Supplementary data to:

*Paired donor and recipient immunophenotyping in allogeneic hematopoietic stem cell transplantation: a cellular network approach*

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Supplemental Table S1. Samples per time point (n) and range of acquisition days (min to max) are shown.

| Timepoint | n   | Min (day) | Max (day) |
|-----------|-----|-----------|-----------|
| preGCSF   | 18  | na        | na        |
| postGCSF  | 21  | na        | na        |
| d3        | 20  | 2         | 4         |
| d7        | 20  | 6         | 7         |
| d14       | 21  | 13        | 15        |
| d28       | 21  | 27        | 45        |
| d60       | 20  | 54        | 73        |
| d90       | 20  | 83        | 132       |
| d180      | 19  | 164       | 222       |
| d360      | 15  | 330       | 454       |
Supplemental table S2

| Abbreviation | Population | Gating |
|--------------|------------|--------|
| T_cells      | T-cells    | Lymphocytes (FSC/SSC), CD3+ |
| CD8_T        | CD8 T cytotoxic cells | CD8+, CD4- |
| CD8_TEMRA    | CD8 terminally differentiated effector memory T-cells | CD8+, CD27-, CD28- |
| CD4_T        | CD4 T helper cells | CD8-, CD4+ |
| CD4_Treg     | Regulatory CD4 T-cells | CD25++, CD127- |
| CD4_Tcon     | Conventional CD4 T-cells | CD25-, CD127+ |
| CD4_TEMRA    | CD4 terminally differentiated effector memory T-cells | CD4+, CD27-, CD28- |
| CD4CD8_T     | CD4CD8 double positive T-cells | CD4+, CD8+ |
| gd_T         | Gamma-delta T-cells | CD8high-, CD4+, TCRgd+ |
| B_cells      | B-cells    | Lymphocytes (FSC/SSC), CD3-, CD19+ |
| Naive_B      | Naïve B-cells | CD27-, IgD+ |
| NonSwitch_B  | Non-Switch memory B-cells | CD27+, IgD+ |
| Mem_B        | Memory B-cells | CD27+, IgD- |
| Plasmablasts | Plasmablasts | CD27+, CD38+ |
| NK_cells     | NK cells  | Lymphocytes (FSC/SSC), CD3-, CD19-, CD16+/− |
| brightNK     | CD56bright NK cells | CD56++ |
| dimNK        | CD56dim NK cells | CD56+ |
| Mono         | Monocytes | Monocytes (FSC/SSC), CD14+, HLA-DR+ |
| cMono  | - Classical monocytes | CD16- |
|--------|----------------------|-------|
| ncMono | - Nonclassical monocytes | CD16+ |
| **DC** | **Dendritic cells** | Lymphocytes (FSC/SSC), CD3-, CD19-, CD27-, CD56-, HLA-DR+ |
| **pDC** | - Plasmocytoid DC | CD123+ |
| **mDC** | - Myeloid DC | CD123- |
| **Neutro** | **Neutrophils** | Granulocytes (FSC/SSC), CD16+ |
| **Eosino** | **Eosinophils** | Granulocytes (FSC/SSC), CD16- |
| **Baso** | **Basophils** | Lymphocytes (FSC/SSC), CD3-, CD19-, CD123+, HLA-DR |

### B

| Antigen | Fluorochrome |
|---------|--------------|
| CD25    | APC          |
| CD16    | APC-750      |
| CD4     | BV510        |
| CD127   | PE           |
| TCRgd   | PC7          |
| HLA-DR  | PC5.5        |
| CD28    | BV785        |
| CD45    | BUV395       |
| CD3     | A700         |
| CD14    | PE           |
| CD56    | Pe Dazzle    |
| CD123   | PC7          |
| IgD     | FITC         |
| CD8     | Alexa488     |
| CD27    | BV421        |
| CD19    | BV605        |
| CD38 | BV650 |
|------|------|

