**Supporting Tables and Figure**

**Table A Clinical details of T-ALL cases studied**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Karyotype** | **Age (y)** | **Sex** | **Disease**  **status** | **Risk status** | **Blast infiltration of BM (%)** | **Immunophenotype at diagnosis** |
| 1 | iso 9, +4 | 15 | M | Relapse | Risk | >90 | CD2+, cCD3+, CD7+, CD99+, TdT+ |
| 2 | t(11;14) | 2 | M | Relapse | Low | >90 | CD2+, cCD3+, CD5+, CD4+, CD7+, CD8+, CD99+ |
| 3 | t(9;16),+7, -9 | 5 | M | Relapse | Risk | >95 | CD2+, cCD3+, CD5+, CD7weak, CD8+, CD99+ |
| 4 | 46 XY | 17 | M | Diagnosis | Risk | >95 | CD1a+, CD2+, cCD3+, CD4+, CD5+, CD7+, CD8+, CD34+, CD99+, TdT+ |
| 5 | t(1;14) | 10 | M | Diagnosis | Low | >90 | CD2+, cCD3+, CD5+, CD7+, CD13+, CD99+, TdTweak |
| 6 | del 6q | 15 | M | Diagnosis | Risk | >90 | CD1aweak, CD2+, cCD3+, CD5+, CD7+, CD13, CD34+, CD99+, TdT+ |
| 7 | +1, del 6, del11 | 2 | F | Diagnosis | N/A | >90 | CD2+, CD7+, CD5+, cCD3+, CD99+, TCR+, TdT- |
| 8 | 46 XY | 3 | M | Diagnosis | Risk | >95 | CD2+, cCD3+, CD7+, CD5+, CD4+, CD8+, CD99+, TCR-, TCR-, TdTweak |
| 9 | complex | 6 | M | Diagnosis | Low | 87 | CD2+, cCD3+, sCD3-, CD7+, CD99+, TdT+ |

**Table B Immunophenotypic and karyotypic analyses of NSG BM**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Patient** | **Subfraction** | **Immunophenotype \*** | | | **% FISH +** |
|  | **Inoculated** | **%CD7+** | **%CD34+** | **%CD99+** |  |
| 2 | Unsorted | 75.82 | 3.03 | 95.54 | 100 |
|  | CD34+/CD99+ | 89.15 | 4.00 | 89.11 | 100 |
|  | CD34+/CD99- | 84.37 | 2.0 | 83.60 | 100 |
|  | CD34-/CD99+ | 96.24 | 0.06 | 96.47 | 100 |
|  | CD34-/CD99- | 95.22 | 0 | 93.53 | 100 |
|  |  |  |  |  |  |
| 3 | Unsorted | 29.78 | 4.74 | 68.04 | 80 |
|  | CD34+/CD99+ | 18.43 | 6.12 | 53.80 | 84 |
|  | CD34+/CD99- | 24.49 | 2.42 | 65.40 | 75 |
|  | CD34-/CD99+ | 14.02 | 0.06 | 44.06 | 70 |
|  | CD34-/CD99- | 9.42 | 0.08 | 39.10 | - |
|  |  |  |  |  |  |
| 4 | Unsorted | 92.27 | 71.90 | 98.67 | - |
|  | CD34+/CD99+ | 93.76 | 73.20 | 98.39 | - |
|  | CD34+/CD99- | 13.26 | 46.70 | 26.03 | - |
|  | CD34-/CD99+ | 86.75 | 9.02 | 91.24 | - |
|  | CD34-/CD99- | 24.27 | 3.11 | 14.92 | - |
|  |  |  |  |  |  |
| 6 | Unsorted | 89.00 | 62.38 | 72.70 | 72 |
|  | CD34+/CD99+ | 72.13 | 74.44 | 88.10 | - |
|  | CD34+/CD99- | 65.17 | 58.41 | 70.70 | - |
|  | CD34-/CD99+ | 83.9 | 54.20 | 96.50 | 71 |
|  | CD34-/CD99- | 83.2 | 61.47 | 62.02 | 85 |
|  |  |  |  |  |  |
| 9 | Unsorted | 99.93 | 2.1 | 89.33 | 91 |
|  | CD34+/CD99+ | 86.46 | 3.4 | 74.27 | 66 |
|  | CD34+/CD99- | 53.85 | 0.17 | 55.09 | 80 |
|  | CD34-/CD99+ | 99.08 | 1.15 | 86.49 | 100 |
|  | CD34+/CD99- | 59.13 | 0 | 63.82 | 81 |

