

Philadelphia-like acute lymphoblastic leukemia is associated with minimal residual disease persistence and poor outcome. First report of the minimal residual disease-oriented GIMEMA LAL1913

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Ph-like ALL is associated with MRD persistence and poor outcome. First report from the MRD-oriented GIMEMA LAL1913 trial

Supplementary Material and Methods

MRD assessment

- *Time points*

MRD was defined positive if $\geq 10^{-4}$ for at least one IG-TR marker; it was evaluated at weeks 4 (time point (TP) 1), 10 (TP2), 16 (TP3) and 22 (TP4) with MRD results at week 10 (TP2) representing the earliest decisional TP.

- *IG/TR gene rearrangement detection*

Genomic DNA samples at diagnosis were screened by PCR amplification using the BIOMED-1 primer sets for Ig kappa deleting element gene rearrangements IGK-Kde, complete and incomplete TRD and TRG gene rearrangements.¹ Complete and incomplete IGH rearrangements were identified using 5 IGHV and 7 IGHD family primers in combination with one JH consensus primer according to BIOMED-2.² Similarly, for incomplete and complete TRB gene rearrangements, the respective BIOMED-2 multiplex PCR primer sets were used.² For TRD/A gene rearrangements, multiplex PCR primer sets were used.³ The products obtained from Ig and TCR gene rearrangements were further examined by heteroduplex analysis to discriminate between amplifications derived from monoclonal or polyclonal lymphoid cell populations.^{4,5} Biclinal or biallelic PCR products were separated either by cutting out amplicons from the polyacrylamide gel or by DNA cloning.

- *Sequencing and gene analysis*

The PCR products were directly sequenced using the Big Dye Terminator Cycle Sequencing Reaction Kit and analyzed using an automatic ABI PRISM 3130 DNA genetic analyzer (Applied Biosystems, Foster City, CA). The IGH, IGK, TRA TRB, TRD and TRG nucleotide sequences obtained were aligned to the IgBLAST data base (<http://www.ncbi.nlm.nih.gov/igblast/>, National Cancer for Biotechnology Information, Bethesda, MD) and to the international ImMunoGeneTics information system (www.imgt.org, Initiator and Coordinator: Marie-Paule Lefranc, Montpellier, France).

- *RQ-PCR*

Tests for residual disease were conducted by RQ-PCR amplification using TaqMan technology. The PCR was performed in 96-well reaction plates; ABI 7300 was the reference instrument (Applied Biosystems) with germline TaqMan fluorescent probes and clone-specific primers for all identified rearrangements.^{6,7} The germline probes/primers and the clone specific primers were designed for each target using the Primer Express (Applied Biosystems) program. The efficiency of our RQ-PCR assay was evaluated by calculating the slope values of the standard curve made by serially diluting the diagnostic DNA specimen in DNA obtained from mononuclear cells (MNC) from a pool of five healthy donors. The serial dilutions ranged from 10^{-1} to 10^{-5} and were tested in triplicate. MRD PCR targets were tested for specificity and sensitivity to select, for each patient, one target with a sensitivity of at least 10^{-4} and a quantitative range of at least 10^{-4} , optimized for each rearrangement tested, both by increasing the annealing temperature and/or designing new primers. For normalization of the quantitative results, ALB - as the reference gene - was always amplified, so that all data were within a certain confidence interval and acceptability. RQ-PCR analyses were performed and interpreted according to the guidelines developed within the "EuroMRD Consortium".⁸

BCR/ABL1-like predictor

This tool is based on the quantification of the 9 previously identified transcripts - *SOCS2*, *IFITM1*, *CD99*, *TP53INP1*, *IFITM2*, *IGJ*, *NUTD4*, *CD97*, *SEMA6A* - and of *CRLF2*⁹ by Q-RT-PCR (SybrGreen method, QuantStudio5 Real-time PCR System, Thermo Fisher Scientific, Waltham, MA) and expression values were computed as $2^{(-\Delta Ct)}$. Patients with a score ≥ -0.3 were classified as Ph-like ALL.

Screening of recurrent mutations and deletions

Sequencing libraries were prepared from 100 ng genomic DNA by using the Truseq custom amplicon kit (Illumina, San Diego, CA). After library quality check, samples were pooled equimolarly and sequenced on an Illumina MiSeq in paired-end reads of 300 bp each by using a MiSeq Reagent Kit v2.

and were analyzed using the Variant Studio Software, considering only variants satisfying the following criteria: i) exonic variants; ii) quality of 100; iii) GQX equal to 100; iv) missense and truncating variants; v) read depth >100. All variants recognized as single nucleotide

polymorphisms (SNPs) were excluded, unless a prognostic value was previously demonstrated or they were previously reported in Ph-like ALL.²¹ Furthermore, SNPs predicted as deleterious by the PolyPhen-2 tool were annotated. Sanger sequencing was also performed to validate selected variants. Exon 6 of *IL7R* was also sequenced by Sanger since the coverage of this hotspot was insufficient and chromatograms were visually inspected for the presence of INDELS by using Mutation Surveyor v4.0.9 (SoftGenetics, State College, PA).

Targeted RNA-sequencing and FISH analysis

After library quality check, samples were pooled equimolarly and sequenced on an Illumina MiSeq in paired-end reads of 76 bp each by using a MiSeq Reagent Kit v3. Fusion call was performed by using TopHat v1.1 and RNA-sequencing Alignment v2.0 software integrated in BaseSpace Sequence Hub (<https://basespace.illumina.com/apps/>).

