Dynamic clonal progression in xenografts of acute lymphoblastic leukemia with intrachromosomal amplification of chromosome 21

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Received: May 15, 2017.
Accepted: February 8, 2018.
Pre-published: February 15, 2018.
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Supplementary methods

Determination of xenograft experimental end points. Mice were checked daily for signs of ill health and routinely weighed once a week or more often if indicated. Mice were culled at end stage disease as defined by: weight gain of >20%, weight loss reaching 20% at any time or >10% maintained for 72 hours compared with weight at the start of the experiment, starey coat, porphyrin staining of eyes and nose or sunken eyes, persistent skin tenting, immobility, unresponsive or very aggressive behaviour, loss of upright stance, laboured respiration, blood staining or other discharge, signs of anaemia including extreme paleness of feet, tail and ears.

Immunophenotyping of xenografts cells. Proportions of human and mouse cells were determined by flow cytometry using anti-human CD10 FITC or CD19 PE in combination with anti-murine-CD45, PE-Cy7 and anti-TER119 PE-Cy7, as previously described.(1) Selected xenografts were further characterised using a panel of anti-human CD19-APC, CD10-PE, CD34-Cy5.5 and CD38-FITC together with anti-mouse CD45-Cy7-A. Cells were initially gated on a lymphocyte population defined by forward and side scatter and on the anti-mouse CD45 negative population. For each sample, identical markers were used to divide the gated cells into human CD19, CD10, CD45 and CD38 negative and positive populations based on median fluorescence intensity (MFI) of the unstained cells. MFI and % positive cells (supplementary table 5) refer to the total gated population with the exception of a minority of samples where the proportion of cells staining positively for mouse CD45 was high and a significant proportion of gated cells, negative for all human markers, was assumed to be of mouse origin and gated out. All antibodies were obtained from BD Biosciences (Oxford, UK) and stained cells were analysed with a BD FACSCanto II cell analyser and processed with FlowJo (Oregon, USA) software.

Lentiviral transduction, In-vitro culture and in-vitro, in-vivo and ex-vivo imaging of xenograft cells. SLIEW lentivirus was produced as previously described(1) with the modification that HEK293FT cells (Thermo Fisher Scientific) and EndoFectin (GeneCopoeia) transfection reagent were used. Lentiviral stocks were concentrated by ultracentrifugation for 2 hours at an RCF of 83018 and re-suspended in Serum free expansion medium (SFEM) (Stemcell Technologies) supplemented with 10% FCS before titrating in 293FT cells. For each patient analysed, 1.2x10^7 xenograft cells in SFEM supplemented with 10% FCS and 10ng/ml IL-7, were transduced at a multiplicity of infection of 1.0 in delta T25 tissue culture flasks. Following overnight incubation medium was replaced and approximately 72 hours after transduction 0.5x10^6 cells were analysed for EGFP expression by FACS, 1.5x10^6 cells were transplanted into each of two NSG mice and 6x10^6 cells were re-suspended in αMEM with 10% FCS for in-vitro co-culture. Co-cultures were established by plating 2x10^6 transduced xenograft cells in
1ml of medium on MS-5 cells (DSMZ # ACC441) (83,000 cells plated/well in 12 well plates) that had been irradiated with 50 Gy 24hrs after plating. EGFP expression of co-cultured cells was assessed using an EVOS fluorescent inverted microscope (Life technologies). 2D and 3D bioluminescent whole body imaging of mice transplanted with transduced xenograft cells was performed with an IVIS Spectrum (Caliper Life Sciences, Hopkington, MA, USA) 10 minutes after intraperitoneal injection of 100ul of D-luciferin (30mg/ml, VivoGlo, Promega). Immediately following final whole body imaging mice were killed and organs dissected and imaged ex-vivo. Quantification of luminescent signals and 3D reconstructions were performed using Living image version 4.3.1 software (Caliper Life Sciences).

The proportion of leukaemia cells expressing SLIEW in spleen preparations was measured by immunostaining and FACS by determining numbers of EGFP+ve cells in the human CD19+ve and/or CD10+ve, murine CD45/TER119−ve population.

**Histopathology.**

After transfer to 10% neutral buffered formalin immediately after dissection, tibias and sternums were decalcified, paraffin embedded, sectioned and stained with haematoxylin and eosin (H&E) or human antibodies, according to standard histopathological techniques, by the department of Cellular Pathology, Newcastle upon Tyne Hospitals NHS Foundation Trust. Mouse heads preserved in formalin were sectioned and stained as previously described.(2)

**Transmission Electron Microscopy (TEM).** TEM was performed by the Electron Microscopy Unit, Faculty of Medical Sciences, Newcastle University. Small trephines of bone were fixed in 5% glutaraldehyde in 3% PFA in phosphate buffer overnight. Samples were then rinsed in phosphate buffer before being placed in EDTA for a minimum of 24hrs at 40°C. After rinsing in phosphate buffer the samples were placed in 1% osmium tetroxide for 2hrs at RT. They were then rinsed in buffer, dehydrated through a graded series of acetone and embedded in TAAB epoxy resin (medium). After polymerisation at 60°C the samples were sectioned on an ultramicrotome and ultrathin sections (70nm) were picked up on copper grids, stained with uranyl acetate and lead citrate and viewed on a Philips CM100 TEM at 100kV.

**RNA Sequencing.**

RNA was extracted from cells isolated from xenograft spleens and purified over Ficol using an RNeasy kit (Qiagen). Illumina RNA sequencing was performed by Aros Applied Biotechnology, Eurofins Genomics Group, Aarhus, DK.

**Analysis of RAS pathway mutations.** Whole exome sequencing (WES) was performed on selected xenografts derived from patient 1 using library preparation and sequencing techniques as previously
described (3). To filter mouse sequences from xenograft samples, the programme, Xenome v 1.0.1, was used to simultaneously align reads to version GRCm38 and hg19 of the mouse and human genomes, respectively (4). Default settings of xenome index and classify were used to designate reads as of human or mouse origin, ambiguous, both or neither, with only unambiguous human reads used for subsequent analysis. Reads were aligned using Burrows-Wheeler Aligner (BWA) version 0.7.12 (5, 6) and MuTect1.7 (7) was used for calling somatic single nucleotide variants (SNVs) following Broad Best practices (8, 9). Calls were derived from jointly realigned and recalibrated tumour normal (patient remission sample) BAM files and annotated with Ensembl Variant Effect Predictor (VEP) version 83 (10). Patient BAM files were re-analysed manually in Integrated Genome Viewer (11) to assess levels of SNV affecting the RAS pathway, predicted to have an oncogenic role (mutations located within coding regions and predicted to affect protein function using SIFT, Polyphen2 and Mutation Taster) and identified by WES in xenografts but not previously in the patient (KRAS, chr12:25380276, T->A; NRAS, chr1:115258744, C->T).

To validate clonal SNV affecting NF1, NRAS and KRAS and extend analysis to remaining patient 1 xenografts, affected exons were amplified and analysed by sanger-sequencing (Durham Genome Centre, Durham UK). Sequence traces were examined manually using FinchTV (Information Technologies, Inc, MO, USA). Target sequence amplification and Sanger-sequencing was also used to analyse an NRAS mutation in xenografts, previously identified in patient 3. Levels of a FLT3 30 nucleotide internal tandem duplication (ITD) identified in patient 5 were assessed in xenografts and a relapse sample using targeted sequencing in combination with electrophoretic analysis of amplicon size using a 2100 Bioanalyzer (Agilent technologies, CA USA). All primer sequences used for exon amplification have been previously published (3).
Supplementary Figure 1. FACS analysis demonstrates immunophenotypic heterogeneity between xenografts. Expression of CD19, CD34, CD10 and CD38 are shown for bone marrow (BM) and/or spleen samples for xenografts from patients 1-4 as indicated. Scales are bi-exponential and units are arbitrary.
Supplementary figure 2. FACS analysis of iAMP21-ALL cells isolated from xenografts transduced with SLIEW lentivirus prior to transplant. FACS plots showing levels of EGFP expression (arbitrary units) in control (green trace) or transduced cells (purple infill). Live cells were gated on forward and side scatter as shown in the right hand box. In all cases the proportion of cells with GFP levels outside the control range (marker by M1) were less than 1%.
Supplementary Figure 3. Serial bioluminescent imaging of all NSG mice transplanted with pSLIEW transduced iAMP21-ALL xenograft derived cells. Measurement of luciferase activity demonstrates variation in the rate of spread of leukaemia from the site of injection to other bones and organs. Spleen involvement (marked by red arrows) was most obvious in 1aSLIEW, 1bSLIEW, 2aSLIEW, and 2bSLIEW but undetectable in 4a/bSLIEW. All captured images are shown. Scale is radiance (p/sec/cm²/sr).
Supplementary Figure 4. Histological sections of control and SLIEW transduced iAMP21-ALL xenograft bone marrows.

