**Supplemental Table 1. Cellular and Soluble Measures of Immune Activation, Immune Exhaustion, and Inflammation**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Immune Phenotype** | **CTN-055 CHOICES** | | | **PLHIV reference** | | **p-value1** | **p-value2** |
| **OUD+/HEP+**  **Median (IQR)** | **OUD+/HEP-**  **Median (IQR)** | | **OUD-/HEP-**  **Median (IQR)** | |  |
| **CD4 (%)** | 25.3 (10.8-49.5) | | 24.5 (14.8-30.2) | | 31.1 (25.3-43.6) | 0.125 | 0.17 |
| **CD4+CD38+ (%)** | 34.6 (28.2-53.8) | | 39.4 (29.2-49.7) | | 28.6 (18.9-45.4) | 0.070 | 0.19 |
| **CD4+HLADR+ (%)** | 4.8 (3.3-13.2) | | 6.3 (4.9-12.9) | | 5.9 (4.7-11.0) | 0.78 | 0.71 |
| **CD4+CD38+HLADR+ (%)** | 2.5 (1.5-5.7) | | 2.7 (2.3-4.1) | | 1.9 (1.4-3.9) | 0.23 | 0.41 |
| **CD4+CD28+ (%)** | 98.6 (93.5-99.8) | | 94.5 (84.4-99.4) | | 92.2 (87.0-97.9) | 0.06 | 0.066 |
| **CD4+CD57+ (%)** | 1.2 (0.2-6.0) | | 6.6 (0.53-14.9) | | 5.9 (2.1-12.6) | 0.22 | 0.12 |
| **CD4+PD-1+ (%)** | 23.35 (16.1-38.3) | | 21.4 (17.7-39) | | 26.6 (18.2-35.2) | 0.82 | 0.97 |
| **CD8 (%)** | 50.5 (31.2-72.1) | | 58 (52.2-64.6) | | 53 (40.1-57.9) | 0.35 | 0.41 |
| **CD8+CD38+ (%)** | 26.9 (16.4-36.9) | | 23.9 (19.3-38.3) | | 10.6 (5.4-22) | **0.0013** | **0.0077** |
| **CD8+HLADR+ (%)** | 13.5 (6.7-29.2) | | 16.9 (11.6-23.9) | | 9.6 (8.0-12.8) | 0.065 | 0.09 |
| **CD8+CD38+HLADR+ (%)** | 7.8 (2.2-14.4) | | 6.7 (6.1-10.4) | | 3.4 (2.2-6.4) | **0.013** | **0.037** |
| **CD8+CD28+ (%)** | 73.0 (53.4-79.1) | | 51.1 (38.6-64.8) | | 49.9 (39.5-59.6) | 0.24 | 0.11 |
| **CD8+CD57+ (%)** | 19.3 (10.4-21.2) | | 24.9 (18.3-33.4) | | 31.8 (16.8-35.3) | 0.084 | 0.06 |
| **CD8+CD28-CD57+ (%)** | 16.9 (9.1-20.4) | | 22.7 (12.3-31.6) | | 29.9 (12.3-32.5) | 0.13 | 0.12 |
| **CD8+PD-1+ (%)** | 24.3 (17.3-41.4) | | 20.0 (15.5-32.7) | | 20.5 (9.6-27.2) | 0.23 | 0.36 |
| **CD14++CD16- (%)** | 76.3 (51.7-82.6) | | 69.2 (57.7-79.9) | | 87.9 (81.7-90.9) | **0.0002** | **0.0020** |
| **CD14++CD16+ (%)** | 15.0 (9.8-32.4) | | 10.1 (7.2-32.9) | | 5.0 (2.2-10.6) | **0.0025** | **0.012** |
| **CD14dimCD16++ (%)** | 0.7 (0.59-1.2) | | 1.3 (1.2-1.8) | | 0.32 (0.14-0.73) | **0.0001** | **0.0004** |
| **CD14++CD16-CD163 MFI3** | 827 (480-1023) | | 507 (278-668) | | 463 (282-640) | **0.19** | **0.054** |
| **CD14++CD16+CD163 MFI3** | 1191 (750-1366) | | 706 (277-970) | | 682 (314-917) | **0.16** | **0.06** |
| **CD14dimCD16+CD163 MFI3** | 239 (120-652) | | 195 (57.6-322) | | 186 (110-358) | 0.71 | 0.4733 |
| **Plasma IL-6 (pg/ml)** | 19.0 (4.8-25.1) | | 15.8 (5.9-50.7) | | 14.9 (3.6-38.6) | 0.93 | 0.9442 |
| **Plasma sCD14 (pg/ml)** | 1,685,399 (1.22-20.9 x 106) | | 1,273,013 (1.09-1.74 x 106) | | 1,354,604 (1.21-1.69 x 106) | 0.74 | 0.2650 |
| **Plasma sCD163 (ng/ml)** | 789.2 (615.7-1171.4) | | 410.6 (342.2-730.8) | | 430.0 (351-524.4) | 0.08 | **0.017** |