DNA clones for *ABL1*, *ABL2*, *CSF1R*, *FGFR1*, *PDGFRB*, *JAK2*, and *TSLP* tyrosin-kinases (TKs) were selected from the genomic databases “Ensembl” (Genome Browser, GRCh37) and “UCSC” (University of California, Santa Cruz, Genome Browser Feb. 2009, GRCh37/hg19), and were labelled by nick translation using spectrum orange and spectrum green dUTP (Abbott Molecular, Chicago, IL) (Supplemental Table 4). *CRLF2* was studied with ZytoLight[®] SPEC CRLF2 Dual Color Break Apart Probe (ZytoVision GmbH, Bremerhaven, Germany). A clone for *IL7R* was used as internal control. Analysis was done out using a fluorescence microscope Olympus BX61 (Olympus, Milan, Italy) equipped with a high sensitive camera JAI (Copenhagen, Denmark) and driven by CytoVision 4.5.4 software (Genetix, New Milton, Hampshire, UK). At least 100 interphase nuclei were analyzed in each experiment. A two-step diagnostic workflow was carried out to study first, *CRLF2* and then, in negative cases, the other TKs. Partner genes were investigated in cases with *ABL1* or *PDGFRB* involvement.

Supplemental results

Genetic features of B-NEG ALL cohort

NGS experiments focused on the most frequently mutated genes of the JAK/STAT and RAS pathway cascades. The median read depth per amplicon was 3467 reads per sample (IQR: 1124–5086), detailed in Supplemental Table 5. Considering the whole cohort, we found 24 JAK/STAT pathway mutations in 16 patients (17%), mainly affecting *JAK2* - mutated in 8 cases (8.8%) - and

JAK1 - mutated in 6 cases (6.6%). *IL7R*, *JAK3* and *CRLF2* mutations were less common, being documented in 3, 2 and 2 samples, respectively. Subclonal mutations (n=13) accounted for 54.2% of the total.

Overall, we detected a total of 59 RAS pathway mutations in 41 cases (45.1%), with 8 cases displaying >1 mutated gene and 7 cases with >1 mutation targeting the same gene. The most frequently affected genes were *NRAS* and *KRAS* (39 mutations): *NRAS* was mutated in 18 (19.8%) and *KRAS* in 15 (16.5%) cases, *FLT3* proved mutated in 9 samples (9.9%) and *PTPN11* in 8 cases (8.8%). Notably, a considerable proportion of mutations (23/59, 38.9%) were detected at the subclonal level (variant-allele frequency <15%). Lastly, in 9 cases the JAK/STAT and RAS cascades were simultaneously affected.

In the entire B-NEG ALL cohort, we found that the most frequently deleted genes were *CDKN2A/2B*, *IKZF1*, *PAX5* and *BTG1*, in 35 (40.2%), 32 (36.7%), 20 (22.9%) and 17 (19.5%) cases, respectively. Sixty-two % of *IKZF1*-deleted samples were *IKZF1+ CDKN2A/2B and/or PAX5*. The remaining gene deletions were detected in <15% of cases.

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Supplemental Table 1. Clinical feature of the cohort of study.

| | Whole B-NEG ALL cohort (n=105) |
|---|---------------------------------------|
| Age (median [range]) | 38.7 [18.2-64.7] |
| WBC x 10⁹/L (median [range]) | 5.1 [0.23-347] |
| Hb g/dL (median [range]) | 9.4 [3.7-15.7] |
| Plts x 10⁹/L (median [range]) | 56 [7.5-630] |
| Gender (%) | |
| M | 61 (58.1) |
| F | 44 (41.9) |
| Risk (%) | |
| Standard risk | 62 (64.6) |
| No Standard risk | 34 (35.4) |
| CR (%) | |
| No CR | 13 (12.6) |
| CR | 90 (87.4) |

Supplemental Table 2. Clinical feature of the cohort of study in comparison with the whole B-NEG ALL cohort enrolled in the protocol.

| | Whole B-NEG cohort enrolled in the protocol (n=115) | Cohort studied for the <i>BCR/ABL1</i>-like predictor (n=88) | <i>p</i>-value |
|---|--|---|-----------------------|
| Age (median [range]) | 39.08 [18.18-64.71] | 37.5 [18.18-64.59] | Ns |
| WBC x 10⁹/L (median [range]) | 4.72 [0.23-347] | 5.62 [0.23-347] | Ns |
| Hb g/dL (median [range]) | 9.00 [3.7-15.7] | 9.4 [3.7-15.7] | Ns |
| Plts x 10⁹/L (median [range]) | 55.5 [7.5-630] | 56.5 [7.5-630] | |
| Gender (%) | | | |
| M | 67 (58.3) | 53 (60.2) | Ns |
| F | 48 (41.7) | 35 (39.8) | |
| Risk (%) | | | |
| Standard risk | 69 (65.1) | 48 (60.8) | Ns |
| No Standard risk | 37 | 31 (39.2) | |
| CR (%) | | | |
| No CR | 14 (12.5) | 12 (14.0) | Ns |
| CR | 98 (87.5) | 74 (86.0) | |

Supplemental Table 3. List of the studies performed in each sample and summary of the main genetic features.