Sections of NSG control and xenograft mouse tibias stained with H&E and anti-human CD19, CD45 and Ki67. 4aSLIEW, 4bSLIEW, 3aSLIEW, 1aSLIEW, 1bSLIEW and 2aSLIEW have packed homogeneous cells similar to those seen in iAMP21-ALL patient trephines (Figure 2G and Supplementary Figure 5), that stain positively for human CD19 and Ki67 and for CD45 in 4aSLIEW, 4bSLIEW, 1aSLIEW and 1bSLIEW only (morphology type A). 3bSLIEW and 2bSLIEW show loss of cellularity and evidence for apoptosis and stain negatively for CD19, CD45 and Ki67 (morphology type B). In each case the image shown is representative of all bone marrow in the section analysed. Scale bars are 50µm.
Supplementary Figure 5. iAMP21-ALL patient trephines. H&E stained sections through iAMP21-ALL trephine sections showing morphology similar to that of xenograft type A (Figure 2 and Supplementary Figure 4) with packed homogeneous cells and loss of vasculature and megakaryocytes. Patient karyotypes and demographic details are provided in supplementary table 2. In each case the image shown is representative of all bone marrow in the trephine section. Scale bars are 50µm.
Supplementary Figure 6. Transmission electron microscopy (TEM) of control and SLIEW transduced iAMP21-ALL xenograft bone marrows.

TEM of ultra-thin sections of xenograft bone marrow; 4a\textsuperscript{SLIEW}, 4b\textsuperscript{SLIEW}, 1a\textsuperscript{SLIEW} and 1b\textsuperscript{SLIEW} have homogeneous cells with high nuclear to cytoplasmic ratio (VL morphology), compared with the control section, they lack vascular structures and cellular heterogeneity. 2a\textsuperscript{SLIEW}, 2b\textsuperscript{SLIEW}, 3a\textsuperscript{SLIEW} and 3b\textsuperscript{SLIEW} show loss of cellularity and evidence for apoptosis, such as chromatin clumping and nuclear fragmentation (AP morphology). Each image is representative of 6 regions captured from a single tibia section. Scale bars are 10µm.
Supplementary Figure 7. SLIEW transduced Xenograft CNS histological sections.

A. H&E stained low powered image of a coronal section through the brain and skull of 1aSLIEW with major structures and areas of leukaemia infiltrate marked with arrows. Scale bar is 1 mm. Leukaemic deposit on the surface of the brain in the region marked in box 1 is shown in detail in B (stained with H&E) and in C (stained with anti-human CD45). Scale bars are 100µm. D-G H&E stained detail of the dural sinus region, marked by box 2 in A, showing varying degrees of leukaemic infiltrate of the leptomeninges marked by arrows, in xenografts from; 4bSLIEW (D), 2aSLIEW (also representative of 2bSLIEW) (E), 3aSLIEW (also representative of 3bSLIEW) (F) and 1aSLIEW (also representative of 1bSLIEW) (G). Scale bars are 100µm.
Supplementary figure 8. Histological sections of additional xenografts showing both A and B type morphology. Upper and lower left hand panels show low magnification image of H&E stained sections of fibia and sternum and of the fore limb of xenografts derived from a relapsed iAMP21-ALL (1°5Rb) and a high hyperdiploid ALL respectively (1°17a). Right hand panels show high magnification H&E and human anti-CD19 stained images of regions marked in the left hand panels by arrows. Regions 1 and 2 of the 1°5Rb section and region 1 of the 1°17a section show A-type morphology. Region 3 and 4 of 1°5Rb and region 2 of 1°17a show B type morphology. Scale bars are 1500um and 50um for low and high magnification images respectively.
Supplementary Figure 9. Histological sections of additional xenografts showing A type morphology only. High magnification images of bone marrow sections stained with H&E and CD19 taken from tibias, fibias or sternum of additional primografts. The regions shown are representative of all bone marrow observed in a single section of 1 or 2 bones. Scale bars are 50µm.
Supplementary Figure 10. Histological sections of additional xenografts showing patches of A-type morphology infiltrating apparently normal mouse bone marrow. Left hand panels show low magnification images of human anti-CD19 stained sections of iAMP21 xenografts showing areas of positively and negatively stained cells. Right hand panels show high magnification H&E and anti-CD19 staining for the same sections. In all three sections infiltrating leukaemia cells are organized into clumps with non-infiltrated areas resembling normal mouse bone marrow. The images shown are representative of a single section of 1 or 2 bones. Scale bars 300 and 50um for low and high magnification images respectively.
Supplementary Figure 11. SNP6.0 chromosome 21 CN profiles for all patient and xenograft samples analysed.

Samples from presentation (P), remission (R) and xenografts. With the exception of patient 1, profiles were indistinguishable between patient leukemia and/or across xenograft samples.
**Supplementary Tables**

**Supplementary Table 1.** Karyotypes and demographic details of iAMP21-ALL patient samples used to create xenografts

| Patient registration number | ID this study | Presentation (P)/relapse (R) | Karyotype* | Age (years) | Sex | Previously published patient ID |
|-----------------------------|--------------|-----------------------------|------------|-------------|-----|-------------------------------|
|                             |              |                             |            |             |     |                               |
| 23229                       | 1            | P                           | 47,XX,+10,der(21)r(21)[?][10] | 10          | F   | 437                           |
| 19578                       | 2            | P                           | 48,XY,+X,?t(6;20)[p1;q1],?t(7;9)[p1;p2], i(9)[q10],+12,der(21)[q?][9] | 11          | M   | 426                           |
| 21567                       | 3            | P                           | 52,XX,+9,-12,-12,+7mar[cp4] | 8           | F   | 430                           | 14 |
| 23317                       | 4            | R                           | 45,X,del(X)[q22],del(1)[p13],der(9;17)[q10;q10],del(10)[q22],del(11)[q14], add(21)[q22][4]/46,idem,+mar[4] | 17          | F   | 439                           |
| 24259                       | 5            | P                           | 46,XX,t(2;16)[p17q;q273]-21,+mar[5] | 13          | F   | 447                           | 7  |

**Samples which failed to engraft**

| Patient registration number | ID this study | Presentation (P)/relapse (R) | Karyotype* | Age (years) | Sex | Previously published patient ID |
|-----------------------------|--------------|-----------------------------|------------|-------------|-----|-------------------------------|
|                             |              |                             |            |             |     |                               |
| 19578**                     | 2            | R                           | Karyotype not available no RUNX1 amplification | 11          | M   | 426                           | 45 |
| 9028                        | 6            | P                           | 46,X,add(X)[q26-q28],77, der(21)dup(21)[q?][8] | 10          | F   | 511                           | 28 |
| 9864                        | 7            | P                           | 47,XY,dup(21)[q?],+dup(21)[q?][6] | 10          | M   | 512                           | 18 |

Age and white blood cell count (WBC) are at diagnosis. *Normal population excluded from abnormal karyotypes.  **Sample was from a case which relapsed in the CNS with no evidence for iAMP21 by interphase FISH of the bone marrow indicating that the blast count was low in this sample. Previously published IDs refer to previous publications; A - Harrison et al 2014(12) B - Ryan et al 2016(3).
Supplementary Table 2. Karyotypes and demographic details of iAMP21-ALL with bone marrow trephines used for histological comparison with iAMP21-ALL derived xenografts.

