**Supplemental information index**

Selective graft-versus-leukemia depends on magnitude and diversity of the alloreactive T cell response

Cornelis van Bergen et al.

**Supplemental data**



**Supplemental experimental procedures**

**Supplemental Table 1: Patient characteristics**

| patient ID | age at alloSCT | don→pat gender[A] | disease | donor[B] | HLA-A | HLA-B | HLA-C | conditioning regimen[C] | stem cell source[D] | state pre DLI[E] | GvHD | GvHD treatment | follow up (years) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6268 | 42 | f→f | AML-M4 | sd | 02:01<br>24:02 | 35:01<br>44:02 | 04:01<br>05:01 | MA cyclophosphamide TBI | PBSC | MC | no | no | 8.4 |
| 7103 | 53 | m→m | MDS | sd | 02:01 | 07:02<br>44:02 | 05:01<br>07:02 | NMA fludarabin busulphan Alemtuzumab | PBSC | BM blasts cytoreduction | no | no | 5.6 |
| 3356 | 45 | m→f | CML-CP | sd | 02:01<br>24:02 | 07:02<br>13:01 | 06:02<br>07:02 | MA cyclophosphamide TBI | PBSC | BCR-ABL pos | no | no | 15.4 |
| 4461 | 23 | f→m | CML-CP | sd | 02:01 | 07:02<br>44:02 | 05:01<br>07:02 | MA cyclophosphamide TBI | PBSC | BCR-ABL pos | no | no | 12.7 |
| 5835 | 50 | m→m | CML-CP | sd | 29:02 | 44:03<br>51:01 | 14:02<br>16:01 | MA cyclophosphamide TBI | PBSC | BCR-ABL pos | no | no | 9.1 |
| 5866 | 55 | f→f | CML-CP | sd | 02:01<br>11:01 | 35:01<br>51:01 | 04:01<br>14:02 | MA cyclophosphamide TBI | PBSC | BCR-ABL pos | no | no | 9.9 |
| 5596 | 66 | m→m | AML | mud | 01:01<br>02:01 | 08:01<br>51:01 | 07:01<br>15:02 | NMA fludarabin busulphan Alemtuzumab horse ATG | PBSC | bm blasts (smouldering) | grade II | dermovate, prednisone, cyclosporine | 8.3 |
| 7995 | 63 | m→m | AML | sd | 01:01 | 35:02<br>52:01 | 04:01<br>12:02 | NMA fludarabin busulphan Alemtuzumab | PBSC | MC | grade II | prednisone, cyclosporin, mycophenolate mofeti | 4.9 |
| 5852 | 56 | m→f | MDS | mud | 02:01 | 07:02<br>44:02 | 05:01<br>07:02 | NMA fludarabin busulphan horse ATG | PBSC | MC | grade II, skin+mouth | dermovate, prednisone, cyclosporin | 9.7 |
| 6181 | 48 | m→f | CML-CP | sd | 11:01<br>24:02 | 44:02<br>51:01 | 05:01<br>15:02 | MA cyclophosphamide TBI | bone marrow | BCR-ABL pos | grade III, skin+mouth | dermovate, prednisone, cyclosprine | 8.6 |
| 4716 | 36 | f→m | AML-M1 | sd | 02:01<br>24:02 | 40:01<br>44:02 | 03:04<br>05:01 | MA cyclophosphamide TBI | PBSC | bm blasts (smouldering) | grade IV, deceased | prednisone, cyclosporin, mycophenolate mofetil | 0.2 |
| | | | | | | | | | | | | **median:** | **8.6** |

[A]f=female
m=male

[B]sd=sibling donor
mud=matched unrelated donor

[C]MA=myeloablative
NMA=non myeoloablative
TBI=total body irradiation

[D]peripheral blood stem cells

[E]MC (mixed chimerism) by STR-PCR
BCR-ABL by real time PCR

**Supplemental Table 2: T cell receptor-β sequencing analysis**

CD8 T cells were purified from patient samples obtained before and after DLI. Two control samples were prepared by defined mixtures of 23 cultures T cells clones each expressing a unique TCR-β sequence. These samples served to compare the input cellular fraction of every T cell clone with the observed fraction of clonotypic sequence reads. After mRNA isolation, ARTISAN PCR was performed for gene-specific cDNA synthesis and amplification (1). TCR-β libraries were barcoded, pooled and paired-end sequencing with a read length of 125 base pairs was performed on an Illumina HiSeq2500 device. Raw reads were processed by using MiXCR algorithms to obtain TCR-β V-gene and J-gene alignments followed by assembly into clonotypes (2). Sequences of oligonucleotides are given in 'Supplemental experimental procedures: Primers used for cDNA synthesis, amplification and sequencing of TCR-β'.

**Table: Input of CD8 T cells, raw HiSeq output and MiXCR output.**

| sample ID | Patient ID | days after DLI | CD8[A] T-cell input *$10^3$ | Raw read count *$10^6$ | % of >= Q30 Bases (PF) | Mean Quality Score (PF) | MiXCR TCR-β reads *$10^6$ | MiXCR clonotypes | Simpson's diversity index |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 7103 | 0 | 12 | 10.35 | 86.6 | 34.3 | 3.11 | 2,038 | 0.85 |
| 2 | | 52 | 479 | 2.49 | 85.4 | 34.0 | 0.98 | 2,290 | 0.75 |
| 3 | 3356 | 0 | 382 | 4.00 | 86.3 | 34.2 | 0.94 | 6,212 | 0.92 |
| 4 | | 56 | 518 | 8.49 | 87.6 | 34.5 | 1.03 | 5,957 | 0.93 |
| 5 | 4461 | 0 | 410 | 3.45 | 88.3 | 34.7 | 0.98 | 3,041 | 0.82 |
| 6 | | 84 | 195 | 5.04 | 86.5 | 34.2 | 1.56 | 8,008 | 0.89 |
| 7 | 5835 | 0 | 339 | 3.09 | 83.0 | 33.4 | 0.64 | 4,581 | 0.94 |
| 8 | | 72 | 187 | 6.06 | 87.9 | 34.6 | 0.64 | 4,202 | 0.96 |
| 9 | 5866 | 0 | 530 | 12.30 | 85.7 | 34.0 | 2.60 | 1,320 | 0.84 |
| 10 | | 99 | 160 | 2.96 | 86.0 | 34.1 | 0.78 | 2,060 | 0.94 |
| 11 | 5596 | 0 | 6 | 4.86 | 86.2 | 34.2 | 1.11 | 3,337 | 0.79 |
| 12 | | 49 | 158 | 7.63 | 87.2 | 34.3 | 1.07 | 6,763 | 0.98 |
| 13 | 7995 | 0 | 523 | 5.14 | 87.7 | 34.5 | 3.11 | 6,187 | 0.14 |
| 14 | | 62 | 331 | 4.94 | 85.7 | 34.0 | 1.31 | 513 | 0.89 |
| 15 | 5852 | 0 | 409 | 3.80 | 82.8 | 33.3 | 1.15 | 1,753 | 0.88 |
| 16 | | 49 | 239 | 6.92 | 87.9 | 34.6 | 0.72 | 3,892 | 0.95 |
| 17 | 6181 | 0 | 143 | 8.58 | 85.0 | 33.8 | 0.83 | 678 | 0.78 |
| 18 | | 84 | 260 | 4.28 | 84.2 | 33.6 | 1.61 | 3,022 | 0.72 |
| 19 | 4716 | 0 | 506 | 4.44 | 84.6 | 33.8 | 1.47 | 5,563 | 0.92 |
| 20 | | 23 | 89 | 9.00 | 87.0 | 34.3 | 1.56 | 9,209 | 0.98 |
| median | | | 296 | 5.0 | | | 1.09 | 3,615 | 0.89 |
| min | | | 6 | 2.5 | | | 0.64 | 513 | 0.14 |
| max | | | 530 | 12.3 | | | 3.11 | 9,209 | 0.98 |

[A] CD8 T cells were purified by magnetic bead isolation from PBMCs.