1Non-parametric test for two group comparison (CTN-055 CHOICES versus PLHIV reference; Mann Whitney Test)

2Non-parametric test for three group comparison (OUD+/HEP+; OUD+/HEP-; OUD-/HEP-; Kruskal-Wallis Test)

**3**Background corrected (isotype control) MFI for CD163 presented

**Supplemental Table 2. PBMC Cytokine and Chemokine Response to LPS**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Cytokine/Chemokine3 (pg/ml)** | **CTN-055 CHOICES** | | | | **PLHIV reference** | **p-value1** | **p-value2** |
| **OUD+/HEP+**  **Median (IQR)** | **OUD+/HEP-**  **Median (IQR)** | | | **OUD-/HEP-**  **Median (IQR)** |  |
| **IFNα2** | Below detection4 | | Below detection4 | Below detection4 | | not done | not done |
| **IFNγ** | 14 (1-8) | | 1 (1-22) | 12 (6-18) | | 0.045 | 0.099 |
| **IL-10** | 23 (16-43) | | 43 (6-83) | 118 (81-221) | | **<0.0001** | **0.0003** |
| **IL-12p70** | Below detection4 | | Below detection4 | Below detection4 | | not done | not done |
| **IL-13** | 14 (0) | | 14 (1-5) | 2.54 (1-10) | | **0.016** | **0.05** |
| **IL-15** | Below detection4 | | Below detection4 | Below detection4 | | not done | not done |
| **IL-1α** | 76 (50-101) | | 31 (20-123) | 146 (90-251) | | **0.0045** | **0.017** |
| **IL-1β** | 405 (133-804) | | 167 (82-496) | 409 (279-686) | | 0.091 | 0.096 |
| **IL-2** | Below detection4 | | Below detection4 | Below detection4 | | not done | not done |
| **IL-4** | Below detection4 | | Below detection4 | Below detection4 | | not done | not done |
| **IL-6** | 2226 (1854-2776) | | 2545 (1055-5403) | 9067 (7597-12087) | | **<0.0001** | **0.0002** |
| **IL-8** | 11851 (7120-16455) | | 11623 (7264-25139) | 29982 (22199-35038) | | **<0.0001** | **0.0002** |
| **MCP-1** | 15 (1-194) | | 15 (1-484) | 5095 (1-2977) | | 0.74 | 0.2 |
| **TNF-α** | 979 (609-1379) | | 1137 (310-2384) | 2129 (1572-3685) | | **0.001** | **0.0056** |

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2Non-parametric test for three group comparison (OUD+/HEP+; OUD+/HEP-; OUD-/HEP-; Kruskal-Wallis Test)

3Background (media alone) corrected responses shown

4Cytokine responses below the level of detection for the assay (3.2 pg/ml) for all donors tested are reported as “Below detection” and statistical comparisons were not performed

