**Supplemental material – table of content**

1 – Direct acyclic graph assumptions.

2 – Accessible donor data

3 – Comparison of pre- and post-transplant FOXP3 mRNA levels.

4 – subgroup analysis comparing immunosuppressive drug administration and FOXP3 splice variant levels in recipients who have had prior kidney transplantations.

**Supplemental Table 1** – Direct acyclic graph assumptions and sources.

|  |  |  |
| --- | --- | --- |
| Potential confounding variable | Articles reporting association to FOXP3 | Articles reporting association to inflammatory response to stimuli |
| Recipient age | (1-3) | (4-6) |
| Recipient sex | (7) | (4, 8, 9) |
| Donor type | (10) | (11) |
| Prior kidney transplantation | (10) | (12) |
| Immunosuppressive therapy | (13-19) | (20-22) |
| Number of comorbidities | (23-25) | (26-28) |

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**Supplemental Table 2 –** AccessibleDonor data.

Table footnotes: Continuous values are reported as median [interquartile range], and categorical values are reported as number (percent).

|  |  |
| --- | --- |
| Donor Characteristics | Statistic |
| Age (years), N = 336 | 55 [46 to 64] |
| Male sex, N (%), N = 343 | 111 (45%) |
| Cold ischemic time (minutes), N= 282 | 780 [585 to 1050] |

**Supplemental Table 3 –** Logarithmic values of normalized forkhead box P3 (FOXP3) splice variant levels in kidney transplant recipients according to day of sample collection.

Table footnotes: Data are presented as median [interquartile range]. Comparisons were performed with Wilcoxon rank sum test. Tx: transplantation. Total FOXP3: detects the two most abundant splice variants, FOXP3fl and FOXP3d2. Pre-mRNA FOXP3: detects pre-mRNA that contain introns. FOXP3fl: detects mature FOXP3 mRNA that includes all exons. FOXP3d2: detects mature FOXP3 mRNA that skip exon 2.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Logarithmic value of | Pre-Tx | First day post-TX | 29 days post-Tx | Comparison of pre-TX and first day post-Tx | Comparison of first day and day 29 post-Tx | Comparison of pre-TX and day 29 post-Tx |
| Total FOXP3 | -3.31 [-3.52 to -3.10] | -3.65 [-3.96 to -3.37] | -3.58 [-3.97 to -3.33] | P < 0.01 | P = 0.05 | P < 0.01 |
| Pre-mRNA FOXP3 | -4.29 [-4.49 to -4.10] | -4.46 [-4.68 to -4.18] | -4.36 [-4.62 to -4.13] | P < 0.01 | P < 0.01 | P = 0.05 |
| FOXP3fl | -3.66 [-3.88 to -3.41] | -4.00 [-4.33 to -3.73] | -3.89 [-4.24 to -3.63] | P < 0.01 | P < 0.01 | P < 0.01 |
| FOXP3d2 | -3.47 [-3.63 to -3.32] | -3.75 [-3.98 to -3.52] | -3.72 [-3.97 to -3.45] | P < 0.01 | P = 0.05 | P < 0.01 |

**Supplemental Table 4** – Subgroup analysis comparing immunosuppressive drug administration and FOXP3 splice variant levels in recipients who have had prior kidney transplantations and for first-time transplant recipients.

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | First-time transplant recipient | Prior kidney transplant recipient | P-value |
| Basiliximab, N (%) | 383 (87 %) | 37 (57 %) | < 0.01 |
| Rituximab, N (%) | 67 (15 %) | 27 (42 %) | < 0.01 |
| Thymoglobulin, N (%) | 51 (12 %) | 28 (43 %) | < 0.01 |
| Corticosteroids, N (%) | 102 (23 %) | 36 (55 %) | < 0.01 |
| Logarithmic values of FOXP3 levels first post-transplant day  Total FOXP3  Pre-mRNA FOXP3  FOXP3fl  FOXP3d2 | -3.66 [-3.89 to -3.39]  -4.43 [-4.69 to -4.12]  -3.97 [-4.27 to -3.75]  -3.74 [-3.98 to -3.53] | -4.03 [-4.57 to -3.68]  -4.41 [-4.76 to -4.23]  -4.25 [-4.80 to -3.95]  -3.99 [-4.47 to -3.61] | < 0.01  0.40  < 0.01  < 0.01 |
| Logarithmic values of FOXP3 levels 29 days post-transplant  Total FOXP3  Pre-mRNA FOXP3  FOXP3fl  FOXP3d2 | -3.57 [-3.84 to -3.30]  -4.34 [-4.62 to -4.11]  -3.85 [-4.16 to -3.63]  -3.66 [-3.95 to -3.44] | -3.83 [-4.47 to -3.52]  -4.44 [-4.70 to -4.08]  -4.14 [-4.52 to -3.88]  -3.89 [-4.27 to -3.69] | < 0.01  0.40  < 0.01  < 0.01 |