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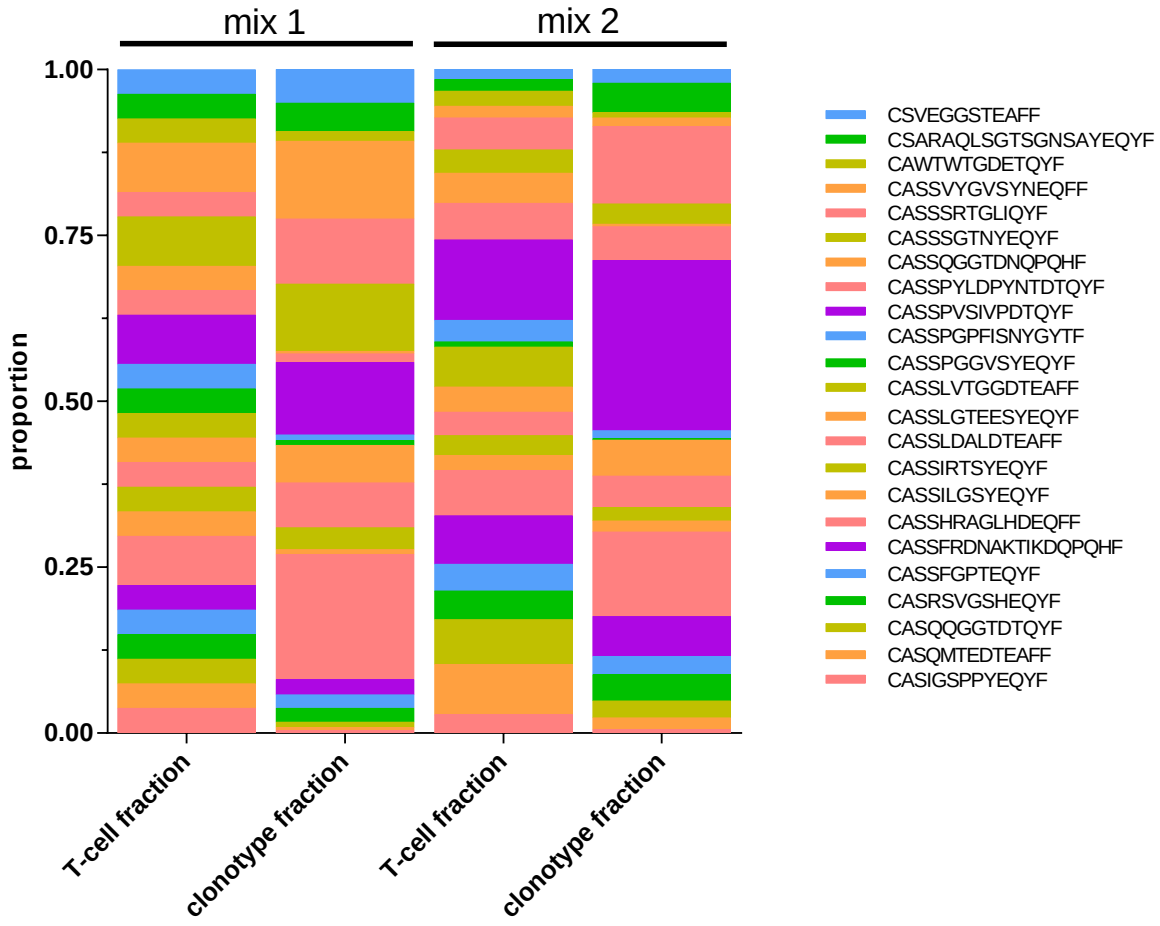
**Expected and observed abundance of clonotypic TCR-β sequences in control samples of 2 defined mixtures of T cell clones**




Cultured T cell clones with known TCR-β were mixed in predefined proportions and analyzed in parallel with primary purified CD8 T cells. Per mix, the fraction of input T-cells is plotted next to the clonotype fraction as calculated from HiSeq data by MiXCR algorithms. Significant correlations were observed between T-cell fraction and clonotype fraction: mix 1: $R^2$=0.67 p<0.0001, mix 2: $R^2$=0.51 p=0.0001.

References


1. Koning MT, Kielbasa SM, Boersma V, Buermans HP, van der Zeeuw SA, van Bergen CA, Cleven AH, Kluin PM, Griffioen M, Navarrete MA, et al. ARTISAN PCR: rapid identification of full-length immunoglobulin rearrangements without primer binding bias. *British journal of haematology.* 2016:bjh.14180.
2. Bolotin DA, Poslavsky S, Mitrophanov I, Shugay M, Mamedov IZ, Putintseva EV, and Chudakov DM. MiXCR: software for comprehensive adaptive immunity profiling. *Nature methods.* 2015;12(5):380-1.

**Supplemental Table 3: Cloning and selection of HLA-DR$^{pos}$ CD8 T cells and tested stimulator cells**

| patient | days after DLI | isolated cells | cloning format (well) | cloning efficiency % (growing clones) | tested target cells[B] EBV-LCL pat | EBV-LCL don | monoDC pat | monoDC don | CD40L-B pat | CD40L-B don | FB[C] pat |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6268 | 0 | 5760 | 384 | 3 (157) | ■ | ■ | | | | | |
| | 56 | 14976 | | 10 (1534) | ■ | ■ | | | | | |
| | 105 | 11520 | | 13 (1462) | ■ | ■ | | | | | |
| 7103 | 0 | 3072 | 384 | 10 (75) | ■ | ■ | | | ■ | | |
| | 17 | 6604 | | 10 (192) | ■ | ■ | | | ■ | | |
| | 24 | 6374 | | 11 \|(192) | ■ | ■ | | | ■ | | |
| | 52 | 6604 | | 31 (384) | ■ | ■ | | | ■ | | |
| 3356 | 0 | 480 | 96 | 10 (50) | ■ | ■ | ■ | ■ | ■ | ■ | |
| | 56 | 2080 | | 9 (191) | ■ | ■ | ■ | ■ | ■ | ■ | |
| | 122 | 864 | | 11 (96) | ■ | ■ | ■ | ■ | ■ | ■ | |
| 4461 | 0 | 923 | 96 | 13 (123) | ■ | ■ | ■ | ■ | ■ | ■ | |
| | 28 | 1212 | | 7 (90) | ■ | ■ | ■ | ■ | ■ | ■ | |
| | 84 | 960 | | 4 (36) | ■ | ■ | ■ | ■ | ■ | ■ | |
| | 182 | 1152 | | 11 (131) | ■ | ■ | ■ | ■ | ■ | ■ | |
| 5835 | 0[A] | 384 | 96 | 20 (78) | ■ | ■ | ■ | ■ | ■ | ■ | |
| | 44[A] | 2016 | | 6 (129) | ■ | ■ | ■ | ■ | ■ | ■ | |
| | 72[A] | 480 | | 16 (78) | ■ | ■ | ■ | ■ | ■ | ■ | |
| 5866 | 0 | 864 | 96 | 15 (126) | ■ | ■ | ■ | ■ | ■ | ■ | |
| | 43 | 3396 | | 8 (272) | ■ | ■ | ■ | ■ | ■ | ■ | |
| | 99 | 3168 | | 3 (94) | ■ | ■ | ■ | ■ | ■ | ■ | |
| **cloning efficiency, median (range)** | | | | **8.6% (2.4%-20.3%)** | | | | | | | |
| **growing clones, median (range)** | | | | **128 (36-1534)** | | | | | | | |
| 5596 | 0 | 480 | 384 | 8 (36) | ■ | ■ | | | ■ | ■ | ■ |
| | 42[A] | 4702 | | 5 (237) | ■ | ■ | | | ■ | ■ | ■ |
| | 49[A] | 9310 | | 2 (206) | ■ | ■ | | | ■ | ■ | ■ |
| 7995 | 62 | 16282 | 384 | 3 (518) | ■ | ■ | | | | | ■ |
| | 78 | 16282 | | 6 (936) | ■ | ■ | | | | | ■ |
| 5852 | 45 | 2000 | 96 | 10 (132) | ■ | ■ | | | | | |
| 6181 | 0 | 384 | 96 | 7 (25) | ■ | ■ | ■ | ■ | | ■ | |
| | 49 | 1440 | | 14 (202) | ■ | ■ | ■ | ■ | | ■ | |
| | 84 | 960 | | 12 (115) | ■ | ■ | ■ | ■ | | ■ | |
| 4716 | 0 | 480 | 384 | 11 (52) | ■ | ■ | ■ | ■ | | | ■ |
| | 23 | 16454 | | 2 (287) | ■ | ■ | ■ | ■ | | | ■ |
| **cloning efficiency, median (range)** | | | | **6.5% (1.7%-14.0%)** | | | | | | | |
| **growing clones, median (range)** | | | | **202 (25-936)** | | | | | | | |