| Record ID | BCR/ABL1 like predictor | BCR/ABL1-like prediction | Score | CRLF2 expression | Mutation analysis | RAS pathway status | JAK/STAT pathway status | MLPA analysis | IKZF1 | CDKN2A/B | PAX5 | IKZF1+CDKN2A and/or PAX5 | BTG1 | EBF1 | CDKN2A/2B and/or RB1 | TK/cytokine receptor fusions (RNAseq and/or FISH analysis) |
|-----------|-------------------------|--------------------------|--------|------------------|-------------------|--------------------|-------------------------|---------------|-------|----------|------|--------------------------|------|------|----------------------|--|
| B-ALL_1 | Yes | BCR/ABL1-like | 3.073 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | Δ | no-Δ | EBF1-PDGFRB |
| B-ALL_2 | NA | | | | Yes | M clonal | WT | Yes | Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | NA |
| B-ALL_3 | Yes | BCR/ABL1-like | 0.928 | Low | Yes | M | WT | Yes | Δ | Δ | Δ | Yes | no-Δ | no-Δ | Δ | No |
| B-ALL_4 | Yes | BCR/ABL1-like | 0.347 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | Δ | no-Δ | Δ | No |
| B-ALL_5 | Yes | non-BCR/ABL1-like | -1.041 | Low | Yes | M clonal | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_6 | Yes | non-BCR/ABL1-like | -1.588 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_7 | Yes | BCR/ABL1-like | 1.216 | High | Yes | WT | M clonal | Yes | Δ | Δ | no-Δ | Yes | no-Δ | no-Δ | Δ | No |
| B-ALL_8 | NA | | | | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | NA |
| B-ALL_9 | Yes | non-BCR/ABL1-like | -0.331 | Low | Yes | WT | WT | Yes | Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_10 | Yes | non-BCR/ABL1-like | -1.701 | Low | Yes | M clonal | M | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_11 | Yes | non-BCR/ABL1-like | -1.439 | Low | Yes | WT | WT | Yes | no-Δ | Δ | Δ | | Δ | no-Δ | Δ | No |
| B-ALL_12 | Yes | non-BCR/ABL1-like | -1.459 | High | Yes | WT | WT | Yes | no-Δ | Δ | no-Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_13 | Yes | non-BCR/ABL1-like | -1.498 | Low | NA | | | NA | | | | | | | | No |
| B-ALL_14 | Yes | non-BCR/ABL1-like | -1.529 | Low | Yes | M clonal | WT | Yes | no-Δ | Δ | no-Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_15 | Yes | non-BCR/ABL1-like | -1.586 | Low | Yes | M | WT | Yes | Δ | Δ | no-Δ | Yes | no-Δ | no-Δ | Δ | No |
| B-ALL_16 | Yes | BCR/ABL1-like | 0.788 | Low | Yes | WT | WT | Yes | Δ | Δ | Δ | Yes | Δ | no-Δ | Δ | BCR/JAK2 |
| B-ALL_17 | Yes | non-BCR/ABL1-like | -0.720 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_18 | Yes | non-BCR/ABL1-like | -1.416 | Low | Yes | M | M | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_19 | Yes | non-BCR/ABL1-like | -0.627 | High | Yes | M | WT | Yes | no-Δ | Δ | no-Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_20 | Yes | non-BCR/ABL1-like | -0.624 | Low | Yes | M clonal | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_21 | Yes | non-BCR/ABL1-like | -1.483 | Low | Yes | M clonal | WT | Yes | Δ | Δ | Δ | Yes | no-Δ | no-Δ | Δ | No |
| B-ALL_22 | Yes | BCR/ABL1-like | 0.157 | Low | Yes | M | WT | Yes | Δ | no-Δ | no-Δ | | Δ | no-Δ | Δ | NUP214/ABL1 |
| B-ALL_23 | NA | | | | Yes | WT | WT | Yes | Δ | Δ | Δ | Yes | no-Δ | no-Δ | Δ | NA |
| B-ALL_24 | NA | | | | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | NA |
| B-ALL_25 | Yes | non-BCR/ABL1-like | -0.600 | High | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_26 | Yes | BCR/ABL1-like | 3.128 | High | Yes | M | M clonal | Yes | Δ | no-Δ | Δ | Yes | no-Δ | Δ | no-Δ | No |
| B-ALL_27 | Yes | non-BCR/ABL1-like | -1.324 | High | NA | | | NA | | | | | | | | No |
| B-ALL_28 | Yes | non-BCR/ABL1-like | -1.169 | Low | Yes | WT | WT | Yes | no-Δ | Δ | Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_29 | Yes | non-BCR/ABL1-like | -0.999 | Low | NA | | | NA | | | | | | | | No |
| B-ALL_30 | NA | | | | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | NA |
| B-ALL_31 | Yes | BCR/ABL1-like | 2.382 | High | Yes | WT | M clonal | Yes | Δ | no-Δ | Δ | Yes | no-Δ | Δ | no-Δ | IGH/CRLF2 |
| B-ALL_32 | Yes | BCR/ABL1-like | 5.720 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | NA |
| B-ALL_33 | Yes | non-BCR/ABL1-like | -1.153 | Low | Yes | M clonal | WT | Yes | Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_34 | Yes | BCR/ABL1-like | 0.725 | Low | Yes | M | WT | Yes | Δ | no-Δ | no-Δ | | Δ | no-Δ | no-Δ | NUP214/ABL1 |
| B-ALL_35 | Yes | non-BCR/ABL1-like | -1.295 | High | Yes | M clonal | M clonal | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No only by FISH |
| B-ALL_36 | Yes | BCR/ABL1-like | 0.205 | High | Yes | WT | M clonal | Yes | Δ | Δ | no-Δ | Yes | no-Δ | no-Δ | Δ | P2RY8/CRLF2 |
| B-ALL_37 | Yes | BCR/ABL1-like | 0.386 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | Δ | Δ | no-Δ | No |
| B-ALL_38 | Yes | non-BCR/ABL1-like | -1.264 | Low | Yes | WT | WT | Yes | Δ | Δ | no-Δ | Yes | no-Δ | no-Δ | Δ | No |
| B-ALL_39 | Yes | non-BCR/ABL1-like | -1.520 | Low | Yes | WT | WT | Yes | no-Δ | Δ | no-Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_40 | Yes | non-BCR/ABL1-like | -1.541 | Low | Yes | M clonal | WT | Yes | no-Δ | Δ | no-Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_41 | Yes | BCR/ABL1-like | 0.726 | Low | Yes | M | M clonal | Yes | Δ | Δ | no-Δ | Yes | no-Δ | no-Δ | Δ | No |
| B-ALL_42 | NA | | | | Yes | M clonal | WT | Yes | no-Δ | no-Δ | no-Δ | | Δ | no-Δ | Δ | NA |
| B-ALL_43 | Yes | non-BCR/ABL1-like | -0.677 | Low | NA | | | NA | | | | | | | | No |
| B-ALL_44 | Yes | BCR/ABL1-like | 1.587 | High | Yes | WT | WT | Yes | Δ | no-Δ | Δ | Yes | Δ | no-Δ | Δ | ZC3HAV1/ABL2 |
| B-ALL_45 | Yes | BCR/ABL1-like | 0.262 | Low | Yes | WT | M clonal | NA | | | | | | | | No |
| B-ALL_46 | Yes | BCR/ABL1-like | 2.449 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_47 | NA | | | | Yes | M clonal | WT | Yes | Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | NA |
| B-ALL_48 | Yes | non-BCR/ABL1-like | -1.191 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_49 | NA | | | | Yes | M clonal | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | NA |
| B-ALL_50 | Yes | non-BCR/ABL1-like | -1.417 | Low | Yes | M clonal | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_51 | Yes | non-BCR/ABL1-like | -1.537 | High | NA | | | NA | | | | | | | | No only by FISH |
| B-ALL_52 | Yes | BCR/ABL1-like | 1.013 | Low | Yes | WT | WT | Yes | no-Δ | Δ | Δ | | Δ | no-Δ | Δ | No |
| B-ALL_53 | Yes | non-BCR/ABL1-like | -0.497 | Low | Yes | WT | WT | Yes | no-Δ | Δ | Δ | | Δ | no-Δ | Δ | No |
| B-ALL_54 | Yes | non-BCR/ABL1-like | -1.636 | Low | Yes | M clonal | WT | Yes | no-Δ | Δ | Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_55 | Yes | BCR/ABL1-like | 0.544 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | Δ | no-Δ | no-Δ | No |
| B-ALL_56 | Yes | non-BCR/ABL1-like | -1.071 | Low | Yes | WT | WT | Yes | Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_57 | Yes | non-BCR/ABL1-like | -1.468 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_58 | Yes | non-BCR/ABL1-like | -1.180 | Low | Yes | M clonal | WT | Yes | no-Δ | Δ | no-Δ | | no-Δ | no-Δ | Δ | No |