\* Immunophenotype of human CD45+ cells removed from murine BM. All samples analyzed were negative for CD19.

**Table C Details of secondary NSG transplants**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Patient** | **1o NSG source** | **Total CD45+ cells inoculated (x104)** | **No. CD99+ cells inoculated (x104)\*** | **% Engraftment** |
| 2 | Unsorted | 12-240 | 11.5-233 | 46-92 |
|  | CD34+/CD99+ | 0.48 | 0.43 | 69 |
|  | CD34+/CD99- | 2.12 | 1.77 | 66 |
|  | CD34-/CD99+ | 78.3 | 75.6 | 73 |
|  | CD34-/CD99- | 92.9 | 86.9 | 87 |
|  |  |  |  |  |
| 3 | Unsorted | 1.75 | 1.19 | 63 |
|  | CD34+/CD99+ | 0.15 | 0.08 | 32 |
|  | CD34+/CD99- | 0.57 | 0.37 | 20 |
|  | CD34-/CD99+ | 6.25 | 2.76 | 52 |
|  | CD34-/CD99- | 0.23 | 0.09 | 41 |
|  |  |  |  |  |
| 4 | Unsorted | 10-97 | 9.87-95 | 58-98 |
|  | CD34+/CD99+ | 0.48-9.77 | 0.47-9.61 | 89 |
|  | CD34+/CD99- | 0.87 | 0.22 | 44 |
|  | CD34-/CD99+ | 123 | 112 | 78 |
|  | CD34-/CD99- | 7.28 | 1.09 | 47 |
|  |  |  |  |  |
| 6 | Unsorted | 18.1 | 13.2 | 5 |
|  | CD34+/CD99+ | 4.56 | 4.01 | 3 |
|  | CD34+/CD99- | 0.17 | 0.12 | 6 |
|  | CD34-/CD99+ | 0.62 | 0.60 | 9 |
|  | CD34-/CD99- | 0.29 | 0.18 | 6 |
|  |  |  |  |  |
| 9 | Unsorted | 10-100 | 8.93-89.3 | 48-97 |
|  | CD34+/CD99+ | 0.80 | 0.59 | 59 |
|  | CD34+/CD99- | 0.18 | 0.09 | 10 |
|  | CD34-/CD99+ | 65.0 | 56.3 | 98 |
|  | CD34+/CD99- | 0.68 | 0.43 | 15 |
|  |  |  |  |  |

\* Number CD99+ cells inoculated calculated based on immunophenotype of cells recovered from BM of primary NSG mice (detailed in Supplementary Table 2).