[A]Indicated are the days after the second DLI.

[B]Shaded boxes indicate that cells in column header were tested.

[C]FB were tested after 4 days incubation with 200 IU/ml IFN-γ

**Supplemental Table 4: Response diversity**

| patient | specificity | clone | HLA restriction | MiHA frequency | clones | TCR-β V-gene[A] | TCR-β J-gene[A] | TCR-β CDR3 region | TCR-β % pre DLI[B] | TCR-β % post DLI[B] |
|---|---|---|---|---|---|---|---|---|---|---|
| 7103 | 1 | a | A*02 | 0.54 | 1 | 7-9 | 2-7 | CASSLTRQGNEQYF | 0.00 | 1.46 |
| | | b | | | 1 | 7-9 | 2-5 | CASSLVAGETQYF | 0.00 | 2.68 |
| 3356 | 2 | | A*02 | 0.41 | 2 | 12-3 | 1-5 | CASSFRDNAKTIKDQPQHF | 0.00 | 0.01 |
| | 3 | | A*02 | 0.04 | 1 | 11-2 | 2-1 | CASSFRRTMNEQFF | 0.00 | 0.00 |
| | 4 | | A*24 | 0.38 | 1 | 7-9 | 2-1 | CASSSSTVSYEQYF | 0.00 | 0.08 |
| | 5 | | B*07 | 0.69 | 2 | 6-2 | 2-1 | CASSWTSGPGGNEQFF | 0.00 | 0.09 |
| | 6 | | B*07 | 0.25 | 2 | 7-9 | 2-1 | CASSLRRGGTDEQFF | 0.00 | 0.05 |
| | 7[3)] | | B*07 | 0.71 | 1 | 30 | 1-2 | CAWNVGQALYGYTF | 0.00 | 0.03 |
| | 8 | | B*07 | 1.00 | 5 | 19 | 1-1 | CASSTGQGVGTEAFF | 0.00 | 0.01 |
| | 9 | | B*07 | 0.29 | 1 | 7-9 | 2-4 | CASSSRTGLIQYF | 0.00 | 0.01 |
| | 10 | | B*13 | 0.31 | 2 | 7-9 | 2-7 | CASSLVGRGHEQYF | 0.00 | 0.47 |
| | 11 | | B*13 | 0.04 | 1 | 7-8 | 2-7 | CASSPKQDFSSYEQYF | 0.00 | 0.00 |
| 4461 | 12 | | A*02 | 0.75 | 1 | 9 | 2-1 | CASSVSSSGDEQFF | 0.00 | 0.20 |
| | 13 | | A*02 | 0.13 | 2 | 12-3 | 2-5 | CASSFASGGGRGQYF | 0.00 | 0.35 |
| | 14 | | B*07 | 0.88 | 1 | 7-2 | 2-1 | CASSFREIGNEQFF | 0.00 | 0.12 |
| | 15 | | B*44 | 0.29 | 1 | 24-1 | 2-1 | CATSPTGGFDEQFF | 0.00 | 0.00 |
| 5835 | 16 | | B*44 | 0.98 | 1 | 12-3 | 2-3 | CASSLAGQGPDTQYF | 0.00 | 0.00 |
| | 17 | a | C*14 | 0.61 | 1 | 28 | 2-3 | CASSLTGAPGGQPQHF | 0.00 | 0.06 |
| | | b | | | 1 | 6-5 | 1-5 | CASSLVRFSGTDTQYF | 0.00 | 0.00 |
| | 18 | a | C*14 | 0.04 | 1 | 7-8 | 2-5 | CASSPMAGTSRTTDTQYF | 0.00 | 0.02 |
| | | b | | | 1 | 7-8 | 2-3 | CASSSYRDREETQYF | 0.00 | 0.02 |
| 5866 | 19 | | B*35 | 0.53 | 1 | 5-5 | 2-7 | CASSLGTEESYEQYF | 0.00 | 9.27 |
| 5596 | 20 | | A*01 | 0.67 | 2 | 5-1 | 2-3 | CASSLAGLAGGRDTQYF | 0.00 | 0.02 |
| | 21 | | A*01 | 0.67 | 1 | 6-2 | 1-2 | CATLGQHYGYTF | 0.00 | 0.03 |
| | 22 | | A*02 | 0.43 | 1 | 27 | 1-2 | CASSPGPFISNYGYTF | 0.00 | 0.14 |
| | 23 | | A*02 | 0.63 | 3 | 7-6 | 2-2 | CASSLGQGAGELFF | 0.02 | 6.58 |
| | 24 | | A*02 | 0.04 | 1 | 10-2 | 2-7 | CATSPGHEQYF | 0.00 | 0.55 |
| | 25[C] | | A*02 | 0.29 | 1 | nd[1)] | nd | nd | nd | nd |
| | 26[C] | | A*02 | 0.38 | 1 | nd | nd | nd | nd | nd |
| | 27 | a | B*08 | 0.68 | 6 | 4-1 | 2-5 | CASSLRQGGYEQYF | 0.00 | 0.00 |
| | | b | | | 1 | 6-1 | 2-7 | CASSPRTSGGLQETQYF | 0.00 | 0.46 |
| | 28 | | B*08 | 0.68 | 3 | 14 | 1-1 | CASSLGLGAFF | 0.00 | 0.14 |
| | 29 | a | B*08 | 0.46 | 1 | 9 | 1-1 | CASDLRPYTGELFF | 0.00 | 0.00 |
| | | b | | | 1 | 9 | 2-2 | CASSPEGNTEAFF | 0.00 | 0.00 |
| | 30 | | B*51 | 0.96 | 1 | 28 | 2-1 | CASSPRANSNEQFF | 0.00 | 0.00 |
| 7995 | 32 | a | A*01 | 0.96 | 5 | 5-6 | 2-7 | CASSLWGAPSYEQYF | 0.00 | 0.00 |
| | | b | | | 1 | 20-1 | 2-5 | CRASGGLWETQYF | 0.00 | 0.00 |
| | 33 | | B*35 | 0.00 | 2 | 14 | 2-5 | CASSQVRQALGQETQYF | 0.00 | 0.00 |
| | 34 | a | B*35 | 0.67 | 15 | 24-1 | 2-1 | CASSPNPSTDTQYF | 0.00 | 2.33 |
| | | b | | | 1 | 7-9 | 2-3 | CATSDLPRQGGTYNEQFF | 0.00 | 4.12 |
| | 35[C] | | C*04 or C*12 | 0.96 | 1 | 27 | 2-2 | CASSLWASGRAGELFF | 0.00 | 0.51 |

[A]TCR-β V-gene and J-gene according to IMGT nomenclature

nd: Not determined due to loss of T cell clone.