| | | | | | | | | | | | | | | | | |
|-----------|-----|--------------------|--------|------|-----|----------|----------|-----|------|------|------|-----|------|------|------|-------------|
| B-ALL_59 | Yes | non-BCR/ABL1 -like | -1.202 | High | Yes | WT | WT | NA | | | | | | | | IGH/CRLF2 |
| B-ALL_60 | Yes | non-BCR/ABL1 -like | -1.390 | Low | Yes | WT | WT | Yes | Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_61 | Yes | BCR/ABL1 -like | 2.722 | Low | NA | | | NA | | | | | | | | No |
| B-ALL_62 | Yes | BCR/ABL1 -like | 0.335 | High | NA | | | NA | | | | | | | | No |
| B-ALL_63 | NA | | | | Yes | WT | WT | Yes | Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | NA |
| B-ALL_64 | Yes | BCR/ABL1 -like | -0.043 | Low | Yes | WT | WT | NA | | | | | | | | NA |
| B-ALL_65 | NA | | | | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | NA |
| B-ALL_66 | NA | | | | Yes | M clonal | WT | Yes | no-Δ | Δ | no-Δ | | no-Δ | no-Δ | Δ | NA |
| B-ALL_67 | NA | | | | Yes | M clonal | M | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | NA |
| B-ALL_68 | Yes | non-BCR/ABL1 -like | -1.119 | Low | Yes | WT | M | Yes | Δ | Δ | Δ | Yes | no-Δ | no-Δ | Δ | No |
| B-ALL_69 | Yes | non-BCR/ABL1 -like | -1.298 | Low | Yes | M clonal | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_70 | NA | | | | Yes | WT | WT | Yes | Δ | Δ | Δ | Yes | Δ | no-Δ | Δ | NA |
| B-ALL_71 | NA | | | | Yes | M clonal | WT | Yes | no-Δ | Δ | no-Δ | | no-Δ | no-Δ | Δ | NA |
| B-ALL_72 | NA | | | | Yes | M clonal | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | NA |
| B-ALL_73 | Yes | BCR/ABL1 -like | 0.048 | Low | Yes | M clonal | WT | Yes | Δ | no-Δ | no-Δ | | no-Δ | Δ | no-Δ | BCR/JAK2 |
| B-ALL_74 | Yes | non-BCR/ABL1 -like | -1.196 | Low | Yes | M clonal | WT | Yes | Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_75 | Yes | non-BCR/ABL1 -like | -0.492 | Low | Yes | M clonal | WT | Yes | no-Δ | no-Δ | Δ | | Δ | no-Δ | no-Δ | No |
| B-ALL_76 | Yes | BCR/ABL1 -like | 1.971 | Low | NA | | | NA | | | | | | | | NA |
| B-ALL_77 | Yes | non-BCR/ABL1 -like | -1.562 | Low | Yes | M clonal | WT | Yes | no-Δ | Δ | Δ | | Δ | Δ | Δ | No |
| B-ALL_78 | Yes | non-BCR/ABL1 -like | -1.172 | Low | NA | | | NA | | | | | | | | No |
| B-ALL_79 | Yes | non-BCR/ABL1 -like | -1.486 | Low | Yes | M clonal | WT | NA | | | | | | | | No |
| B-ALL_80 | Yes | non-BCR/ABL1 -like | -1.248 | High | Yes | M clonal | M clonal | Yes | Δ | Δ | no-Δ | Yes | no-Δ | no-Δ | Δ | No |
| B-ALL_81 | Yes | BCR/ABL1 -like | 1.150 | High | Yes | WT | WT | Yes | Δ | Δ | no-Δ | Yes | Δ | no-Δ | Δ | No |
| B-ALL_82 | Yes | non-BCR/ABL1 -like | -1.522 | Low | Yes | M clonal | WT | Yes | no-Δ | Δ | no-Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_83 | Yes | non-BCR/ABL1 -like | -1.672 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_84 | NA | | | | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | NA |
| B-ALL_85 | Yes | non-BCR/ABL1 -like | -0.400 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_86 | Yes | non-BCR/ABL1 -like | -1.150 | Low | NA | | | NA | | | | | | | | No |
| B-ALL_87 | NA | | | | Yes | M clonal | WT | Yes | Δ | Δ | no-Δ | Yes | no-Δ | no-Δ | Δ | NA |
| B-ALL_88 | Yes | non-BCR/ABL1 -like | -1.235 | Low | Yes | WT | WT | Yes | no-Δ | Δ | Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_89 | Yes | non-BCR/ABL1 -like | -1.103 | Low | Yes | M clonal | M | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_90 | Yes | non-BCR/ABL1 -like | -1.027 | Low | NA | | | NA | | | | | | | | No |
| B-ALL_91 | Yes | non-BCR/ABL1 -like | -1.310 | Low | Yes | M clonal | M | Yes | Δ | Δ | Δ | Yes | no-Δ | no-Δ | Δ | No |
| B-ALL_92 | Yes | BCR/ABL1 -like | -0.112 | High | NA | | | NA | | | | | | | | No |
| B-ALL_93 | Yes | non-BCR/ABL1 -like | -1.232 | Low | NA | | | NA | | | | | | | | No |
| B-ALL_94 | Yes | non-BCR/ABL1 -like | -1.398 | Low | Yes | WT | WT | Yes | Δ | Δ | no-Δ | Yes | no-Δ | no-Δ | Δ | No |
| B-ALL_95 | Yes | non-BCR/ABL1 -like | -1.411 | Low | NA | | | NA | | | | | | | | No |
| B-ALL_96 | Yes | BCR/ABL1 -like | 6.371 | High | Yes | WT | M clonal | Yes | Δ | no-Δ | Δ | Yes | Δ | Δ | no-Δ | NUP214/ABL1 |
| B-ALL_97 | Yes | BCR/ABL1 -like | 3.432 | High | Yes | WT | M clonal | Yes | Δ | no-Δ | no-Δ | | Δ | no-Δ | no-Δ | IGH/CRLF2 |
| B-ALL_98 | Yes | non-BCR/ABL1 -like | -1.563 | Low | Yes | M clonal | WT | Yes | no-Δ | Δ | no-Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_99 | Yes | non-BCR/ABL1 -like | -0.835 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_100 | Yes | BCR/ABL1 -like | -0.180 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_101 | Yes | non-BCR/ABL1 -like | -1.420 | Low | Yes | M clonal | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_102 | Yes | non-BCR/ABL1 -like | -1.534 | Low | Yes | M clonal | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |
| B-ALL_103 | Yes | non-BCR/ABL1 -like | -1.658 | Low | Yes | WT | WT | Yes | no-Δ | Δ | Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_104 | Yes | non-BCR/ABL1 -like | -1.557 | Low | Yes | WT | WT | Yes | no-Δ | Δ | no-Δ | | no-Δ | no-Δ | Δ | No |
| B-ALL_105 | Yes | non-BCR/ABL1 -like | -1.623 | Low | Yes | WT | WT | Yes | no-Δ | no-Δ | no-Δ | | no-Δ | no-Δ | no-Δ | No |