**Supplemental Table S2.** Antigens (A) and fluorochromes (B) that were used for flow cytometric evaluation of different subpopulations are shown.
### Supplemental table S3

| cell_type     | Statistics | preGCSF | postGCSF | d3    | d7    | d14   | d28   | d60   | d90   | d180  | d360  |
|---------------|------------|---------|----------|-------|-------|-------|-------|-------|-------|-------|-------|
| T cells       | median     | 1067.30 | 2114.20  | 4.18  | 7.40  | 18.00 | 168.40| 309.00| 417.50| 682.19| 712.22|
| T cells       | IQR        | 491.82  | 1011.73  | 21.51 | 11.39 | 51.00 | 219.52| 638.43| 634.60| 645.30| 861.52|
| CD4 T         | median     | 641.40  | 1281.38  | 0.40  | 0.95  | 2.80  | 76.40 | 101.20| 98.79 | 169.00| 212.49|
| CD4 T         | IQR        | 292.51  | 599.80   | 3.72  | 4.90  | 28.26 | 106.00| 145.85| 129.55| 109.48| 152.74|
| CD4 Tcon      | median     | 549.10  | 1083.13  | 0.21  | 0.74  | 2.60  | 68.80 | 89.00 | 85.52 | 150.40| 170.21|
| CD4 Tcon      | IQR        | 263.25  | 535.20   | 3.35  | 4.25  | 22.47 | 100.60| 135.13| 118.25| 106.72| 142.40|
| CD4 Treg      | median     | 67.90   | 139.00   | 0.00  | 0.21  | 0.60  | 6.20  | 9.90  | 12.50 | 18.69 | 27.00 |
| CD4 Treg      | IQR        | 45.43   | 84.00    | 0.49  | 0.70  | 3.81  | 10.00 | 17.21 | 14.37 | 14.80 | 16.35 |
| CD4 TEMRA     | median     | 3.77    | 7.60     | 0.00  | 0.00  | 0.20  | 0.60  | 3.90  | 4.60  | 9.79  | 7.34  |
| CD4 TEMRA     | IQR        | 11.45   | 23.40    | 0.14  | 0.20  | 0.40  | 5.80  | 16.38 | 19.90 | 19.75 | 37.04 |
| CD8 T         | median     | 224.40  | 466.40   | 0.21  | 1.23  | 6.90  | 64.80 | 138.00| 239.00| 528.22| 453.23|
| CD8 T         | IQR        | 190.00  | 550.00   | 1.02  | 4.84  | 25.20 | 115.09| 337.50| 541.14| 595.63| 759.95|
| CD8 TEMRA     | median     | 28.92   | 38.80    | 0.00  | 0.31  | 1.00  | 5.20  | 11.40 | 19.30 | 42.60 | 29.59 |
| CD8 TEMRA     | IQR        | 70.49   | 101.20   | 0.20  | 1.05  | 2.40  | 16.00 | 23.40 | 31.90 | 96.37 | 73.98 |
| CD4CD8 T      | median     | 8.50    | 18.80    | 0.00  | 0.20  | 0.12  | 0.20  | 1.20  | 1.90  | 3.34  | 3.56  |
| CD4CD8 T      | IQR        | 8.01    | 10.80    | 0.20  | 0.20  | 0.40  | 2.00  | 1.60  | 2.58  | 4.73  | 4.33  |
| gd T          | median     | 8.40    | 13.00    | 0.93  | 1.20  | 1.56  | 1.60  | 2.10  | 4.00  | 4.60  | 6.01  |
| gd T          | IQR        | 8.50    | 16.10    | 1.40  | 2.20  | 3.80  | 3.60  | 5.11  | 6.00  | 10.40 | 11.29 |
| B cells       | median     | 166.80  | 351.80   | 4.90  | 2.83  | 4.20  | 6.20  | 25.80 | 46.11 | 71.00 | 155.75|
| B cells       | IQR        | 109.58  | 441.98   | 6.12  | 5.30  | 4.20  | 6.84  | 49.05 | 40.83 | 89.77 | 100.74|
| Plasmablasts  | median     | 1.40    | 3.00     | 0.00  | 0.00  | 0.00  | 0.20  | 0.53  | 0.94  | 1.56  | 1.34  |
| Plasmablasts  | IQR        | 2.35    | 3.80     | 0.00  | 0.00  | 0.20  | 0.20  | 1.21  | 1.61  | 3.10  | 2.22  |
| Naive B       | median     | 96.76   | 245.20   | 1.00  | 0.96  | 2.00  | 2.20  | 19.10 | 38.70 | 57.85 | 133.06|
| Naive B       | IQR        | 92.51   | 365.40   | 4.29  | 2.70  | 2.78  | 3.60  | 45.02 | 41.72 | 77.66 | 79.89 |
| NonSwitch B   | median     | 13.16   | 32.71    | 0.20  | 0.00  | 0.00  | 0.20  | 0.20  | 0.53  | 0.67  | 1.11  |
| NonSwitch B   | IQR        | 8.19    | 28.40    | 0.21  | 0.21  | 0.22  | 0.20  | 0.41  | 0.60  | 1.38  | 1.44  |