[B]Numbers represent percentages of MiHA specific TCR-β sequences within the total TCR-β repertoire of purified CD8 T cells.

[C]T cell clone could not be tested in tissue specificity experiments due to insufficient in vitro expansion.

**Supplemental Table 4: Response diversity (continued)**

| patient | specificity | | HLA restriction | MiHA frequency | clones | TCR-β V-gene[A] | TCR-β J-gene[A] | TCR-β CDR3 region | TCR-β % pre DLI[B] | TCR-β % post DLI[B] |
|---|---|---|---|---|---|---|---|---|---|---|
| 5852 | 36 | a | A*02 | 0.50 | 3 | 19 | 2-7 | CASSILGSYEQYF | 0.02 | 0.02 |
| | | b | | | 1 | 3-1 | 1-5 | CASSQGGTDNQPQHF | 0.00 | 0.21 |
| | 38 | | A*02 | 0.50 | 2 | 7-2 | 1-1 | CASSLVTGGDTEAFF | 0.00 | 0.03 |
| | 39 | a | A*02 | 0.04 | 1 | 30 | 2-5 | CASSPVSIVPDTQYF | 0.30 | 0.27 |
| | | b | | | 1 | 5-1 | 2-3 | CAWTWTGDETQYF | 0.01 | 0.04 |
| | 40 | a | A*02 | 0.13 | 2 | 7-2 | 1-1 | CASSHRAGLHDEQFF | 0.14 | 0.52 |
| | | b | | | 2 | 12-3 | 2-1 | CASSLDALDTEAFF | 0.05 | 0.06 |
| | 41 | | A*02 | 0.98 | 1 | 5-6 | 2-7 | CASSFGPTEQYF | 0.03 | 0.78 |
| | 42 | | A*02 | 0.08 | 1 | nd | nd | nd | nd | nd |
| | 43 | | B*07 | 0.67 | 1 | 9 | 2-1 | CASSVYGVSYNEQFF | 0.01 | 0.96 |
| | 44 | | B*07 | 0.60 | 1 | 6-1 | 2-7 | CASIGSPPYEQYF | 0.00 | 0.01 |
| | 45 | a | B*07 | 0.37 | 2 | 7-9 | 2-7 | CASSIRTSYEQYF | 0.02 | 0.14 |
| | | b | | | 1 | 7-9 | 2-7 | CASSSGTNYEQYF | 0.03 | 0.84 |
| | 46 | | B*07 | 0.53 | 1 | 6-1 | 2-7 | CASSRGRGQRDEQYF | 0.00 | 0.01 |
| | 47 | | B*07 | 0.13 | 3 | 20-1 | 2-7 | CSARAQLSGTSGNSAYEQYF | 0.05 | 0.00 |
| | 48 | | B*07 | 0.04 | 2 | 9 | 2-5 | CASSAYNAGGYQETQYF | 0.00 | 0.02 |
| | 50 | | B*44 | 0.71 | 1 | nd | nd | nd | 0.00 | 0.39 |
| | 51 | | B*44 | 0.04 | 1 | 7-2 | 2-3 | CASSQVPLAGGRADTQYF | nd | nd |
| | 52 | | C*05 | 0.96 | 2 | 13 | 2-7 | CASSGTGDEQYF | 0.01 | 5.55 |
| | 53 | | C*05 or C*07 | 0.04 | 3 | 3-1 | 2-7 | CASSPGGVSYEQYF | 0.00 | 0.50 |
| | 54 | | C*05 or C*07 | 0.08 | 1 | 19 | 1-1 | CASQMTEDTEAFF | 0.02 | 0.04 |
| | 55[C] | | nd | nd | 2 | nd | nd | nd | nd | nd |
| 6181 | 56 | a | A*11 | 0.63 | 3 | 10-3 | 1-5 | CASSEGQGAYEQYF | 0.00 | 0.00 |
| | | b | | | 1 | 11-2 | 2-7 | CAVGHSNQPQHF | 0.01 | 1.58 |
| | 57 | | B*44 | 0.88 | 2 | 5-1 | 1-1 | CASSLPLQGAREMNTEAFF | 0.15 | 52.35 |
| | 58 | | B*51 | 0.51 | 13 | 7-9 | 1-2 | CASSLVSGTDYGYTF | 0.01 | 1.77 |
| | 60 | | B*51 | 0.04 | 6 | 9 | 2-7 | CASSVAGTGTDEQYF | 0.01 | 1.91 |
| 4716 | 61 | a | A*02 | 0.58 | 1 | 10-3 | 1-1 | CAISESAAGLNTEAFF | 0.00 | 0.02 |
| | | b | | | 1 | 6-5 | 1-6 | CASRTAAHSPLHF | 0.00 | 0.11 |
| | 62 | | A*24 | 0.58 | 1 | 6-1 | 2-7 | CASVPPYTGNYEQYF | 0.00 | 0.00 |
| | 63 | | B*40 | 0.58 | 1 | 3-1 | 2-7 | CASSQDLTAEQYF | 0.00 | 0.06 |
| | 64 | a | A*02 | 0.43 | 1 | 13 | 1-2 | CASRRTLEGSYTF | 0.00 | 0.11 |
| | | b | | | 1 | 24-1 | 2-1 | CASSPYLDPYNTDTQYF | 0.00 | 0.04 |
| | | c | | | 1 | 27 | 2-3 | CATSDLTSPSLTF | 0.00 | 0.02 |
| | 65 | | A*02 | 0.04 | 1 | 5-6 | 2-3 | CASSLALAIATDTQYF | 0.00 | 0.01 |
| | 66 | | B*40 | 0.30 | 1 | 11-3 | 2-3 | CASSSRLAATDTQYF | 0.00 | 0.00 |
| | 67 | a | B*40 | 0.83 | 3 | 27 | 2-7 | CASSGTGTEAFF | 0.01 | 0.01 |
| | | b | | | 1 | 27 | 1-1 | CASSWTGDEQYF | 0.93 | 0.31 |
| | 68 | | B*44 | 0.04 | 1 | 7-6 | 1-2 | CASSQGQGYNYGYTF | 0.00 | 0.07 |
| | 69 | a | B*44 | 0.88 | 1 | 7-6 | 1-2 | CASSPGQSNEQYF | 0.01 | 1.55 |
| | | b | | | 1 | 7-6 | 2-7 | CASSQGQGITMATF | 0.00 | 0.00 |
| | 70 | | B*44 | 0.96 | 2 | 7-6 | 2-1 | CASSLESRRVSYNEQFF | 0.00 | 0.64 |
| | 71 | | B or C | 0.00 | 1 | 6-5 | 1-2 | CASSYSIGQSNYGYTF | 0.14 | 0.06 |

[A]TCR-β V-gene and J-gene according to IMGT nomenclature nd: Not determined due to loss of T cell clone.

[B]Numbers represent percentages of MiHA specific TCR-β sequences within the total TCR-β repertoire of purified CD8 T cells.

[C]T cell clone could not be tested in tissue specificity experiments due to insufficient in vitro expansion.