Supplemental Table 4. FISH probes.

| Kinase | Mapping | Genomic clones | | |
|---------------|---------|--|-------------|-------------|
| | | Centromeric | Spanning | Telomeric |
| <i>ABL2</i> | 1q25 | RP11-177A2 | | RP11-345I18 |
| <i>IL7R</i> | 5p13 | RP11-974M7 | | |
| <i>TSLP</i> | 5q22 | | RP11-746A23 | |
| <i>PDGFRB</i> | 5q32 | LSI PDGFRB Dual Color, Break Apart (Vysis, Abbott) | | |

| | | | | |
|-----------------|--------|--------------|-------------|-------------|
| <i>CSF1R</i> | 5q32 | RP11-100O5 | | RP11-432O16 |
| <i>FGFR1</i> | 8p11 | RP11-359P11 | | RP11-513D5 |
| | | RP11-495O10 | | RP11-265K5 |
| <i>JAK2</i> | 9p24 | RP11-39K24 | | RP11-125K10 |
| <i>ABL1</i> | 9q34 | RP11-57C19 | | RP11-83J21 |
| | | | | |
| Partners | | | | |
| <i>TNIP1</i> | 5q33.1 | RP11-915J10 | | RP11-632E9 |
| <i>EBF1</i> | 5q33.3 | RP11-1019K12 | | RP11-583A20 |
| <i>NUP214</i> | 9q34 | RP11-143H20 | RP11-544A12 | |

Supplemental Table 5. Median coverage per sample itemized by amplicon. Amplicons are indicated by chromosome and start/end coordinates according to GRCh37/hg19.