| Mem B         | median     | 28.64   | 60.52    | 0.70  | 0.50  | 0.40  | 0.45  | 0.60  | 1.79  | 3.56  | 5.56  |
| Mem B         | IQR        | 21.47   | 57.23    | 1.39  | 0.61  | 0.60  | 0.58  | 1.50  | 1.33  | 2.59  | 5.83  |
| NK cells      | median     | 122.58  | 243.64   | 0.43  | 0.95  | 33.40 | 128.60| 107.90| 113.03| 97.68 | 105.24|
| Subpopulation | Median Cell Count (µl) | IQR       |
|---------------|------------------------|-----------|
| NK_cells      | 83.86                  | 162.09    |
|               | 0.65                   | 3.28      |
|               | 49.08                  | 227.80    |
|               | 90.84                  | 63.37     |
|               | 67.94                  | 93.45     |
| brightNK      | 5.60                   | 10.46     |
|               | 0.00                   | 0.00      |
|               | 2.80                   | 47.00     |
|               | 26.53                  | 25.00     |
|               | 18.80                  | 8.68      |
| dimNK         | 114.80                 | 229.62    |
|               | 0.40                   | 0.70      |
|               | 31.80                  | 102.60    |
|               | 77.10                  | 74.79     |
|               | 65.19                  | 101.91    |
| Mono          | 80.92                  | 156.57    |
|               | 0.65                   | 1.53      |
|               | 47.60                  | 148.60    |
|               | 46.46                  | 24.91     |
|               | 53.23                  | 89.00     |
| cMono         | 102.67                 | 332.60    |
|               | 0.20                   | 0.20      |
|               | 24.00                  | 94.80     |
|               | 90.34                  | 89.50     |
|               | 109.47                 | 69.00     |
| ncMono        | 101.53                 | 315.20    |
|               | 0.00                   | 0.00      |
|               | 22.40                  | 91.89     |
|               | 85.72                  | 84.30     |
|               | 99.68                  | 66.00     |
| pDC           | 22.59                  | 176.20    |
|               | 1.11                   | 1.17      |
|               | 11.40                  | 50.40     |
|               | 23.48                  | 52.00     |
|               | 44.72                  | 31.82     |
| mDC           | 102.67                 | 332.60    |
|               | 0.11                   | 0.17      |
|               | 11.40                  | 50.40     |
|               | 23.48                  | 52.00     |
|               | 44.72                  | 31.82     |
| Eosino        | 50.60                  | 258.00    |
|               | 8.30                   | 7.60      |
|               | 8.00                   | 26.70     |
|               | 51.20                  | 46.78     |
|               | 75.65                  | 111.03    |
| Neutro        | 2059.02                | 11060.00  |
|               | 17.70                  | 4.10      |
|               | 79.80                  | 1056.21   |
|               | 1050.46                | 1030.54   |
|               | 1012.38                | 1290.95   |
| Baso          | 16.65                  | 17.66     |
|               | 2.45                   | 0.41      |
|               | 2.00                   | 17.60     |
|               | 8.27                   | 16.19     |
|               | 8.76                   | 9.13      |