**Supplemental Table 5: Expression of MiHA-encoding genes according to EMBL-EBI Expression Atlas** **www.ebi.ac.uk/gxa**

| | tissue[A] | genes targeted in patient with selective GvL reactivity[B] | | | | | | | | | genes targeted in patients with GVHD[B] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *HMHA1* | *CCL4* | *TMEM8A* | *MOB3A* | *PNP* | *NCAPD3* | *ZDHHC6* | *POLE* | *RWDD4* | *NDC80* | *APOBEC* | *GSTP1* | *GEMIN4* | *TTK* | *WNK1* | *ERAP1* | *ARHGDIB* | *BCAT2* | *PDCD11* | *C19orf48* | *KDM5D* | *ZNFX1* |
| hematopoietic | bone marrow | 46 | 84 | 17 | 24 | 190 | 10 | 11 | 17 | 9 | 13 | 19 | 249 | 6 | 10 | 20 | 16 | 760 | 4 | 3 | 33 | 6 | 12 |
| | lymph node | 45 | 25 | 23 | 50 | 37 | 9 | 26 | 8 | 9 | 14 | 5 | 207 | 8 | 6 | 25 | 30 | 605 | 10 | 9 | 17 | 0 | 12 |
| | spleen | 56 | 28 | 22 | 36 | 29 | 7 | 26 | 11 | 8 | 3 | 2 | 176 | 6 | 0.9 | 21 | 26 | 413 | 10 | 7 | 11 | 18 | 17 |
| | *mean* | *49* | *46* | *21* | *37* | *85* | *9* | *21* | *12* | *9* | *10* | *9* | *211* | *7* | *6* | *22* | *24* | *593* | *8* | *6* | *20* | *8* | *14* |
| | *sd* | *5* | *27* | *3* | *11* | *74* | *1* | *7* | *4* | *0* | *5* | *7* | *30* | *1* | *4* | *2* | *6* | *142* | *3* | *2* | *9* | *7* | *2* |
| non-hematopoietic | adipose tissue | 3 | 4 | 15 | 8 | 4 | 4 | 14 | 5 | 10 | 0.8 | 0.7 | 112 | 3 | 0.8 | 41 | 49 | 74 | 11 | 4 | 7 | 0 | 9 |
| | adrenal gland | 3 | 16 | 13 | 8 | 18 | 4 | 17 | 2 | 45 | 0 | 0 | 151 | 4 | 0 | 15 | 13 | 50 | 31 | 4 | 10 | 0 | 8 |
| | animal ovary | 0.6 | 2 | 8 | 15 | 2 | 4 | 19 | 5 | 33 | 0 | 1 | 232 | 11 | 0 | 20 | 18 | 17 | 21 | 8 | 22 | 0 | 9 |
| | appendix[C] | 34 | 30 | 28 | 32 | 51 | 8 | 25 | 6 | 9 | 7 | 5 | 191 | 7 | 4 | 26 | 34 | 421 | 9 | 9 | 15 | 0 | 15 |
| | bladder | 7 | 12 | 28 | 20 | 29 | 6 | 21 | 3 | 14 | 4 | 3 | 350 | 7 | 2 | 24 | 29 | 246 | 34 | 6 | 10 | 15 | 12 |
| | cerebral cortex | 2 | 18 | 11 | 7 | 10 | 4 | 13 | 1 | 6 | 0 | 0 | 69 | 3 | 0 | 32 | 12 | 30 | 7 | 4 | 4 | 9 | 9 |
| | colon | 8 | 5 | 36 | 8 | 22 | 5 | 20 | 6 | 12 | 3 | 7 | 155 | 4 | 4 | 15 | 28 | 53 | 18 | 6 | 13 | 10 | 10 |
| | duodenum | 12 | 6 | 33 | 9 | 97 | 5 | 34 | 5 | 9 | 4 | 5 | 249 | 5 | 3 | 8 | 46 | 54 | 12 | 4 | 13 | 22 | 8 |
| | endometrium | 3 | 8 | 13 | 14 | 11 | 5 | 23 | 4 | 11 | 2 | 1 | 271 | 8 | 0.7 | 18 | 19 | 53 | 13 | 8 | 15 | 0 | 9 |
| | esophagus | 0.8 | 5 | 28 | 13 | 62 | 5 | 15 | 3 | 13 | 3 | 2 | 1048 | 9 | 4 | 39 | 13 | 49 | 11 | 7 | 12 | 12 | 6 |
| | gall bladder | 5 | 38 | 29 | 15 | 60 | 5 | 23 | 3 | 14 | 1 | 1 | 368 | 7 | 0.7 | 21 | 22 | 137 | 10 | 4 | 6 | 16 | 14 |
| | heart | 0 | 1 | 14 | 5 | 5 | 2 | 8 | 2 | 10 | 0 | 0 | 156 | 4 | 0 | 29 | 9 | 47 | 21 | 2 | 3 | 0 | 7 |
| | kidney | 3 | 3 | 22 | 5 | 88 | 3 | 28 | 2 | 16 | 0 | 0 | 155 | 3 | 0 | 54 | 13 | 44 | 16 | 4 | 8 | 0 | 8 |
| | liver | 0 | 54 | 8 | 3 | 30 | 2 | 10 | 3 | 7 | 0.7 | 0.7 | 13 | 2 | 0 | 11 | 12 | 51 | 3 | 2 | 2 | 3 | 4 |
| | lung | 13 | 15 | 25 | 18 | 19 | 4 | 18 | 4 | 9 | 0.6 | 2 | 407 | 8 | 0 | 27 | 23 | 204 | 16 | 5 | 8 | 0 | 15 |
| | pancreas | 0 | 3 | 23 | 3 | 6 | 1 | 4 | 1 | 6 | 0 | 0.9 | 66 | 2 | 0 | 4 | 3 | 6 | 14 | 2 | 13 | 2 | 2 |
| | placenta | 7 | 16 | 60 | 13 | 96 | 7 | 20 | 5 | 9 | 7 | 2 | 307 | 5 | 4 | 20 | 39 | 91 | 14 | 5 | 18 | 5 | 9 |
| | prostate | 2 | 4 | 30 | 12 | 8 | 62 | 19 | 5 | 13 | 4 | 0 | 145 | 7 | 0.6 | 17 | 16 | 35 | 21 | 5 | 47 | 17 | 7 |
| | salivary gland | 5 | 3 | 20 | 6 | 4 | 2 | 10 | 3 | 13 | 0 | 0.7 | 92 | 4 | 0 | 7 | 9 | 45 | 14 | 4 | 9 | 0 | 5 |
| | skin | 0.9 | 0 | 4 | 13 | 12 | 8 | 20 | 11 | 9 | 3 | 2 | 334 | 19 | 3 | 34 | 26 | 40 | 8 | 11 | 18 | 0 | 9 |
| | small intestine | 12 | 9 | 31 | 10 | 43 | 5 | 36 | 6 | 9 | 4 | 3 | 210 | 4 | 3 | 10 | 43 | 72 | 12 | 4 | 13 | 35 | 8 |
| | stomach | 10 | 9 | 32 | 10 | 28 | 4 | 23 | 5 | 10 | 2 | 3 | 408 | 5 | 2 | 15 | 25 | 56 | 17 | 4 | 12 | 17 | 9 |
| | testis | 2 | 2 | 53 | 9 | 5 | 10 | 16 | 15 | 12 | 12 | 0.6 | 38 | 40 | 29 | 39 | 8 | 14 | 12 | 15 | 18 | 11 | 14 |
| | thyroid | 1 | 5 | 20 | 8 | 13 | 8 | 22 | 2 | 22 | 0.6 | 1 | 475 | 8 | 0 | 17 | 14 | 65 | 14 | 6 | 29 | 0 | 13 |
| | *mean* | *6* | *11* | *24* | *11* | *30* | *7* | *19* | *4* | *13* | *2* | *2* | *250* | *7* | *3* | *23* | *22* | *81* | *15* | *6* | *14* | *7* | *9* |
| | *sd* | *7* | *13* | *13* | *6* | *30* | *12* | *7* | *3* | *9* | *3* | *2* | *208* | *8* | *6* | *12* | *13* | *89* | *7* | *3* | *9* | *9* | *3* |
| | ***ratio hem/nonhem*** | ***9*** | ***4*** | ***1*** | ***3*** | ***3*** | ***1*** | ***1*** | ***3*** | ***1*** | ***4*** | ***5*** | ***1*** | ***1*** | ***2*** | ***1*** | ***1*** | ***7*** | ***1*** | ***1*** | ***2*** | ***1*** | ***1*** |
| | | **hemat** | **rel hem** | **broad** | **broad** | **broad** | **broad** | **broad** | **broad** | **broad** | **rel hem** | **rel hem** | **broad** | **broad** | **broad** | **broad** | **broad** | **hemat** | **broad** | **broad** | **broad** | **broad** | **broad** |

[A]Tissues were separated between hematopoietic (bone marrow, lymph node, spleen) and non-hematopoietic.