| Amplicon ID | Chromosome | Start | End | Median read depth per sample | Target Exon | CCDS |
|---|------------|----------|----------|------------------------------|-------------|---------|
| CRLF2 + CRLF2_UserDefined (47131292)_140707109 | chrX | 1314870 | 1315034 | 3188.50 | EX6 | 75945.1 |
| CRLF2 + CRLF2_UserDefined (47229427)_140707061 | chrX | 1325306 | 1325512 | 1763.50 | EX3 | 75945.1 |
| CRLF2 + CRLF2_UserDefined (47229427)_140707062 | chrX | 1325306 | 1325512 | 5066.00 | EX3 | 75945.1 |
| CRLF2_Cds_17104068_UserDefined (47229428)_140707063 | chrX | 1331429 | 1331547 | 6125.00 | EX1 | 75945.1 |
| CRLF2_Cds_17104572_UserDefined (47229425)_140707058 | chrX | 1317399 | 1317601 | 1386.00 | EX5 | 75945.1 |
| CRLF2_Cds_17104572_UserDefined (47229425)_140707059 | chrX | 1317399 | 1317601 | 5341.50 | EX5 | 75945.1 |
| CRLF2_Cds_17104572_UserDefined (47229425)_140707059 | chrX | 1317399 | 1317601 | 272.50 | EX5 | 75945.1 |
| CRLF2_Cds_17105645_UserDefined (47229426)_140707060 | chrX | 1321252 | 1321425 | 97.00 | Ex4 | 75945.1 |
| CRLF2_Cds_17108029_UserDefined (47229374)_140706942 | chrX | 1327679 | 1327821 | 138.50 | Ex2 | 75945.1 |
| FLT3 + FLT3 + FLT3_UserDefined (47229379)_140706972 | chr13 | 28608004 | 28608564 | 3867.50 | EX13,14,15 | 31953.1 |
| FLT3 + FLT3 + FLT3_UserDefined (47229379)_140706973 | chr13 | 28608004 | 28608564 | 7544.00 | EX13,14,15 | 31953.1 |
| FLT3 + FLT3 + FLT3_UserDefined (47229379)_140706974 | chr13 | 28608004 | 28608564 | 8473.00 | EX13,14,15 | 31953.1 |
| FLT3 + FLT3 + FLT3_UserDefined (47229379)_140706975 | chr13 | 28608004 | 28608564 | 5510.00 | EX13,14,15 | 31953.1 |
| FLT3 + FLT3_UserDefined (47132139)_140706976 | chr13 | 28609612 | 28610200 | 1858.50 | EX11,12 | 31953.1 |
| FLT3 + FLT3_UserDefined (47132139)_140706977 | chr13 | 28609612 | 28610200 | 2586.50 | EX11,12 | 31953.1 |
| FLT3 + FLT3_UserDefined (47132139)_140706978 | chr13 | 28609612 | 28610200 | 4771.50 | EX11,12 | 31953.1 |
| FLT3 + FLT3_UserDefined (47132139)_140706979 | chr13 | 28609612 | 28610200 | 4137.00 | EX11,12 | 31953.1 |
| FLT3 + FLT3_UserDefined (47229380)_140706980 | chr13 | 28623501 | 28623931 | 379.00 | EX7,8 | 31953.1 |
| FLT3 + FLT3_UserDefined (47229380)_140706981 | chr13 | 28623501 | 28623931 | 5173.00 | EX7,8 | 31953.1 |
| FLT3 + FLT3_UserDefined (47229380)_140706982 | chr13 | 28623501 | 28623931 | 6367.00 | EX7,8 | 31953.1 |
| FLT3_Cds_16768078_UserDefined (47132129)_140706943 | chr13 | 28611302 | 28611445 | 7255.00 | EX10 | 31953.1 |
| FLT3_Cds_16768110_UserDefined (47229408)_140707032 | chr13 | 28622392 | 28622600 | 2194.00 | EX9 | 31953.1 |
| FLT3_Cds_16768110_UserDefined (47229408)_140707033 | chr13 | 28622392 | 28622600 | 3821.00 | Ex9 | 31953.1 |
| FLT3_Cds_16768173_UserDefined (47229409)_140707034 | chr13 | 28624212 | 28624379 | 6360.00 | EX6 | 31953.1 |
| FLT3_Cds_16768912_UserDefined (47131202)_140707037 | chr13 | 28635984 | 28636226 | 3693.50 | EX3 | 31953.1 |
| FLT3_Cds_16768912_UserDefined (47131202)_140707038 | chr13 | 28635984 | 28636226 | 6962.50 | EX3 | 31953.1 |
| FLT3_Cds_16768942_UserDefined (47229413)_140707040 | chr13 | 28674585 | 28674667 | 150.00 | Ex1 | 31953.1 |