| Patient registration number | ID this study | Presentation (P)/relapse (R) | Karyotype** | Age (years) | Sex | Previously published patient ID |
|-----------------------------|--------------|-----------------------------|-------------|-------------|-----|---------------------------------|
| 23229*                      | 1            | P                           | 47,XX,+10,der(21)r(21)[q?][10] | 10          | F   | 437                             |
| 24259*                      | 5            | P                           | 46,XX,t(2;16)(p17?;q23),-21,+mar[5] | 13          | F   | 447                             |
| 5754                        | 8            | P                           | 46,XY,ider(21)[q10]dup(21)[q?][6] | 9           | M   | 478                             |
| 21795†                      | 9            | P                           | 46,XX[20]   | 8           | F   |                                 |
| 23982                       | 10           | P                           | 46,XX,add(21)[q2?][6] | 8           | F   | 444                             |
| 25821                       | 11           | P                           | 46,XX,del(?)[q2?],del[8][q22],add[15][q26],der(21)[cp10] | 25          | F   |                                 |
| 27421†                      | 12           | P                           | N/A         | 14          | F   |                                 |

Age was at diagnosis. *Patients 1 and 5 were also used to create xenografts. †iAMP21 identified by interphase FISH. **Normal population excluded from abnormal karyotypes. N/A, not available. Previously published IDs refer to previous publications; A - Harrison et al 2014(12) B - Ryan et al 2016(3).

Supplementary Table 3. Karyotypes and demographic details of additional B-ALL patient samples used to create xenografts examined histologically.

| Patient registration number | ID this study | Genetic subtype /Presentation (P) or relapse (R) | Karyotype** | Age (years) | Sex | Previously published patient ID |
|-----------------------------|--------------|-----------------------------------------------|-------------|-------------|-----|---------------------------------|
| 22322                       | 12           | iAMP21/P                                      | 46~47,XX,+?X,add(7)[p22],del(11)[q23],del(13)[q12q14],dup(21)[q22],+r | 8           | F   | 433                             |
| 20724*                      | 13           | iAMP21/P                                      | 46,XY,del(?)[(1;13)[q2?5;q12],-del(9)[p2?1],-11,-13,-15,del(16)[q10],-17,-21,-3mar[5]/45,XY,rob(15;21)[q10][q10][q10][cp10] | 3           | M   | 429                             |
| 23299                       | 14           | iAMP21/P                                      | 46,XY,der(1)[(1;13)[q2?5;q12],-del(9)[p2?1],-11,-13,-15,del(16)[q10],-17,-21,-3mar[5]/45,XY,rob(15;21)[q10][q10][q10][cp10] | 10          | M   | 595                             |
| 22340                       | 15           | iAMP21/P                                      | 46,XY,ider(21)?inv dup(21)[q1q2][9] | 10          | F   | 434                             |
| 24259*                      | 5            | iAMP21/R                                      | N/A         | 13          | M   | 447                             |
| 10420†                      | 16           | ETV6-RUNX1/P                                  | 46,XY,del(?)[13q23],add(8p),der(12)?(12;22)[p?q?],+21,-22,der(22)[q?22] | 3           | M   |                                 |
| 2058                        | 17           | Hyperdiploid/P                                | 52,XX,+X,+9,del(9)[p13],+14,+18,+21,+21 | 4           | F   |                                 |

Age was at diagnosis. *iAMP21 identified by interphase FISH. †ETV6-RUNX1 fusion identified by FISH. **Normal population excluded from abnormal karyotypes. N/A, not available. Previously published IDs refer to previous publications; A - Harrison et al 2014(12) B - Ryan et al 2016(3).
## Supplementary Table 4. Details of xenograft generation and ex-vivo analysis of tumour load.

| Xenograft id /sex of host | time to cull (weeks) | spleen weight/tumour load ** | CD19 | CD10 | +ve cells Bone marrow | +ve cells Spleen |
|---------------------------|----------------------|-----------------------------|------|------|-----------------------|-----------------|
| Patient 1; CD19 92%, CD10 94%, CD34 93%. | | | | | | |
| primary mice; 1.6x10^6 cells transplanted / mouse | | | | | | |
| 1°1a/M | 38 | 1.13g/NA | +ve | +ve | 40% | 36% |
| 1°1b/M | 30 | 1.43g/NA | +ve | +ve | 60% | 23% |
| secondary mice; 2x10^6 1°1B bone marrow cells transplant / mouse | | | | | | |
| 2°1a/F | 35 | 0.8g/6x10^4 | +ve | +ve | 50% | 32% |
| 2°1b/F | 24 | 0.33g/NA | +ve | +ve | 42% | 33% |
| tertiary mice; 2x10^6 2°1A spleen cells transplanted / mouse | | | | | | |
| 3°1a/F | 22 | 1.77g/8.9x10^5 | +ve | +ve | Ficol 90% | Ficol 95% |
| 3°1b/F | 23 | 1.6g/1.4x10^5 | +ve | +ve | Ficol 98% | Ficol 95% |
| 3°1c/M | 28 | enlarged*/*3.7x10^5 | +ve | +ve | NA | Ficol 96% |
| 3°1d/M | 28 | enlarged*/*1.8x10^5 | +ve | +ve | NA | Ficol 94% |
| 3°1e/M | 27 | 1.6g/8x10^4 | +ve | +ve | Ficol 92% | Ficol 95% |
| 3°1f/M | 27 | 1.78g/1.5x10^5 | +ve | +ve | Ficol 96% | Ficol 95% |
| 3°1g/M | 12 | 0.66g/2.5x10^4 | +ve | +ve | Ficol 99% | |
| Patient 2; CD19 85%, CD10 83%, CD34 80%. | | | | | | |
| primary mouse 3.3x10^5 transplanted | | | | | | |
| 1°2a/NA | 29 | 0.285g/NA | +ve | +ve | 40% | 27% |
| secondary mice; 1x10^6 1°2A spleen cells transplanted / mouse | | | | | | |
| 2°2a/F | 8 | 0.34g/NA | +ve | +ve | 6% | 5% |
| 2°2b/F | 22 | 0.73g/2.4x10^5 | +ve | +ve | 18% | Ficol 85% |
| 2°2c/F | 22 | 1.3g/1x10^5 | +ve | +ve | NA | NA |
| 2°2d/F | 29 | 1.0g/1.4x10^5 | +ve | +ve | Ficol 97% | |
| 2°2e/F | 29 | 1.5g/6x10^4 | +ve | +ve | NA | |
| 2°2f/F | 23 | 1.2g/1.5x10^5 | +ve | +ve | 51% | Ficol 85% |
| 2°2g/M | 25 | 1.3g/2.5x10^5 | +ve | +ve | 48% | Ficol 85% |
| Patient 3; CD19 85%, CD10, 84%, CD34 83%. | | | | | | |
| primary mice; 1x10^6 cells transplanted / mouse. | | | | | | |
| 1°3a/F | 25 | enlarged*/*NA | +ve | +ve | 21% | Ficol 87% |
| 1°3b/F | 35 | 3.6g/4x10^5 | +ve | +ve | NA | Ficol 99% |
| Secondary mice; 1x10^6 1°3B spleen cells transplanted / mouse. | | | | | | |
| 2°3a/M | 16 | 2.53g/4x10^5 | +ve | +ve | 38% | Ficol 92% |
| 2°3b/M | 16 | 0.28g/3.6x10^5 | +ve | +ve | 29% | Ficol 97% |
| 2°3c/M | 15 | 2.4g/7x10^4 | +ve | +ve | 36% | Ficol 95% |
| 2°3d/M | 16 | 3.6g/3.2x10^5 | +ve | +ve | 34% | Ficol 95% |
| 2°3e/M | 16 | 1.3g/6x10^4 | +ve | +ve | 32% | Ficol 95% |
| Patient 4; NA | | | | | | |
| Primary mice; 2x10^5 cells transplanted / mouse. | | | | | | |
| 1°4a/F | 22 | enlarged*/*NA | +ve | +ve | NA | Ficol 95% |
| 1°4b/F | 20 | 1.3g/1.4x10^5 | +ve | +ve | NA | Ficol 97% |
| 1°4c/F | 20 | 1.3g/9.7x10^5 | +ve | +ve | 95% | Ficol 96% |
Patient 5; CD19 56%, CD10 59%, CD34 62%.