[B]Numbers represent fragments per kilobase of transcript per million mapped reads. The threshold for expression is 0.5.

[C]No difference in ratios is observed when appendix is excluded from the analysis

Example: Value of HMHA1 in bone marrow: 46 fragments were mapped per 1000 basepairs of the HMHA1 transcript per 1 million mapped reads.

Mean expression level of bone marrow, lymph node and spleen is divided by the mean expression level of 24 other tissue types.

High ratios represent selective expression in hematopoietic tissues, low ratios represent tissue type independent expression

**Classification of ratio hem/nonhem:**

| | |
|---|---|
| **1 - 3** | **expressed in all tissues** |
| **4 - 6** | **more frequent in hematopoietic tissues** |
| **7 - 9** | **selective expression in hematopoeitic tissues** |

**Supplemental experimental procedures: Mixed models statistical analysis**

Linear mixed-effects models are extensions of standard linear regression models including random effects to model correlation structures between dependent measurements. Parameters describing both fixed and random effects are estimated with a maximum likelihood approach. The estimated parameters can be used to calculate model-based predictions of outcome values for different values of the predictor variables, as shown in the formulas and graphs below. As stimulators were tested EBV-LCL and fibroblasts (FB). FB stimulation was tested after culturing of FB for 4 days in medium alone, in the presence of 200 IU/mL IFN-γ (stimFB-IFNγ) or in the presence of IFN-γ in combination with 10 ng/mL TNF- α and 100 IU/mL Interleukin-4 (stimFB-IT4). The data of the experiments with stimFB-IT4 are not presented in the main paper. The statistical model for FB however was built on experiments including this stimulator type and therefore the data are presented here. The effect of various ratios of stimulators was measured by production of IFN-γ by the T-cell clone and is expressed in fg per T cell per 20 h.

Predictors:

Stimulators:

EBV-LCL

steady-state fibroblasts (steady-state FB)

FB treated with IFN-γ (stimFB-IFNγ)

FB treated with IFN-γ, TNF-α and IL-4 (stimFB-IT4)

Ratios (as continuous variable):

0.11 0.33 1.0 3.0 9.0

Groups:

Selective GvL = 0

GvL+GvHD = 1

Outcome: IFN-γ production (fg/T cell/20h)

On the following pages parameter estimates, formulas and graphical representations for each of the stimulators are given.

In formulas: ‘log’ indicates $^{e}$log

‘I’ indicates an indicator variable (1 if condition fulfilled, 0 otherwise).

**Stimulator = EBV-LCL**

Estimated mixed model parameters:

| | Effect | Value | Std.Error | *p* -value |
|---|---|---|---|---|
| B0 | Fixed (intercept) | 1.56 | 0.27 | <0.001 |
| B1 | Ratio | 0.32 | 0.02 | <0.001 |
| B2 | groupGVHD | 0.13 | 0.32 | 0.69 |
| B3 | ratio*groupGVHD | 0.04 | 0.03 | 0.20 |

*: interaction

formula to predict outcomes based on the mixed model:

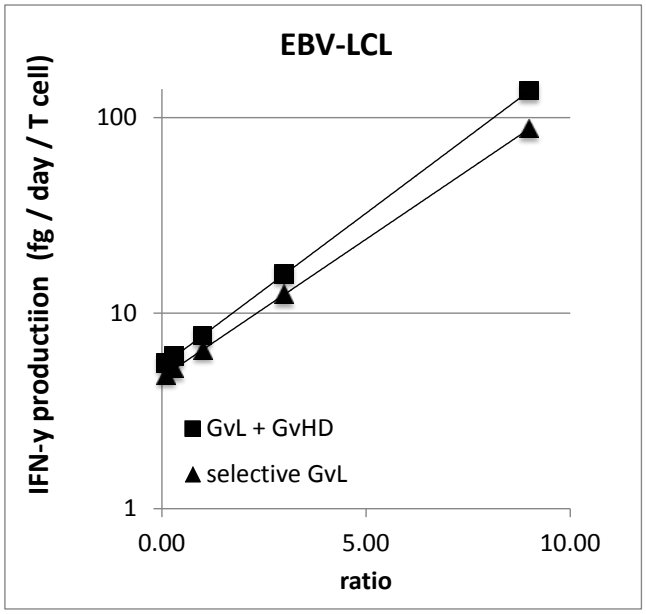
$\log(\text{outcome} + 0.1) = B0 + (B1*\text{ratio}) + B2*(I_{group=GVHD}) + B3*\text{ratio}*(I_{group=GVHD})$

with values for ratio= 0.11 – 9.0

$\log(\text{outcome} + 0.1) = 1.56 + 0.32*\text{ratio} + 0.13*(I_{group=GVHD}) + 0.04*\text{ratio}*(I_{group=GVHD})$

*p*-value=0.26 for the difference in outcome at ratio=9.0

Plotted:



**Stimulator=FB (steady state / IFN-γ / IFN-γ+TNF-α+IL-4)**

Estimated mixed model parameters:

| | Effect | Value | Std.Error | p-value |
|---|---|---|---|---|
| B0 | Fixed (Intercept) | -0.85 | 0.40 | 0.03 |
| B1 | log(ratio) | 0.30 | 0.03 | <0.001 |
| B2 | groupGVHD | 0.87 | 0.46 | 0.097 |
| B3 | stimFB-IFNy | 0.53 | 0.09 | <0.001 |
| | stimFB-IT4 | 0.69 | 0.09 | <0.001 |
| B4 | log(ratio)*groupGVHD | 0.03 | 0.03 | 0.40 |
| B5 | groupGVHD*stimFB-IFNy | 0.32 | 0.11 | 0.003 |
| | groupGVHD*stimFB-IT4 | <0.001 | 0.11 | 1.00 |

*: interaction

**Stimulator = steady-state FB**

formula to predict outcomes based on the mixed model:

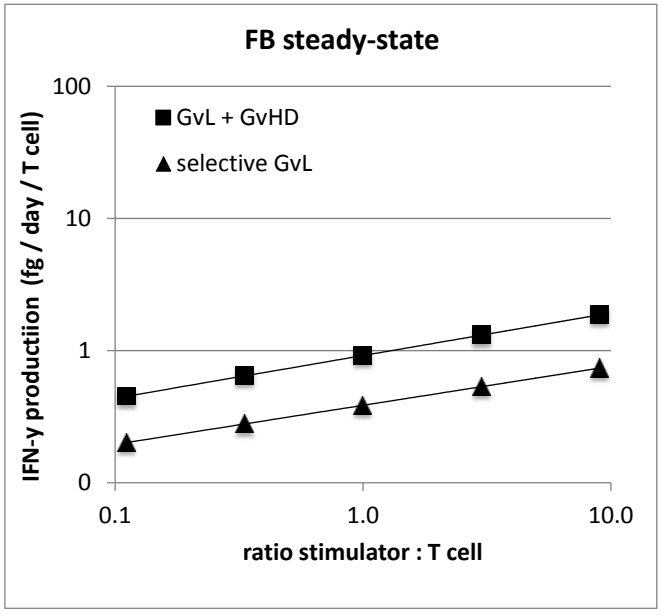
$\log(\text{outcome} + 0.1) = B0 + B1*\log(\text{ratio}) + B2*(I_{group=GVHD}) + B3*(I_{stim=FB\text{-}IFNy}) + B4*\log(\text{ratio})*(I_{group=GVHD}) + B5*(I_{stim=FB\text{-}IFN})*(I_{group=GVHD})$

with values for ratio= 0.11 – 9.0:

$\log(\text{outcome} + 0.1) = -0.85 + 0.30*\log(\text{ratio}) + 0.87*(I_{group=GVHD}) + 0 + 0.03*\log(\text{ratio})*(I_{group=GVHD}) + 0$

*p*-value=0.10 for the difference in outcome at ratio=1.0

Plotted:



**Stimulator = IFN-γ treated FB**

formula to predict outcomes based on the mixed model:

$\log(\text{outcome} + 0.1) = B0 + B1*\log(\text{ratio}) + B2*(I_{group=GVHD}) + B3*(I_{stim=FB\text{-}IFNy}) + B4*\log(\text{ratio})*(I_{group=GVHD}) + B5*(I_{stim=FB\text{-}IFN})*(I_{group=GVHD})$

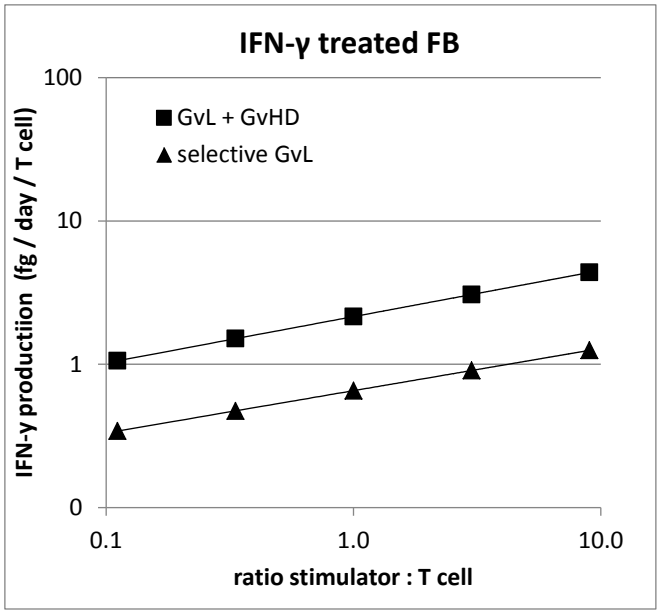
with values for ratio= 0.11 – 9.0

$\log(\text{outcome} + 0.1) = -0.85 + 0.30*\log(\text{ratio}) + 0.87*(I_{group=GVHD}) + 0.53 + 0.03*\log(\text{ratio})*(I_{group=GVHD}) + 0.32*(I_{group=GVHD})$

The *p*-value for difference in outcome between the groups noGVHD and GVHD for FB-IFN-γ was calculated using the FB model but changing the reference category to FB-IFN-γ.

*p*-value=0.03 for the difference in outcome at ratio=1.0

Plotted:



**Stimulator = IFN-γ, TNF-α and IL-4 treated FB**

formula to predict outcomes based on the mixed model:

$\log(\text{outcome} + 0.1) = B0 + B1*\log(\text{ratio}) + B2*(I_{group=GVHD}) + B3*(I_{stim=FB\text{-}IT4}) + B4*\log(\text{ratio})*(I_{group=GVHD}) + B5*(I_{stim=FB\text{-}IT4})*(I_{group=GVHD})$

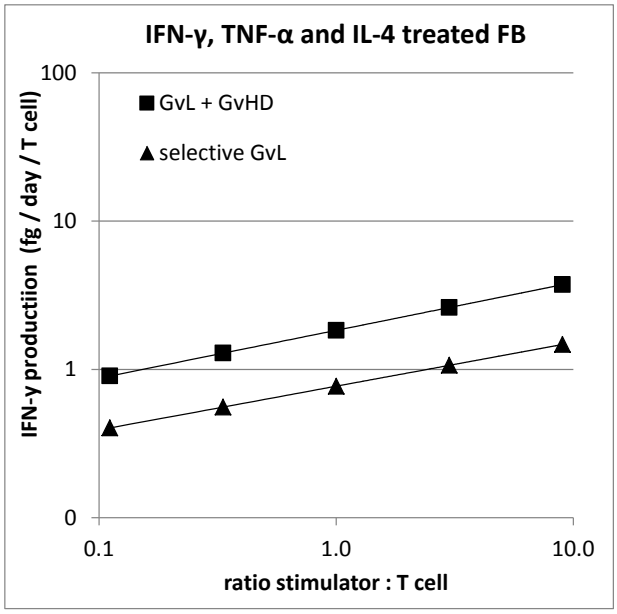
with values for ratio= 0.11 – 9.0

$\log(\text{outcome} + 0.1) = -0.85 + 0.30*\log(\text{ratio}) + 0.87*(I_{group=GVHD}) + 0.69 + 0.03*\log(\text{ratio})*(I_{group=GVHD}) + 0.0004*(I_{group=GVHD})$

The *p*-value for difference in outcome between the groups noGVHD and GVHD for FB-IT4 was calculated using the FB model but changing the reference category to FB-IT4.

*p*-value=0.10 for the difference in outcome at ratio=1.0

Plotted:



**Supplemental experimental procedures: Primers used for amplification and sequencing of MiHA-encoding genes**

| WGAs results | | NCBI Reference Sequence | | | PCR product[B] | Primer sequence 5' ► 3' | |
|---|---|---|---|---|---|---|---|
| SNP ID | gene name | Accession | Length (bp) | CDS[A] | | Forward | Reverse |
| rs1719152 | *CCL4* | NM_002984 | 667 | 80 ► 358 | 32 ► 583 | TGAGTTCTGCAGCCTCACCTCTGA | ACAGTGACAGTGGACCATCCCCA |
| rs2071915 | *TMEM8A* | NM_021259 | 3.656 | 130 ► 2,445 | 231 ► 1,018 | CGGCTACAGCGGGAAGAGCGA | CAGCACTGAAAGCCACTGTCCC |
| | | | | | 737 ► 1,409 | CCCTCCTCTCCCATCCCAGCTAC | AAGGGCGAGGCAGCATTCACG |
| rs11541046 | *MOB3A* | NM_130807 | 3.347 | 319 ► 972 | 361 ► 919 | ACATTCCGCCCCAAGCGCAA | CGATGAGGCCGAACTCCTTGACG |
| rs305087 | *C16ORF99* | mRNA not known, PCR performed on genomic DNA | | | 1 ► 368 | ACGCACACAAACACCATCCTGC | ATCTTCCCTGCCCTCCCTCACC |
| rs1049564 | *PNP* | NM_000270 | 2.438 | 147 ► 1,016 | 132 ► 742 | GCGTCTGCGAGACCATGGAGAA | TGGGGCCTGCCACCATCACATA |
| rs12292394 | *NCAPD3* | NM_015261 | 5.661 | 607 ► 5,103 | 2,163 ► 2,753 | TTCCGAACCCTCAGGGGAGATCA | GCCGAATGTTCCGTGCCAGT |
| rs4918752 | *ZDHHC6* | NM_022494 | 2.187 | 425 ► 1,666 | 744 ► 1,400 | ACAAGGCACCACGTTCACATCACTG | ATTCAGAGGGCAGCAGGCACC |
| rs5745001 | *POLE* | NM_006231 | 8.024 | 210 ► 7,070 | 437 ► 1,418 | GCGCTTAGGCAGTGCAGTGGA | GATGCACTGGGGCGCCTTGT |
| | | | | | 1,373 ► 2322 | CTTCCAGAAGGACAGCCAGGGG | GAGCTGGCCCCTCTGGGAACA |
| | | | | | 2,312 ► 3,261 | GGGGCCAGCTCGGGCCTTTC | ACAGCACGTCCAGCCAGTAGTCA |
| | | | | | 2,973 ► 3,954 | GTCACCCGCTCAGAGAACAGCA | CAGTCGGCGTGAGGTCCTGG |
| | | | | | 3,934 ► 4,637 | CCCAGGACCTCACGCCGACT | GAACTGGGCCAGAGAGCGCAT |
| | | | | | 4,618 ► 5,539 | TGCGCTCTCTGGCCCAGTTCA | CTGGCAGCCTGACCACCCGT |
| | | | | | 5,326 ► 6,031 | TGGAGTTCGATGACCAAGCCACT | TGGGAGTCTTGCAGTCCACAGTGA |
| | | | | | 6,211 ► 7,014 | CAGCGTACATCGTGGCCGTGT | ACGACATGCCGTAGTGCTGGG |
| rs4547833 | *RWDD4A* | NM_152682 | 2.593 | 227 ► 793 | 26 ► 754 | TCGTAGCCCACAGCCCACTGC | CACAACATCAACCCAGTTCCAGCC |
| rs7233405 | *NDC80* | NM_006101 | 2.209 | 183 ► 2,111 | 15 ► 745 | GCGTAATGACGTCAGCGCC | AGCCAAACTAAGGCTGCCAC |
| | | | | | 702 ► 1483 | GCTCCTCATACATGGCCTCAC | CCTTTGGAATTCTCAGCACCT |
| | | | | | 1,400 ► 2,042 | AGCGATTGAAACACAATTAGCAGA | CGAGAGATCTTCTGACATGCATT |
| rs608962 | *TTK* | NM_001166691 | 3.019 | 124 ► 2694 | 124 ► 1,515 | ATGGAATCCGAGGATTTAAGTG | CAGCTCATGTAATCATCCAAG |
| | | | | | 1,343 ► 2,215 | GCAGATTCCGGAGTTAGCC | ACATATCTTTGATTGCTTCTGG |
| | | | | | 2,132 ► 2,963 | ATGCAACCAGATACAACAAGTG | CTTGCTATCCACCCACTATTC |
| rs26654 | *ERAP1* | NM_001040458 | 5.085 | 348 ► 3173 | 140 ► 816 | GTTTACCCTTTCCCCAGCTC | CCGAAAGATTGCCAGCATAG |
| | | | | | 669 ► 1,290 | AGGAAGGGAGCTGGAGAGAG | GAAAGTCGGGAATAGCAGCA |
| | | | | | 1,268 ► 1,973 | TCTTGCTGCTATTCCCGACT | TACATTCCTCCCCCTCACTG |
| | | | | | 1,890 ► 2,583 | GTGGATGTGAAAACCATGATGA | TCTGTACGCACGGCTGATAG |
| rs4703 | *ARHGDIB* | NM_001175 | 1.216 | 105 ► 710 | 69 ► 701 | CCGGACAGAGACGTGAAGC | CCACTCCTTCTTAATCGACAG |
| | | | | | 510 ► 1,216 | GTGGATAAAGCAACATTTATGG | CTCAGCCAGAACACACACAG |
| rs4801775 | *BCAT2* | NM_001190 | 1.616 | 38 ► 1216 | 510 ► 972 | ATCGAAGTGGACAAGGACTG | GAGCCATGTCCAGTAGACTC |
| | | | | | 918 ► 1,472 | CTGAATGGTGTTATCCTGCCTG | GCACGACAAGGAGTAATGGG |
| rs4801853 | *C19ORF48* | NM_199250 | 1.667 | 898 ► 1,251 | 776 ► 1,425 | CAGCCCACCATGGACAGGGGTA | TCTGGCCAGTGACACCCACAG |
| rs238199 | *ZNFX1-AS1* | NR_003604 | 1.020 | n.a. | 9 ► 694 | CAGGGTGGAGAGCACGAG | CCCGCATTCATCCTGGTTCA |
| | | | | | 458 ► 980 | ATTCGTTCTTTCGCGTCTGC | ATGCAGGTAGGCAGTTAGAAAT |
| rs238221 | *ZNFX1* | NM_021035 | 7.371 | 248 ► 6,004 | 2,828 ► 3,300 | GCTAAAATGCTTCTGGCCATGAG | GCAATGGTATGGGCCTCAAG |

[A] first and last bp of the coding sequence [B] first to last bp of the PCR product

**Supplemental experimental procedures: Primers used for cDNA synthesis, amplification and sequencing of TCR-β**

| Step | Orientation | Name | 5'-to-3' DNA-sequence |
|---|---|---|---|
| reverse transcription | TSO | SA.rt | AAGCAGTGGTATCAACGCAGAGTACGCrGrGrG |
| | rev | TRB.rt | CACGTGGTCGGGGWAGAAGC |
| amplification | for | SA.pcr | GAGTTCAGACGTGTGCTCTTCCGATCTAAGCAGTGGTATCAACGCAGAGTACGC |
| | rev | TCRβ.1 | CCTACACGACGCTCTTCCGATCTGTGGGAACACCTTGTTCAGGTCCTC |
| | rev | TCRβ.2 | CCTACACGACGCTCTTCCGATCTGTGGGAACACGTTTTTCAGGTCCTC |
| barcoding PacBio | for | SA.bc-xx | GGTAG [ SAbc.xx ] CTTAAGCAGTGGTATCAACGCAGAGTACG |
| | | SAbc.01 GCGCTCTGTGTGCAGC | SAbc.07 AGAGACACGATACTCA |
| | | SAbc.02 TCATGAGTCGACACTA | SAbc.08 CTGCTAGAGTCTACAG |
| | | SAbc.03 TATCTATCGTATACGC | SAbc.09 AGCACTCGCGTCAGTG |
| | | SAbc.04 ATCACACTGCATCTGA | SAbc.10 TCATGCACGTCTCGCT |
| | | SAbc.05 ACGTACGCTCGTCATA | SAbc.11 AGAGCATCTCTGTACT |
| | | SAbc.06 TGTGAGTCAGTACGCG | SAbc.12 CGCATCGACTACGCTA |
| | rev | TR_D5xx | AATGATACGGCGACCACCGAGATCTACAC [ D5xx ] GACACTCTTTCCCTACACGACGCTCTTCCGATCT |
| | | D501 CGTGAT | D507 GATCTG |
| | | D502 ACATCG | D508 TCAAGT |
| | | D503 GCCTAA | D509 CTGATC |
| | | D504 TGGTCA | D510 AAGCTA |
| | | D505 CACTGT | D511 GTAGCC |
| | | D506 ATTGGC | D512 TACAAG |
| barcoding Illumina HiSeq | for | TR_D7xx | CAAGCAGAAGACGGCATACGAGAT [ D7xx ] GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC |
| | | D701 TTGACT | D704 GGACGG |
| | | D702 GGAACT | D705 CTCTAC |
| | | D703 TGACAT | |
| | rev | TR_D5xx | AATGATACGGCGACCACCGAGATCTACAC [ D5xx ] GACACTCTTTCCCTACACGACGCTCTTCCGATCT |

TSO=template switching oligo W=A/T rG=RNA