**Supplemental Table S3.** Median cell counts per µl and IQR are shown for the evaluated time points and subpopulations.
Supplemental Figure S1

Supplemental Figure S1. Flow cytometry analysis of T-cells in PBMCs. (A) The SSC-A versus CD45 plot was used to exclude debris and gate nucleated leukocytes. (B) The SSC-A versus FSC-A gate further differentiated the granulocyte, monocyte and lymphocyte subsets. (C) T-cells are defined as CD19-CD3+ cells, which were further divided in (D) CD4 T helper cells, CD8 T cytotoxic cells and CD4CD8 double positive T-cells. (E) CD4 T-cells were examined for conventional and regulatory CD4 T-cells. (F) Conventional CD4 T-cells were examined for CD4 terminally differentiated memory T-cells (TEMRA). (G) CD8 T-cells were examined for CD8 terminally differentiated memory T-cells (TEMRA). (H) Gamma-delta T-cells were defined as gamma-delta T-cell-receptor+ within the lymphocyte population.
Supplemental Figure S2. Flow cytometry analysis of B-cells in PBMCs. (A) The SSC-A versus CD45 plot was used to exclude debris and gate nucleated leukocytes. (B) The SSC-A versus FSC-A gate further differentiated the granulocyte, monocyte and lymphocyte subsets. (C) Gate excluding CD19- lymphocytes. (D) Gate excluding CD45-CD19+ lymphocytes. (E) Gate excluding CD3+gamma-delta T-cell-receptor+ CD19+CD45+ cells, resulting in the B-cell gate. (F and G) Characterization of B-cell subpopulations. (F) Sub-gate to differentiate CD27+CD38+ plasmablasts. (G) B-cells excluding plasmablasts could be grouped into naive B-cells defined as CD27-IgD+, memory B-cells defined as CD27+IgD- and non-switched memory B-cells defined as CD27+IgD+.
Supplemental figure S3

Supplemental Figure S3. Flow cytometry analysis of NK cells in PBMCs. (A) The SSC-A versus CD45 plot was used to exclude debris and gate nucleated leukocytes. (B) The SSC-A versus FSC-A gate further differentiated the granulocyte, monocyte and lymphocyte subsets. (C) NK cells were defined as CD3-CD56+ cells, followed by the exclusion of (D) CD19+ cells and (E) CD14+ cells. (F) Two NK cell subsets were identified: CD56 bright NK cells and CD56 dim NK cells.
Supplemental Figure S4
Supplemental Figure S4. Flow cytometry analysis of granulocyte, monocytes and dendritic cells in PBMCs. (A) The SSC-A versus CD45 plot was used to exclude debris and gate nucleated leukocytes. (B) The SSC-A versus FSC-A gate further differentiated the granulocyte, monocyte and lymphocyte subsets. (C) Expression of CD16 was used to identify neutrophils. (D) Eosinophils were defined as HLA-DR+ and CD38low cells within the CD16- granulocyte population. (I) and (J) plots showing the exclusion criteria for dendritic cells, as defined by the absence of CD14+ cells within the monocyte population. (K) Expression of CD123+ was used to identify two subsets of dendritic cells: myeloid dendritic cells and plasmacytoid dendritic cells. In addition, basophils were defined as HLA-DR-CD123+ cells.
Supplemental Figure S5

Supplemental Figure S5. Correlation of cell counts in clinical routine measurements (routine cells/µl) and experimental measurements (experimental cells/µl). Experimental cell counts of Leukocytes and Lymphocytes are calculate from gated subpopulations.
Supplemental Figure S6. Supervised clustering of recipients according to aGvHD grade. Supervised clustering of Z-transformed cell count values for post-transplant time points. T_cells = T cells, CD8_T = CD8 T cytotoxic cells, CD8_TEMRA = CD8 terminally differentiated memory T cells, CD4_T = CD4 T helper cells, CD4_Treg = Regulatory CD4 T cells, CD4_Tcon = Conventional CD4 T cells, CD4_TEMRA = CD4 terminally differentiated memory T cells, CD4CD8_T = CD4CD8 double positive T cells, gd_T = gamma-delta T cells, B_cells = B cells, Naive_B = Naive B cells, NonSwitch_B = Non-switched memory B cells, Mem_B = Memory B cells, Plasmablasts = Plasmablasts, NK_cells = NK cells, brightNK = CD56 bright NK cells, dimNK = CD56 dim NK cells, Mono = Monocytes, cMono = Classical monocytes, ncMono = Non-classical monocytes, DC = Dendritic cells, pDC = Plasmacytoid dendritic cells, mDC = Myeloid dendritic cells, Neutro = Neutrophils, Eosino = Eosinophils, Baso = Basophils, aGvHD = acute GvHD, cGvHD = chronic GvHD. For GvHD prophylaxis, “none” depicts patients who received neither ATG nor post transplantation cyclophosphamide; these patients may still have received ciclosporin A, MTX, mycophenolat mofetil. Onset of aGvHD in Recipient (R) 10 = day + 27, R30 day + 32; onset of cGvHD in R10 day + 242, R13 day + 270, R16 day + 254.