Primary mouse; 1x10^6 cells transplanted.

| Patient | 1.5x10^6 transduced 3°1E spleen cells transplanted / mouse |
|---------|----------------------------------------------------------|
| 1a/F    | 53                                                       |
|         | 0.80g/6x10^8                                           |
|         | +ve                                                     |
|         | +ve                                                     |
|         | NA                                                      |
|         | Ficol 80%                                                |

Transduced with pSLIEW and analysed by in-vivo imaging and histology post-mortem

| Patient 1; 1.5x10^6 transduced 3°1E spleen cells transplanted / mouse |
|----------------------------------------------------------|
| 1a/F | 16 | 0.53g/NA | +ve | NA | NA | Ficol 92% |
| 1b/F | 18 | 0.88g/NA | +ve | NA | NA | Ficol 92% |

| Patient 2; 1.5x10^6 transduced 2°2E spleen cells transplanted / mouse |
|----------------------------------------------------------|
| 2a/F | 16 | 0.40g/NA | +ve | NA | NA | Ficol 86% |
| 2b/F | 14 | 0.55g/2x10^6 | +ve | NA | NA | Ficol 89% |

| Patient 3; 1.5x10^6 transduced 2°3D spleen cells transplanted / mouse |
|----------------------------------------------------------|
| 3a/F | 15 | 0.23g/NA | +ve | NA | NA | Ficol 85% |
| 3b/F | 15 | 0.27g/NA | +ve | NA | NA | Ficol 94% |

| Patient 4; 1.5x10^6 transduced 1°4B spleen cells transplanted / mouse |
|----------------------------------------------------------|
| 4a/F | 15 | 0.83g/NA | +ve | NA | NA | Ficol 89% |
| 4b/F | 14 | 0.81g/NA | +ve | NA | NA | Ficol 79% |

Ficol indicates that the sample was purified by Ficol gradient separation. * Spleen weight not recorded but splenomegaly noted. ** Tumour load refers to the total number of cells isolated (a variable proportion of cells are lost in the course of purification over Ficol). NA data not available.
Supplementary table 5. Xenograft Immunophenotypic data.

| id | %CD19+ve (MFI) | %CD10+ve (MFI) | %CD34+ve (MFI) | %CD38+ve (MFI) |
|----|----------------|----------------|----------------|----------------|
|    | BM | spleen | BM | spleen | BM | spleen | BM | spleen |
| Patient 1; CD19 92%, CD10 94%, CD34 93%. |
| primary mice |
| 1°1a | 100 (10920) | 100 (11171) | 100 (21462) | 100 (18458) | 52 (5224) | 51 (4623) | 18 (567) | 10 (474) |
| 1°1b | 100 (10133) | 100 (12845) | 99 (9642) | 100 (53563) | 44 (2940) | 78 (12640) | 3 (340) | 50 (1579) |
| secondary mice |
| 2°1a | 100 (28751) | 100 | 100 (61831) | 100 | 100 (19668) | 97 (4334) | |
| 2°1b | 100 (21717) | 100 | 100 (59035) | 100 | 96 (17696) | 97 (20089) | 88 (3028) | 91 (3234) |
| tertiary mice |
| 3°1a | 100 | 100 | 100 | 100 | 90 (14720) | 93 (4281) | |
| 3°1b | 100 | 100 | 100 | 100 | 90 (11325) | 72 (3170) | |
| 3°1c | 100 | 100 | 100 | 100 | 82 (11666) | 82 (3113) | |
| 3°1d | 100 | 100 | 100 | 100 | 72 (12212) | 75 (3170) | |
| 3°1e | 100 | 100 | 100 | 100 | 69 (11586) | 73 (3132) | |
| 3°1f | 100 | 100 | 100 | 100 | 77 (12268) | 83 (3095) | |
| 3°1g | 100 (24742) | 100 | 100 (68666) | 100 | 94 (17696) | 89 (12815) | 97 (4831) | 94 (3847) |
| Patient 2; CD19 85%, CD10 83%, CD34 80% |
| primary mouse |
| 1°2a | 100 | 100 | 100 | 100 | |
| secondary mice |
| 2°2a | 100 (14349) | 100 (12815) | 100 (51397) | 100 (38352) | 82 (15065) | 84 (12296) | 85 (3089) | 63 (1881) |
| 2°2b | 100 | 100 | 100 | 100 | 89 (11020) | 39 (1204) | |
| 2°2d | 100 | 100 | 100 | 100 | 88 (10341) | 16 (762) | |
| 2°2e | 100 (9664) | 100 (7629) | 100 (45750) | 100 (30305) | 91 (14283) | 89 (9513) | 29 (1125) | 1 (700) |
| 2°2f | 100 (11711) | 100 (12101) | 100 (61831) | 100 (54881) | 93 (17490) | 92 (14652) | 55 (1902) | 51 (1652) |
| 2°2g | 100 | 100 | 100 | 100 | |
| Patient 3; CD19 85%, CD10, 84%, CD34 83% |
| primary mice |
| 1°3a | 100 | 100 | 100 | 100 | |
| 1°3b | 100 | 100 | 100 | 100 | |
| Secondary mice |
| 2°3a | 100 (21110) | 100 (26956) | 100 (143642) | 100 (124849) | 84 (29236) | 81 (26384) | 19 (1784) | 6 (1320) |
| 2°3b | 100 | 100 | 100 | 100 | 56 (25038) | 1 (1188) | |
| 2°3c | 100 | 100 | 100 | 100 | 82 (27739) | 3 (1290) | |
| 2°3d | 100 | 100 | 100 | 100 | 78 (25038) | 3 (1188) | |
| 2°3e | 100 | 100 | 100 | 100 | 66 (24334) | 1 (1127) | |
| Patient 4; |
| Primary mouse |
| 1°4a | 100 | 100 | 100 | 100 | 60 (7817) | 19 (807) | |
| 1°4b | 100 | 100 | 100 | 100 | 80 (18853) | 66 (2428) | |
| 1°4c | 100 | 100 | 100 | 100 | 35 (8027) | 88 (3532) | |
| Patient 5; CD19 56%, CD10 59%, CD34 62%. |
|------------------------------------------|
| Primary mouse                           |
| 15a 100 100 100 100                     |
| Transduced with pSLiEW and analysed by in-vivo imaging and histology post-mortem |

| Patient 1 |          |          |          |          |
|-----------|----------|----------|----------|----------|
| 1aSLiEW   | 100      | 100      |          |          |
| 1bSLiEW   | 100 (16926) | 100 (57616) | 71 (13235) | 86 (3400) |

| Patient 2 |          |          |          |          |
|-----------|----------|----------|----------|----------|
| 2aSLiEW   | 100      | 100      |          |          |
| 2bSLiEW   | 100      | 100      |          |          |

| Patient 3 |          |          |          |          |
|-----------|----------|----------|----------|----------|
| 3aSLiEW   | 100 (18720) | 100(145780) | 48 (27345) | 0 (1744) |
| 3bSLiEW   | 100      | 100      |          |          |

| Patient 4 |          |          |          |          |
|-----------|----------|----------|----------|----------|
| 4aSLiEW   | 100 (15709) | 100 (36635) | 58 (9156) | 77 (3046) |
| 4bSLiEW   | 100      | 100      |          |          |

Mean fluorescence intensity (MFI). Gray boxes indicate that no sample was available for analysis.
Supplementary table 6. Bone marrow histology of additional xenografts.