5For cytokine responses that ranged from below the level of detection for that assay (3.2 pg/ml) to detectable among donors, values below the level of detection were set at 1 pg/ml to allow for statistical comparisons

**Supplemental Table 3. PBMC Cytokine and Chemokine Response to PHA**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Cytokine/Chemokine3 (pg/ml)** | **CTN-055 CHOICES** | | | **PLHIV reference** | | **p-value1** | **p-value2** |
| **OUD+/HEP+**  **Median (IQR)** | **OUD+/HEP-**  **Median (IQR)** | | **OUD-/HEP-**  **Median (IQR)** | |  |
| **IFNα2** | Below detection4 | | Below detection4 | | Below detection4 | not done | not done |
| **IFNγ** | 309 (162-525) | | 595 (279-2171) | | 771 (173-1081) | 0.55 | 0.27 |
| **IL-10** | 26 (13-171) | | 39 (11-101) | | 274 (147-411) | **<0.0001** | **0.0005** |
| **IL-12p70** | 14 (1-11) | | 14 (1-22) | | 14 (0) | 0.2 | 0.4 |
| **IL-13** | 97 (32-571) | | 278 (160-1025) | | 257 (113-422) | 0.82 | 0.48 |
| **IL-15** | Below detection4 | | Below detection4 | | Below detection4 | not done | not done |
| **IL-1α** | 96 (44-177) | | 65(34-201.9) | | 207 (139-580) | **0.0013** | **0.0073** |
| **IL-1β** | 657 (139-997) | | 131 (101-824) | | 871 (456-1899) | **0.0082** | **0.018** |
| **IL-2** | 106 (44-350) | | 201 (77-664) | | 139 (67-252) | 0.52 | 0.34 |
| **IL-4** | 45 (31-322) | | 123 (52-320) | | 153 (82-188) | 0.66 | 0.81 |
| **IL-6** | 2572 (2048-4008) | | 3502 (972-7127) | | 13003 (9803-15140) | **<0.0001** | **0.0001** |
| **IL-8** | 13573 (7670-18007) | | 16334 (5934-24753) | | 18286 (14297-29482) | 0.04 | 0.096 |
| **MCP-1** | 15 (1-5858) | | 15 (1-2228) | | 17135 (1-3038) | 0.21 | 0.44 |
| **TNF-α** | 1540 (901-2486) | | 2786 (1129-4254) | | 4983 (2984-7473) | **0.0003** | **0.0013** |

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**Supplemental Table 4. Spearman rank correlation analysis**

|  |  |  |
| --- | --- | --- |
|  | **Correlation est. (n)** | **p-value** |
| CD14++CD16- (%) vs. LPS IL-6 (pg/ml) | 0.713 (33) | **<.0001** |
| CD14++CD16- (%) vs. LPS IL-10 (pg/ml) | 0.625 (33) | **<.0001** |
| CD14++CD16- (%) vs. LPS IL-8 (pg/ml) | 0.520 (33) | **0.0019** |
| CD14++CD16- (%) vs. LPS MCP-1 (pg/ml) | 0.180 (33) | 0.3405 |
| CD14++CD16+ (%) vs. LPS TNF-α (pg/ml) | -0.446 (33) | **0.0092** |
| CD14++CD16+ (%) vs. LPS IL-1α (pg/ml) | -0.548 (33) | **0.0010** |
| CD14++CD16+ (%) vs. LPS IL-1β (pg/ml) | -0.452 (33) | **0.0083** |
| CD14++CD16+ (%) vs. sCD163 (ng/ml) | 0.194 (34) | 0.271 |
| CD14++CD16+ (%) vs. sCD14 (pg/ml) | -0.003 (29) | 0.988 |
| CD14++CD16+CD163 MFI vs. sCD163 (ng/ml) | 0.109 (28) | 0.583 |
| sCD163 (ng/ml) vs sCD14 (pg/ml) | 0.024 (31) | 0.897 |
| sCD163 (ng/ml) vs IL6 (pg/ml) | -0.133 (37) | 0.434 |

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