| FLT3_Cds_16769202_UserDefined (47229406)_140707030 | chr13 | 28601205 | 28601398 | 7894.50 | EX17 | 31953.1 |
| FLT3_Cds_16769656_UserDefined (47229411)_140707036 | chr13 | 28631464 | 28631619 | 2173.50 | EX4 | 31953.1 |
| FLT3_Cds_16769738_UserDefined (47229410)_140707035 | chr13 | 28626662 | 28626831 | 567.00 | EX5 | 31953.1 |
| FLT3_Cds_16770190_UserDefined (47131204)_140707025 | chr13 | 28589707 | 28589858 | 460.50 | Ex21 | 31953.1 |
| FLT3_Cds_16770364_UserDefined (47229412)_140707039 | chr13 | 28644608 | 28644769 | 864.00 | EX2 | 31953.1 |
| FLT3_Cds_16770538_UserDefined (47229403)_140707026 | chr13 | 28592584 | 28592746 | 5156.50 | EX20 | 31953.1 |
| FLT3_Cds_16770619_UserDefined (47229405)_140707029 | chr13 | 28598978 | 28599100 | 127.50 | Ex18 | 31953.1 |
| FLT3_Cds_16770758_UserDefined (47229407)_140707031 | chr13 | 28602295 | 28602445 | 244.00 | Ex16 | 31953.1 |
| FLT3_Cds_16772090_UserDefined (47229402)_140707024 | chr13 | 28589274 | 28589413 | 6930.50 | EX22 | 31953.1 |
| FLT3_Cds_16772363_UserDefined (47229401)_140707023 | chr13 | 28588569 | 28588714 | 1914.00 | EX23 | 31953.1 |
| FLT3_Cds_16773285_UserDefined (47229404)_140707027 | chr13 | 28597467 | 28597634 | 4068.50 | EX19 | 31953.1 |
| FLT3_Cds_16773285_UserDefined (47229404)_140707028 | chr13 | 28597467 | 28597634 | 6348.50 | EX19 | 31953.1 |
| FLT3_Cds_16773901_UserDefined (47229453)_140707108 | chr13 | 28578172 | 28578331 | 7226.00 | EX24 | 31953.1 |
| IL7R_Cds_17002546_UserDefined (47229432)_140707067 | chr5 | 35871138 | 35871335 | 2252.00 | Ex4 | 3911.1 |
| IL7R_Cds_17003436_UserDefined (47229431)_140707066 | chr5 | 35867388 | 35867585 | 625.50 | Ex3 | 3911.1 |
| IL7R_Cds_17004661_UserDefined (47229430)_140707065 | chr5 | 35860934 | 35861112 | 4317.50 | Ex2 | 3911.1 |
| IL7R_Cds_17006734_UserDefined (47229429)_140707064 | chr5 | 35857060 | 35857181 | 471.50 | Ex1 | 3911.1 |
| IL7R_Cds_17007143_UserDefined (47229455)_140707115 | chr5 | 35876065 | 35876605 | 109.00 | Ex8 | 3911.1 |
| IL7R_Cds_17007143_UserDefined (47229455)_140707116 | chr5 | 35876065 | 35876605 | 241.00 | Ex8 | 3911.1 |
| IL7R_Cds_17007143_UserDefined (47229455)_140707117 | chr5 | 35876065 | 35876605 | 6207.00 | EX8 | 3911.1 |
| IL7R_Cds_17007407_UserDefined (47229433)_140707068 | chr5 | 35873562 | 35873770 | 4700.00 | Ex5 | 3911.1 |
| IL7R_Cds_17007407_UserDefined (47229433)_140707069 | chr5 | 35873562 | 35873770 | 6925.50 | Ex5 | 3911.1 |
| IL7R_Cds_17008069_UserDefined (47229434)_140707070 | chr5 | 35874531 | 35874664 | 43.50 | Ex6 | 3911.1 |
| IL7R_Cds_17008455_UserDefined (47132221)_140707114 | chr5 | 35875594 | 35875709 | 3216.50 | EX7 | 3911.1 |
| JAK1 + JAK1_UserDefined (47131288)_140706987 | chr1 | 65306908 | 65307304 | 4772.00 | EX17,18 | 41346.1 |
| JAK1 + JAK1_UserDefined (47131288)_140706988 | chr1 | 65306908 | 65307304 | 43.50 | EX17,18 | 41346.1 |
| JAK1_Cds_16667511_UserDefined (47229436)_140707073 | chr1 | 65303595 | 65303807 | 5756.00 | EX21 | 41346.1 |
| JAK1_Cds_16667511_UserDefined (47229436)_140707074 | chr1 | 65303595 | 65303807 | 2611.00 | EX21 | 41346.1 |
| JAK1_Cds_16668160_UserDefined (47131254)_140707075 | chr1 | 65304128 | 65304292 | 2640.50 | EX20 | 41346.1 |