| Xenograft ID | Genetic sub-type | Sample   | Morphology type* |
|--------------|------------------|----------|------------------|
| 1°5Ra        | iAMP21           | femur/sternum | A                |
| 1°5Rb        | iAMP21           | femur/sternum | A/B              |
| 1°13a        | iAMP21           | tibia     | A                |
| 1°13b        | iAMP21           | tibia/sternum | A                |
| 1°14a        | iAMP21           | tibia/sternum | A                |
| 1°14b        | iAMP21           | tibia/sternum | A                |
| 1°14c        | iAMP21           | tibia     | A                |
| 1°15a        | iAMP21           | tibia/sternum | WT/A            |
| 1°15b        | iAMP21           | tibia/sternum | A                |
| 1°16a        | iAMP21           | humerus/sternum | WT/A        |
| 1°16b        | iAMP21           | humerus/sternum | WT/A        |
| 2°17a        | ETV6-RUNX1       | tibia     | A                |
| 1°18a        | Hyperdiploid     | forelimb/sternum | A/B          |

*WT (wild type morphology similar to control NSG mice), A and B (as defined in the main text and shown in figures 2, and Supplementary figures 3 and 7).
**Supplementary Table 7. Copy number abnormalities (CNA) identified by SNP6.0 array analysis in patients and xenografts.**

Y; clonal CNA present

N; CNA not detected

S; sub-clonal CNA present

| Chr | abnormality | genomic position | patient | 1° | 2° | 3° Xenografts | genes |
|-----|-------------|------------------|---------|----|----|----------------|-------|
|     |             |                  |         | a  | b  | a  | b  | a  | b  | c  | d  | e  | f  | g   |       |
| 4   | del (CN 1)  | 153060000-153272000 | Y       | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | FBXW7 |
| 6   | del (CN 1)  | 25950000-26285000   | Y       | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 33 genes TRIM38, HIST1H1A- HIST1H2B1 |
| 6   | del (CN 0)  | 261350000-26255000   | Y       | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 18 genes HIST1H1E- HIST1H2B1 |
| 7   | del (CN 1)  | 49850000-51565000   | Y       | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | VWC2, ZPB8, C7orf72, IKZF1, FIGNL1, DDC, GRB10, COBL |
| 7   | del (CN 1)  | 65800000-66475000   | Y       | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 9 gene TPST1-TYW1 |
| 7   | del (CN 1)  | 1378890000-138447000 | Y       | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | TRIM24, SUVPL, ATP6VOA4 |
| 7   | del (CN 1)  | 1384890000-141624000 | Y       | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 30 genes KIAA1549- OR9A4, |
| 7   | del (CN 1)  | 142060000-142202000 | Y       | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | none |
| 7   | del (CN 1)  | 154559000-155465000 | Y       | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 9 genes DPP6-RBM33 |
| 8   | del (CN 1)  | 323750000-325520000 | N       | N  | N  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | CMTM 8, 7, 6 |
| 9   | del (CN 1)  | 159540000-32310000  | N       | N  | N  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 40 genes SLC24A2- MOB3B, |
| 9   | del (CN 0)  | 196200000-27194000   | N       | N  | N  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | Y  | 37 genes SLC24A2, MLLT3-TEK |
| 10  | trisomy     |                   | Y       | N  | N  | N  | N  | N  | N  | N  | N  | N  | N  |       |
### Patient 2 concordant CNA

#### 2° xenografts

| Chr | Abnormality | genome position | patient | g | d | c | b | a | genes |
|-----|-------------|-----------------|---------|---|---|---|---|---|-------|
| 3   | del (CN 1)  | 35528000-35684000 | Y       | Y | Y | Y | Y | Y | ARPP21 (exons1&2) |
| 6   | del (CN 1)  | 46699000-47018000 | Y       | Y | Y | Y | Y | Y | PLA2G7, LOC100287718, MEP1A, GPR116, GPR110 |
| 7   | del (CN 1)  | 50150000-51210000 | Y       | Y | Y | Y | Y | Y | C7orf72, IKZF1, FIGNL1, DDC, GRB10, COBL |
| 7   | del (CN 1)  | 109732000-158646480 | Y       | Y | Y | Y | Y | Y | many |
| 8   | del (CN 1)  | 53567000-53596000 | Y       | Y | Y | Y | Y | Y | RB1CC1 (exons 9-20) |
| 9   | del (CN 1)  | 0:39500000       | Y       | Y | Y | Y | Y | Y | many |
| 9   | del (CN 0)  | 20365000-22404000 | Y       | Y | Y | Y | Y | Y | 32 genes MLLT3-CDKN2B-AS1 |
| 9   | amp (CN 3)  | 70950000-141067840 | Y       | Y | Y | Y | Y | Y | many |
| 11  | del (CN 1)  | 63950000-64000000 | Y       | Y | Y | Y | Y | Y | STIP1, FERMT3, TRPT1, NUDT22, DNAJC4, VEGFB |
| 11  | del (CN 1)  | 11846000-11930000 | Y       | Y | Y | Y | Y | Y | ETV6 (ex 2) |
| 13  | amp (CN 3)  | 67315000-115150780 | Y       | Y | Y | Y | Y | Y | many |
| 16  | del (CN 1)  | 37810000-38240000 | Y       | Y | Y | Y | Y | Y | CREBBP (ex 1-19) |
| 22  | del (CN 1)  | 22320000-22540000 | Y       | Y | Y | Y | Y | Y | TOP3B |
| X   | WCG         |                 | Y       | Y | Y | Y | Y | Y | |

### Patient 2 discordant CNA

| Chr | Abnormality | genome position | patient | g | d | c | b | a | genes |
|-----|-------------|-----------------|---------|---|---|---|---|---|-------|
| 2   | del (CN 1)  | 74000000-86000000 | N       | N | N | S | N | N | Many TPRKB-ST3GAL5 |
| 12  | trisomy     |                 | Y       | N | N | N | N | N | |
| X   | iso(p)      |                 | Y       | N | N | N | S | Y | many |
| X   | del (CN 1)  | 47248000-47342000 | N       | N | Y | S | S | S | ZNF157, ZNF41 |

### Patient 3 concordant CNA

| Chr | Abnormality | genome position | patient | g | d | c | b | a | genes |
|-----|-------------|-----------------|---------|---|---|---|---|---|-------|
| 4   | amp (CN 3)  | 70125000-70238000 | Y       | Y | Y | Y | Y | Y | UGT2B28 |
| 5   | trisomy     |                 | Y       | Y | Y | Y | Y | Y | |
| 10  | trisomy     |                 | Y       | Y | Y | Y | Y | Y | |
| 12  | del (CN 1)  | 104860000-105042000 | Y       | Y | Y | Y | Y | Y | CHST11 (ex 2&3) |
| 14  | trisomy     |                 | Y       | Y | Y | Y | Y | Y | |
| 17  | trisomy     |                 | Y       | Y | Y | Y | Y | Y | |
| 17  | del (CN 1)  | 62590000-62665000 | Y       | Y | Y | Y | Y | Y | SMURF2 (ex 15-18) |
| X   | del (CN 1)  | 13800000-16040000 | Y       | Y | Y | Y | Y | Y | CSF2RA, IL3RA, SLC25A6, ASMTL-AS1, ASMTL, P2RY8(UTR) |