**Table D Sequence analyses of TCR rearrangements in cells from engrafted NSG mice**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample source** | | **Clonal rearrangement** | | | | | | | | | | |
|  | |  |  | | |  | | |  | | |  |
| Pt. 2 | |  |  | | |  | | |  | | |  |
| Unsorted NSG 1° | | (TRGV2\*01) | tgtgccacc......... | | *aaatccccctcatgg* | | | ............gaaactcttt | | | (TRGJ1\*01) | | |
|  | |  |  | |  | | |  | | |  | | |
| CD34+/CD99+ NSG 1° | | (TRGV9\*01) | tgtgccacc......... | | *aaatccccctcatgg* | | | ............gaaactcttt | | | (TRGJ1\*01) | | |
|  | |  |  | |  | | |  | | |  | | |
| CD34+/CD99- NSG 1° | | (TRGV9\*01) | tgtgccacc......... | | *aaatccccctcatgg* | | | ............gaaactcttt | | | (TRGJ1\*01) | | |
|  | |  |  | |  | | |  | | |  | | |
| CD34-/CD99+ NSG 1° | | (TRGV9\*01) | tgtgccacc......... | | *aaatccccctcatgg* | | | ............gaaactcttt | | | (TRGJ1\*01) | | |
|  | |  |  | |  | | |  | | |  | | |
| CD34-/CD99- NSG 1° | | (TRGV9\*01) | tgtgccacc......... | | *aaatccccctcatgg* | | | ............gaaactcttt | | | (TRGJ1\*01) | | |
|  | |  |  | |  | | |  | | |  | | |
| Pt. 4 | |  |  | |  | | |  | | |  | | |
| Unsorted NSG 1° | | (TRGV9\*01) | tgtgcc............ | | *ccgggagg* | | | ...ttattataagaaactcttt | | | (TRGJ1\*01) | | |
| Unsorted NSG 2° | | (TRGV9\*01) | tgtgcc............ | | *ccgggagg* | | | ...ttattataagaaactcttt | | | (TRGJ1\*01) | | |
|  | |  |  | |  | | |  | | |  | | |
| CD34+/CD99+ NSG 1° | | (TRGV9\*01) | tgtgcc............ | | *ccgggagg* | | | ...ttattataagaaactcttt | | | (TRGJ1\*01) | | |
| CD34+/CD99+ NSG 2° | | (TRGV9\*01) | tgtgcc............ | | *ccgggagg* | | | ...ttattataagaaactcttt | | | (TRGJ1\*01) | | |
|  | |  |  | |  | | |  | | |  | | |
| CD34+/CD99- NSG 1 | | (TRGV9\*01) | tgtgcc............ | | *ccgggagg* | | | ...ttattataagaaactcttt | | | (TRGJ1\*01) | | |
| CD34+/CD99- NSG 2° | | (TRGV9\*01) | tgtgcc............ | | *ccgggagg* | | | ...ttattataagaaactcttt | | | (TRGJ1\*01) | | |
|  | |  |  | |  | | |  | | |  | | |
| CD34-/CD99+ NSG 1° | | (TRGV9\*01) | tgtgcc............ | | *ccgggagg* | | | ...ttattataagaaactcttt | | | (TRGJ1\*01) | | |
| CD34-/CD99+ NSG 2° | | (TRGV9\*01) | tgtgcc............ | | *ccgggagg* | | | ...ttattataagaaactcttt | | | (TRGJ1\*01) | | |
|  | |  |  | |  | | |  | | |  | | |
| CD34-/CD99- NSG 1° | | (TRGV9\*01) | tgtgcc............ | | *ccgggagg* | | | ...ttattataagaaactcttt | | | (TRGJ1\*01) | | |
| CD34-/CD99- NSG 2° | | (TRGV9\*01) | tgtgcc............ | | *ccgggagg* | | | ...ttattataagaaactcttt | | | (TRGJ1\*01) | | |
|  |  | | |  | | |  | | |  | | |

Unique V and J segments are show in parentheses. Randomly deleted bases are represented by full stops. Randomly inserted bases (both N and P nucleotides) are in italics, 1° and 2° indicate primary and secondary NSG mice.



**Figure A Gating strategy for flow cytometric analyses and sorting**

T-ALL blast cells were initially gated on the basis of low forward and side scatter (i), doublets were excluded using a FSC area (integral) vs height (peak) plot (ii), then live 7AAD negative cells selected (iii). Subsequently cells were gated for expression of CD34-APC and CD7-FITC (iv). Sort gates were established using FMO controls and were separated by at least 10 channels, as shown. The proportion of CD99-PE+ cells in each of the sorted population is shown (v-viii). A similar strategy was used to sort CD34/CD99 subpopulations with subsequent analyses of CD7 expression in each subpopulation.