 149Sm |
| CD223(LAG3) | 874501 | 150Nd |
| CD278 (ICOS) | C398.4A | 151Eu |
| CD66b | 80H3 | 152Sm |
| CD45RA | HI100 | 153Eu |
| TIM-3 | F38-2E2 | 154Sm |
| CD27 | L128 | 155Gd |
| CD14 | HCD14 | 156Gd |
| CD134 (OX40) | ACT35 | 158Gd |
| GITR | 621 | 159Tb |
| CD28 | CD28.2 | 160Gd |
| CD152(CTLA-4) | 14D3 | 161Dy |
| FoxP3 | 259D/C7 | 162Dy |
| CD272(BTLA) | MIH26 | 163Dy |
| CD185(CXCR5) | 51505 | 164Dy |
| CD40 | 5C3 | 165Ho |
| CD44 | BJ18 | 166Er |
| CD197 (CCR7) | G043H7 | 167Er |
| Ki-67 | Ki-67 | 168Er |
| CD33 | WM53 | 169Tm |
| CD3 | UCHT1 | 170Er |
| CD20 | 2H7 | 171Yb |
| VISTA | Janssen | 172Yb |
| HLA-DR | L243 | 173Yb |
| TIGIT | MBSA43 | 174Yb |
| CD279(PD-1) | EH12.2H7 | 175Lu |
| CD56 | R19-760 | 176Yb |

| Panel 2 | | |
|---|---|---|
| **Antigen** | **Clone** | **Metal Tag** |
| CD45 | HI30 | 141Pr |
| CD19 | HIB19 | 142Nd |
| CD117(ckit) | 104D2 | 143Nd |
| CD38 | HIT2 | 144Nd |
| CD4 | RPA-T4 | 145Nd |
| CD8 | RPA-T8 | 146Nd |
| CD11c | Bu 15 | 147Sm |
| CD16(FcgRIII) | 3G8 | 148Nd |
| CD34 | 581 | 149Sm |
| CD86 | IT2.2 | 150Nd |
| CD123(IL-3R) | 6H6 | 151Eu |
| CD66b | 80H3 | 152Sm |
| TIM-3 | F38-2E2 | 153Eu |
| CD163 | GHI/61 | 154Sm |
| VISTA | Janssen | 155Gd |
| CD14 | HCD14 | 156Gd |
| FLT3 | BV10A4H2 | 158Gd |
| CD115 | 9-4D2-1E4 | 159Tb |
| CD13 | WM15 | 160Gd |
| | | |
| CD80 | 2D10.4 | 162Dy |
| TGFb | TW4-6H10 | 163Dy |
| Arginase (i) | 14D2C43 | 164Dy |
| Notch2 (i) | MHN2-25 | 165Ho |
| IL-10 (i) | JES3-9D7 | 166Er |
| CD11b | ICRF44 | 167Er |
| pStat6 (i) | 18 | 168Er |
| CD33 | WM53 | 169Tm |
| CD3 | UCHT1 | 170Er |
| CD20 | 2H7 | 171Yb |
| CD15 | W6D3 | 172Yb |
| HLA-DR | L243 | 173Yb |
| IL-1rap | | 174Yb |
| CD274(PDL1) | 29E.2A3 | 175Lu |
| CD56 | R19-760 | 176Yb |

Antibody specificity (antigen), clone and corresponding metal tags used in the CyTOF-based immunophenotyping.