### Patient 3 discordant CNA

| Chr | Abnormality | genome position | patient | g | d | c | b | a | genes |
|-----|-------------|-----------------|---------|---|---|---|---|---|-------|
| 9   | trisomy     |                 | Y       | Y | N | N | N | N | |
| 12  | del (CN 1)  | 0-24500000      | S       | S | N | N | N | N | |
| 12  | del (CN 1)  | 119500000-32460000 | N       | N | S | N | N | N | ETV6 (ex 3-7) many genes BICD1 (exon 1-4) |
| X   | trisomy     |                 | Y       | S | Y | S | Y | S | |
patient 4 concordant CNA

| Chr | Abnormality | genomic position | 1° xenografts | 2° xenografts | genes in region |
|-----|-------------|------------------|---------------|--------------|----------------|
| 1   | complex     | 898000000-121500000 | Y | Y | many |
| 1   | del (CN 0)  | 152550000-152590000 | Y | Y | LCE3, LCE3B, LCE3A |
| 1   | del (CN 1)  | 196720000-196800000 | Y | Y | CFHR3, CFHR1 |
| 1   | del (CN 1)  | 184500000-184600000 | Y | Y | ZNF804A |
| 2   | del (CN 1)  | 142674000-142750000 | Y | y | many including CDKN1A/B |
| 2   | del (CN 1)  | 142674000-142724350 | Y | y | NR3C1 |
| 9   | del (CN 1)  | 0-341500000 | Y | Y | many |
| 9   | del (CN 0)  | 219540000-221190000 | y | N | CDKN1A, CDKN1B, CDKN1B-A51 |
| 9   | del (CN 0)  | 370000000-373500000 | Y | Y | PAX5, ZCCCH7 |
| 10  | del (CN 0)  | 983500000-987000000 | Y | Y | PIK3AP1, LCOR |
| 10  | del (CN 1)  | 808000000-134963460 | Y | Y | many |
| 12  | del (CN 1)  | 118000000-120000000 | Y | Y | ETV6 intragenic |
| 12  | del (CN 0)  | 111760000-112060000 | Y | Y | CUX2, FAM109A, SH2B3, ATXN2 |
| 12  | del (CN 1)  | 133350000-134800000 | Y | Y | many |
| 12  | del (CN 1)  | 111140000-111900000 | Y | Y | many |
| 12  | del (CN 1)  | 373600000-377400000 | N | N | RAC2, CYTH4 |

Patient 4 discordant CNA

| Chr | Abnormality | genomic position | presentation | relapse | a | genes |
|-----|-------------|------------------|--------------|---------|---|-------|
| 4   | del (CN 1)  | 174060000-174260000 | N | N | GALNT7, HMGB2 |
| 4   | amp (CN2)   | 693500000-695000000 | N | Y | UGT2B17 |
| 10  | del (CN 1)  | 900000000-135534000 | N | N | many |
| 10  | del (CN 1)  | 808000000-126400000 | N | N | many |
| 10  | del (CN 0)  | 980150000-980600000 | N | Y | BLNK |
| 14  | amp (CN 3)  | 369000000-439000000 | N | N | 13 genes SLC25A21- LRFN5 |
| 14  | del (CN 1)  | 439000000-107349520 | Y | N | many |
| 16  | del (CN 1)  | 377000000-392000000 | Y | N | CREBBP |
| 17  | amp (CN 1)  | 253000000-274500000 | N | N | many |
| 17  | amp (CN 2)  | 259200000-263000000 | Y | Y | many |
| 22  | del (CN 1)  | 376300000-377400000 | N | N | RAC2, CYTH4 |

Patient 5 concordant CNA

| Chr | Abnormality | genomic position | presentation | relapse | a | genes |
|-----|-------------|------------------|--------------|---------|---|-------|
| 2   | del (CN 1)  | 637760000-750950000 | Y | Y | Y | many |
| 4   | del (CN 1)  | 717600000-719000000 | y | Y | Y | MOB1B, DCK |
| 6   | del (CN 1)  | 261260000-262600000 | y | Y | Y | 16 genes HIST1H1E-HIST1H2BH |

Patient 5 discordant CNA

| Chr | Abnormality | genomic position | presentation | relapse | a | genes |
|-----|-------------|------------------|--------------|---------|---|-------|
| 1   | del (CN 1)  | 234612000-235540000 | N | N | S | 8 genes TARBP1- ARID4B, |
| 2   | del (CN 1)  | 23220000-88740000 | S | N | Y | LOC100506274, LOC339788, LINC00299, ID2, KIDINS220 (ex 1) |
| 3   | del (CN 1)  | 114080000-114680000 | S | N | Y | ZBTB20 |
| 3   | del (CN 1)  | 459000000-482500000 | S | Y | N | 37 genes CCR9-CAMP |
| 5   | del (CN 1)  | 1580880000-1581490000 | N | N | S | EBF1 exons 1-5 |
| 5   | del (CN 1)  | 1583880000-158580000 | N | Y | N | EBF1 exons 10-16 |
| 12  | CN-LOH      | 535000000-133721800 | Y | Y | N | many |
| 12  | del (CN 1)  | 1110500000-112135000 | S | N | Y | 12 genes TCTN1- ACAD10 |
Supplementary Table 8. Regions of chromosome 21 copy number change that occurred in xenografts from patient 1.

| start   | end     | size   | CN change |
|---------|---------|--------|-----------|
| 0       | 16446092| 16446092| 0         |
| 16446092| 17302313| 856221  | 1         |
| 17381183| 17697572| 316389  | -2        |
| 17697723| 18163458| 465735  | -1        |
| 18163544| 18174691| 11147   | 0         |
| 18177172| 20521089| 2343917 | -1        |
| 20521648| 21760268| 1238620 | 0         |
| 21760283| 22125481| 365198  | -2        |
| 22125690| 22517120| 391430  | 0         |
| 22517189| 23343710| 826521  | -2        |
| 23345771| 26533474| 3187703 | 0         |
| 26533786| 28305871| 1772085 | -1        |
| 28306308| 29236061| 929753  | -2        |
| 29243377| 31443709| 2200332 | -1        |
| 31447334| 31680485| 233151  | 0         |
| 31683535| 33949423| 2265888 | -1        |
| 33949543| 48096945| 14147402| 0         |
### Supplementary Table 9. MLPA values for patients and their xenografts. A score of 1 indicates normal copy number. Examples with clear evidence for CN changes between samples are highlighted (loss by shades of red and gain by shades of green).

| Target                      | Patient 1 | Patient 2 |
|-----------------------------|-----------|-----------|
|                             | 2°1b      | 3°1g      |
| 01a_EBF1_ex16               | 1.035     | 0.855     | 1.142 |
| 01b_EBF1_ex14               | 1.086     | 1.114     | 0.952 |
| 01c_EBF1_ex10               | 1         | 0.974     | 1.16  |
| 01d_EBF1_ex3                | 0.965     | 0.958     | 1.127 |
| 02a_IKZF1_ex1               | 0.588     | 0.499     | 0.596 |
| 02b_IKZF1_ex2               | 0.649     | 0.481     | 0.56  |
| 02c_IKZF1_ex3               | 0.616     | 0.451     | 0.598 |
| 02d_IKZF1_ex4               | 0.583     | 0.454     | 0.603 |
| 02e_IKZF1_ex5               | 0.639     | 0.435     | 0.567 |
| 02f_IKZF1_ex6               | 0.609     | 0.566     | 0.529 |
| 02g_IKZF1_ex7               | 0.636     | 0.511     | 0.597 |
| 02h_IKZF1_ex8               | 0.55      | 0.49      | 0.622 |
| 03a_JAK2_ex23               | 0.844     | 0.825     |       |
| 03b_CDKN2A_ex5              | 0.936     | 0.987     | 0     |
| 03c_CDKN2A_ex2a             | 1.022     | 0.897     | 0     |
| 03d_CDKN2B_ex2              | 1.021     | 0.919     | 0     |
| 04a_PAX5_ex10               | 1.032     | 0.97      | 1.255 |
| 04b_PAX5_ex8                | 0.942     | 0.828     | 1.236 |
| 04c_PAX5_ex7                |           |           | 0.966 |
| 04d_PAX5_ex6                | 1.015     | 0.974     | 1.183 |
| 04e_PAX5_ex5                | 0.981     | 1.086     | 0.996 |
| 04f_PAX5_ex2                | 1.149     | 0.795     | 1.171 |
| 04g_PAX5_ex1                | 0.981     | 0.938     | 1.149 |
| 05a_ETV6_ex1A               | 0.97      | 1.048     | 0.969 |
| 05b_ETV6_ex1B               | 1.003     | 1.1       | 0.948 |
| 05c_ETV6_ex2                | 0.201     | 0         | 0.527 |
| 05d_ETV6_ex3                | 0.401     | 0.511     | 0.473 |
| 05e_ETV6_ex5                | 0.616     | 9         | 0.627 |
| 05f_ETV6_ex8                | 0.986     | 0.945     | 1.122 |
| 06a_BTG1_AREA1              | 0.972     | 0.913     | 1.043 |
| 06b_BTG1_AREA2              | 0.893     | 0.919     | 1.106 |
| 06c_BTG1_ex2                | 1.038     | 0.967     | 1.14  |
| 06d_BTG1_ex1                | 1.06      | 0.815     | 1.262 |
| 07a_RB1_ex6                 | 0.955     | 0.874     | 1.137 |
| 07b_RB1_ex14                | 1.015     | 94        | 0.967 |
| 07c_RB1_ex19                | 0.117     | 0         | 0     |
| 07d_RB1_ex24                | 0.118     | 0         | 0     |
| 07e_RB1_ex26                | 0.095     | 0         | 0     |
| 08a_SHOX_area               | 1.01      | 0.918     | 0.995 |
| 08b_CRLF2_ex4               | 0.997     | 0.942     | 1.09  |
| 08c_CS2RA_ex10              | 1.043     | 0.993     | 0.924 |
| 08d_IL3RA_ex1               | 0.98      | 1.038     | 0.852 |
| 08e_P2RY8_ex2               | 0.987     | 0.943     | 1.106 |
| 08f_ZFY_ex4                 | 0         | -1        | -1    |
| Target                                    | patient | secondary xenografts |
|-------------------------------------------|---------|----------------------|
|                                           | 2°2c    | 2°2d     | 2°2b     | 2°2e     | 2°2f     |
| 01a_EBF1_ex16                             | 0.975   | 0.965    | 0.918    | 0.945    | 1.054    | 1.019    |
| 01b_EBF1_ex14                             | 0.948   | 0.949    | 1.025    | 0.953    | 0.84     | 0.901    |
| 01c_EBF1_ex10                             | 0.965   | 0.994    | 1.06     | 1.061    | 1.083    | 1.083    |
| 01d_EBF1_ex1                              | 0.898   | 1.051    | 1.067    | 0.998    | 1.157    | 1.078    |
| 02a_IKZF1_ex1                             | 0.485   | 0.541    | 0.602    | 0.579    | 0.538    | 0.551    |
| 02b_IKZF1_ex2                             | 0.539   | 0.53     | 0.583    | 0.536    | 0.572    | 0.543    |
| 02c_IKZF1_ex3                             | 0.547   | 0.535    | 0.541    | 0.555    | 0.579    | 0.579    |
| 02d_IKZF1_ex4                             | 0.55    | 0.539    | 0.599    | 0.59     | 0.598    | 0.622    |
| 02e_IKZF1_ex5                             | 0.544   | 0.534    | 0.544    | 0.526    | 0.574    | 0.559    |
| 02f_IKZF1_ex6                             | 0.575   | 0.494    | 0.497    | 0.494    | 0.447    | 0.53     |
| 02g_IKZF1_ex7                             | 0.483   | 0.599    | 0.665    | 0.598    | 0.61     | 0.588    |
| 02h_IKZF1_ex8                             | 0.509   | 0.611    | 0.648    | 0.602    | 0.645    | 0.635    |
| 03a_JAK2_ex23                             | 0.565   | 0.426    | 0.432    | 0.409    | 0.368    | 0.396    |
| 03b_CDKN2A_ex5                            | 0       | 0        | 0        | 0        | 0        | 0        |
| 03c_CDKN2A_ex2a                           | 0       | 0        | 0        | 0        | 0        | 0        |
| 03d_CDKN2B_ex2                           | 0       | 0        | 0        | 0        | 0        | 0        |
| 04a_PAX5_ex10                             | 0.512   | 0.591    | 0.622    | 0.587    | 0.669    | 0.625    |
| 04b_PAX5_ex8                              | 0.5     | 0.732    | 0.823    | 0.862    | 0.703    | 0.637    |
| 04c_PAX5_ex7                              | 0.552   | 0.659    | 0.72     | 0.677    | 0.497    | 0.482    |
| 04d_PAX5_ex6                              | 0.512   | 0.592    | 0.662    | 0.633    | 0.629    | 0.653    |
| 04e_PAX5_ex5                              | 0.56    | 0.545    | 0.593    | 0.54     | 0.451    | 0.491    |
| 04f_PAX5_ex2                              | 0.508   | 0.548    | 0.616    | 0.647    | 0.605    | 0.591    |
| 04g_PAX5_ex1                              | 0.516   | 0.556    | 0.595    | 0.639    | 0.624    | 0.632    |
| 05a_ETV6_ex1A                             | 1.344   | 1.016    | 1.047    | 1.027    | 0.942    | 0.919    |
| 05b_ETV6_ex1B                             | 1.307   | 0.939    | 0.948    | 0.97     | 0.875    | 0.891    |
| 05c_ETV6_ex2                              | 0.596   | 0.522    | 0.532    | 0.506    | 0.456    | 0.476    |
| 05d_ETV6_ex3                              | 1.378   | 0.915    | 0.921    | 0.89     | 0.856    | 0.924    |
| 05e_ETV6_ex5                              | 1.39    | 1.069    | 1.047    | 1.076    | 1.146    | 1.123    |
| 05f_ETV6_ex8                              | 1.283   | 1.07     | 1.074    | 1.068    | 1.126    | 1.101    |
| 06a_BTG1_AREA1                            | 1.215   | 0.945    | 0.973    | 0.924    | 0.997    | 0.95     |
| 06b_BTG1_AREA2                            | 1.247   | 1.02     | 1.039    | 1.021    | 1.043    | 1.04     |
| 06c_BTG1_ex2                              | 1.258   | 1.078    | 1.093    | 1.033    | 1.138    | 1.104    |
| 06d_BTG1_ex1                              | 1.141   | 1.229    | 1.391    | 1.395    | 1.256    | 1.307    |
| 07a_RB1_ex6                              | 0.909   | 1.013    | 1.06     | 0.991    | 1.087    | 1.077    |
| 07b_RB1_ex14                             | 0.984   | 0.962    | 0.998    | 0.939    | 0.894    | 0.924    |
| 07c_RB1_ex19                             | 0.93    | 1.031    | 1.094    | 1.012    | 0.919    | 0.926    |
| 07d_RB1_ex24                             | 1.004   | 1.048    | 1.023    | 1.029    | 0.85     | 0.955    |
| 07e_RB1_ex26                             | 0.998   | 0.954    | 0.876    | 0.88     | 0.83     | 0.877    |
| 08a_SHOX_area                             | 1.404   | 1.315    | 1.26     | 1.332    | 1.651    | 1.347    |
| 08b_CRLF2_ex4                             | 1.446   | 1.34     | 1.486    | 1.343    | 1.46     | 1.536    |
| 08c_CSF2RA_ex10                           | 1.496   | 1.268    | 1.205    | 1.111    | 1.351    | 1.221    |
| 08d_IL3RA_ex1                             | 1.353   | 1.384    | 1.506    | 1.433    | 1.328    | 1.232    |
| 08e_P2RY8_ex2                             | 1.252   | 1.52     | 1.717    | 1.657    | 1.893    | 1.637    |
| 08f_ZFY_ex4                               | 1000    | 1000     | 1000     | 1000     | 1000     | 1000     |
| Target               | Patient 1°3b | 2°3c | 2°3a | 2°3e | 2°3b | 2°3d |
|----------------------|-------------|------|------|------|------|------|
| 01a_EBF1_ex16        | 1.356       | 1.432| 1.506| 1.529| 1.449| 1.553| 1.507|
| 01b_EBF1_ex14        | 1.306       | 1.403| 1.319| 1.298| 1.323| 1.319| 1.423|
| 01c_EBF1_ex10        | 1.405       | 1.223| 1.571| 1.596| 1.522| 1.621| 1.512|
| 01d_EBF1_ex1         | 1.363       | 1.511| 1.521| 1.621| 1.574| 1.56 | 1.429|
| 02a_IKZF1_ex1        | 1.01        | 1.047| 1.051| 1.059| 1.041| 1.038| 0.987|
| 02b_IKZF1_ex2        | 0.964       | 0.969| 1.022| 1.083| 1.051| 1.037| 0.999|
| 02c_IKZF1_ex3        | 0.978       | 1.042| 1.053| 1.09  | 1.096| 1.089| 1.063|
| 02d_IKZF1_ex4        | 0.949       | 1.033| 1.034| 1.17  | 1.098| 1.14 | 1.066|
| 02e_IKZF1_ex6        | 1           | 0.95 | 1.019| 1.063| 1.054| 1.048| 0.994|
| 02f_IKZF1_ex7        | 1.109       | 0.901| 0.891| 0.915| 0.916| 0.994| 1.018|
| 02g_IKZF1_ex7        | 0.941       | 1.198| 1.143| 1.164| 1.168| 1.102| 1.077|
| 02h_IKZF1_ex8        | 1.037       | 1.183| 1.153| 1.239| 1.213| 1.185| 1.094|
| 03a_JAK2_ex23        | 0.722       | 0.652| 0.676| 0.719| 0.783| 0.836|
| 03b_CDK2A_ex5        | 1.227       | 1.011| 1.054| 1.154| 1.116| 1.104| 1.009|
| 03c_CDK2A_ex2a       | 1.348       | 1.171| 1.018| 1.037| 1.033| 1.022| 0.964|
| 03d_CDK2B_ex2        | 1.215       | 1.128| 1.076| 1.142| 1.116| 1.074| 1.034|
| 04a_PAX5_ex10        | 1.096       | 1.275| 1.214| 1.239| 1.185| 1.164| 1.121|
| 04b_PAX5_ex8         | 1.334       | 1.676| 1.42 | 1.156| 1.213| 1.138| 1.242|
| 04c_PAX5_ex7         | 1.391       | 1.084| 0.848| 0.919| 0.909| 1.095|
| 04d_PAX5_ex6         | 1.231       | 0.938| 0.983| 1.189| 1.162| 1.19 | 1.113|
| 04e_PAX5_ex5         | 1.309       | 0.798| 0.729| 0.913| 0.97 | 0.923| 0.973|
| 04f_PAX5_ex2         | 1.213       | 1.063| 1.008| 1.106| 1.105| 1.158| 1.05 |
| 04g_PAX5_ex1         | 1.207       | 1.06 | 1.089| 1.145| 1.077| 1.136| 1.046|
| 05a_ETV6_ex1A        | 0.838       | 1.009| 0.912| 0.85 | 0.915| 0.91 | 0.939|
| 05b_ETV6_ex1B        | 0.843       | 0.926| 0.882| 0.826| 0.855| 0.877| 0.875|
| 05c_ETV6_ex2         | 0.856       | 1.001| 0.942| 0.868| 0.883| 0.909| 0.944|
| 05d_ETV6_ex3         | 0.868       | 0.541| 0.737| 0.758| 0.791| 0.792| 0.864|
| 05e_ETV6_ex5         | 0.86        | 0.709| 1.106| 1.017| 1.083| 1.02 | 1.04 |
| 05f_ETV6_ex8         | 0.85        | 0.68 | 1.028| 0.964| 1.008| 1.007| 0.974|
| 06a_BTG1-AREA1       | 1.003       | 0.933| 0.941| 0.95 | 0.948| 0.977| 0.922|
| 06b_BTG1-AREA2       | 1.136       | 1.031| 0.989| 1.013| 0.988| 1.064| 0.976|
| 06c_BTG1_ex2         | 0.91        | 1.085| 1.098| 1.081| 1.065| 1.132| 1.045|
| 06d_BTG1_ex1         | 0.953       | 1.322| 1.114| 1.295| 1.231| 1.304| 1.086|
| 07a_RB1_ex6          | 1.079       | 1.013| 1.026| 1.093| 0.992| 1.049| 0.985|
| 07b_RB1_ex14         | 0.998       | 0.938| 0.871| 0.817| 0.841| 0.863| 0.917|
| 07c_RB1_ex19         | 0.875       | 1.016| 0.929| 0.914| 0.911| 0.922| 0.962|
| 07d_RB1_ex24         | 0.926       | 0.987| 0.918| 0.953| 0.91 | 0.954| 0.978|
| 07e_RB1_ex26         | 0.933       | 0.885| 0.855| 0.884| 0.835| 0.913| 0.958|
| 08a_SHOX_area        | 1.305       | 1.204| 1.202| 1.374| 1.186| 1.18 | 1.279|
| 08b_CRLF2_ex4        | 1.325       | 1.191| 1.445| 1.454| 1.209| 1.239| 1.437|
| 08c_CSFR2A_ex10      | 0.579       | 0.451| 0.35 | 0.379| 0.216| 0.195| 0.408|
| 08d_IL3RA_ex1        | 0.583       | 0.412| 0.301| 0.377| 0.24 | 0.199| 0.402|
| 08e_P2RY8_ex2        | 0.562       | 0.453| 0.514| 0.282| 0.237| 0.441|
| 08f_ZFY_ex4          | -1          | -1   | -1   | -1   | -1   | -1   | -1   |
| Target | patient | primary xenograft |
|--------|---------|------------------|
| 01a_EBF1_ex16 | 0.777 | 0.536 |
| 01b_EBF1_ex14 | 0.771 | 0.554 |
| 01c_EBF1_ex10 | 0.928 | 1.006 |
| 01d_EBF1_ex1 | 0.865 | 0.988 |
| 02a_IKZF1_ex1 | 0.97 | 1.025 |
| 02b_IKZF1_ex2 | 1.005 | 1.004 |
| 02c_IKZF1_ex3 | 0.956 | 1.003 |
| 02d_IKZF1_ex4 | 0.966 | 1.028 |
| 02e_IKZF1_ex5 | 1 | 1.011 |
| 02f_IKZF1_ex6 | 1.09 | 0.994 |
| 02g_IKZF1_ex7 | 0.905 | 0.975 |
| 02h_IKZF1_ex8 | 0.964 | 1.013 |
| 03a_JAK2_ex23 | 1.004 | 0.542 |
| 03b_CDKN2A_ex5 | 0.998 | 0.564 |
| 03c_CDKN2A_ex2a | | |
| 03d_CDKN2B_ex2 | 1.017 | 0.979 |
| 04a_PAX5_ex10 | 0.904 | 1.032 |
| 04b_PAX5_ex8 | 0.971 | 0.93 |
| 04c_PAX5_ex7 | 1.143 | 0.948 |
| 04d_PAX5_ex6 | 0.98 | 1.05 |
| 04e_PAX5_ex5 | 1.013 | 1.018 |
| 04f_PAX5_ex2 | 1 | 1.062 |
| 04g_PAX5_ex1 | 0.988 | 1.042 |
| 05a_ETV6_ex1A | 1.025 | 0.995 |
| 05b_ETV6_ex1B | 1 | 1.002 |
| 05c_ETV6_ex2 | 1.019 | 1.014 |
| 05d_ETV6_ex3 | 1 | 1.035 |
| 05e_ETV6_ex5 | 1 | 1.033 |
| 05f_ETV6_ex8 | 0.991 | 1.02 |
| 06a_BTG1_AREA1 | 1.022 | 0.986 |
| 06b_BTG1_AREA2 | 1.031 | 0.938 |
| 06c_BTG1_ex2 | 0.983 | 1.001 |
| 06d_BTG1_ex1 | 1.011 | 0.995 |
| 07a_RB1_ex6 | 1.022 | 1.022 |
| 07b_RB1_ex14 | 1.039 | 1.031 |
| 07c_RB1_ex19 | 1.01 | 0.906 |
| 07d_RB1_ex24 | 1.2 | 0.949 |
| 07e_RB1_ex26 | 0.982 | 0.914 |
| 08a_SHOX_area | 0.984 | 1.019 |
| 08b_CRLF2_ex4 | 1.038 | 1.041 |
| 08c_CSF2RA_ex10 | 1.008 | 1.018 |
| 08d_IL3RA_ex1 | 1 | 1.031 |
| 08e_P2RY8_ex2 | 0.979 | 1.04 |
| 08f_ZFY_ex4 | 0 | -1 |
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