| | | | | | | |
|---|-------|----------|----------|---------|---------|---------|
| JAK1_Cds_16668437_UserDefined (47229435)_140707071 | chr1 | 65301059 | 65301209 | 1186.50 | EX23 | 41346.1 |
| JAK1_Cds_16668664_UserDefined (47229448)_140707097 | chr1 | 65348940 | 65349178 | 2564.00 | EX2 | 41346.1 |
| JAK1_Cds_16668664_UserDefined (47229448)_140707098 | chr1 | 65348940 | 65349178 | 3595.00 | EX2 | 41346.1 |
| JAK1_Cds_16668729_UserDefined (47229446)_140707091 | chr1 | 65332529 | 65332911 | 7072.00 | EX6 | 41346.1 |
| JAK1_Cds_16668729_UserDefined (47229446)_140707092 | chr1 | 65332529 | 65332911 | 6445.50 | EX6 | 41346.1 |
| JAK1_Cds_16668896_UserDefined (47229441)_140707081 | chr1 | 65312312 | 65312439 | 1012.50 | EX13 | 41346.1 |
| JAK1_Cds_16669432_UserDefined (47131256)_140707118 | chr1 | 65300228 | 65300360 | 4055.50 | EX24 | 41346.1 |
| JAK1_Cds_16669868_UserDefined (47131268)_140707093 | chr1 | 65334974 | 65335177 | 3677.50 | EX5 | 41346.1 |
| JAK1_Cds_16669993_UserDefined (47229443)_140707083 | chr1 | 65316467 | 65316613 | 4800.50 | EX11 | 41346.1 |
| JAK1_Cds_16670263_UserDefined (47229440)_140707080 | chr1 | 65311176 | 65311343 | 709.00 | EX14 | 41346.1 |
| JAK1_Cds_16670566_UserDefined (47131258)_140707089 | chr1 | 65330450 | 65330675 | 3741.00 | EX7 | 41346.1 |
| JAK1_Cds_16670566_UserDefined (47131258)_140707090 | chr1 | 65330450 | 65330675 | 4244.00 | EX7 | 41346.1 |
| JAK1_Cds_16670758_UserDefined (47229447)_140707094 | chr1 | 65339033 | 65339226 | 4466.50 | EX4 | 41346.1 |
| JAK1_Cds_16670758_UserDefined (47229447)_140707095 | chr1 | 65339033 | 65339226 | 2156.50 | EX4 | 41346.1 |
| JAK1_Cds_16671022_UserDefined (47229445)_140707087 | chr1 | 65325768 | 65325965 | 5669.50 | EX8 | 41346.1 |
| JAK1_Cds_16671022_UserDefined (47229445)_140707088 | chr1 | 65325768 | 65325965 | 3150.50 | EX8 | 41346.1 |
| JAK1_Cds_16671238_UserDefined (47229444)_140707084 | chr1 | 65321172 | 65321401 | 5527.50 | EX10 | 41346.1 |
| JAK1_Cds_16671238_UserDefined (47229444)_140707085 | chr1 | 65321172 | 65321401 | 3457.00 | EX10 | 41346.1 |
| JAK1_Cds_16671455_UserDefined (47229442)_140707082 | chr1 | 65313195 | 65313378 | 3702.50 | EX12 | 41346.1 |
| JAK1_Cds_16671565_UserDefined (47229439)_140707079 | chr1 | 65310417 | 65310592 | 1402.00 | EX15 | 41346.1 |
| JAK1_Cds_16671602_UserDefined (47131252)_140707072 | chr1 | 65301761 | 65301918 | 4786.00 | EX22 | 41346.1 |
| JAK1_Cds_16671956_UserDefined (47229438)_140707078 | chr1 | 65309727 | 65309918 | 8625.50 | EX22 | 41346.1 |
| JAK1_Cds_16672003_UserDefined (47229437)_140707076 | chr1 | 65305266 | 65305498 | 354.00 | EX19 | 41346.1 |
| JAK1_Cds_16672003_UserDefined (47229437)_140707077 | chr1 | 65305266 | 65305498 | 494.50 | EX19 | 41346.1 |
| JAK1_Cds_16672014_UserDefined (47131262)_140707096 | chr1 | 65344688 | 65344851 | 2996.50 | EX3 | 41346.1 |
| JAK1_Cds_16672393_UserDefined (47131272)_140707099 | chr1 | 65351922 | 65351967 | 2898.00 | EX1 | 41346.1 |
| JAK1_Cds_16672446_UserDefined (47132208)_140707086 | chr1 | 65323319 | 65323482 | 3368.50 | EX9 | 41346.1 |
| JAK2 + JAK2_UserDefined (47229375)_140706944 | chr9 | 5080209 | 5080703 | 3969.00 | EX15,16 | 6457.1 |
| JAK2 + JAK2_UserDefined (47229375)_140706945 | chr9 | 5080209 | 5080703 | 3622.50 | EX15,16 | 6457.1 |
| JAK2 + JAK2_UserDefined (47229375)_140706946 | chr9 | 5080209 | 5080703 | 5232.00 | EX15,16 | 6457.1 |
| JAK2 + JAK2_UserDefined (47229449)_140707100 | chr9 | 5126313 | 5126808 | 3888.50 | EX22,23 | 6457.1 |
| JAK2 + JAK2_UserDefined (47229449)_140707101 | chr9 | 5126313 | 5126808 | 4304.50 | EX22,23 | 6457.1 |
| JAK2 + JAK2_UserDefined (47229449)_140707102 | chr9 | 5126313 | 5126808 | 3657.00 | EX22,23 | 6457.1 |
| JAK2 + JAK2_UserDefined (47229454)_140707110 | chr9 | 5090347 | 5091015 | 4185.50 | EX19,20 | 6457.1 |
| JAK2 + JAK2_UserDefined (47229454)_140707111 | chr9 | 5090347 | 5091015 | 5543.00 | EX19,20 | 6457.1 |
| JAK2 + JAK2_UserDefined (47229454)_140707112 | chr9 | 5090347 | 5091015 | 319.50 | EX19,20 | 6457.1 |
| JAK2 + JAK2_UserDefined (47229454)_140707113 | chr9 | 5090347 | 5091015 | 4606.00 | EX19,20 | 6457.1 |
| JAK2_Cds_17086073_UserDefined (47229385)_140706993 | chr9 | 5050666 | 5050851 | 2780.00 | EX4 | 6457.1 |
| JAK2_Cds_17086073_UserDefined (47229385)_140706994 | chr9 | 5050666 | 5050851 | 125.00 | EX4 | 6457.1 |
| JAK2_Cds_17086074_UserDefined (47229393)_140707010 | chr9 | 5089654 | 5089883 | 314.00 | EX18 | 6457.1 |
| JAK2_Cds_17086074_UserDefined (47229393)_140707011 | chr9 | 5089654 | 5089883 | 2120.00 | EX18 | 6457.1 |
| JAK2_Cds_17086262_UserDefined (47229382)_140706989 | chr9 | 5021968 | 5022233 | 1241.50 | EX1 | 6457.1 |
| JAK2_Cds_17086262_UserDefined (47229382)_140706990 | chr9 | 5021968 | 5022233 | 6433.50 | EX1 | 6457.1 |
| JAK2_Cds_17087236_UserDefined (47229392)_140707009 | chr9 | 5081705 | 5081881 | 178.50 | EX17 | 6457.1 |
| JAK2_Cds_17087477_UserDefined (47229394)_140707012 | chr9 | 5122984 | 5123141 | 2337.50 | EX21 | 6457.1 |
| JAK2_Cds_17087554_UserDefined (47229383)_140706991 | chr9 | 5029763 | 5029926 | 4415.00 | EX2 | 6457.1 |
| JAK2_Cds_17087712_UserDefined (47229389)_140707003 | chr9 | 5069905 | 5070072 | 1260.50 | EX10 | 6457.1 |
| JAK2_Cds_17087860_UserDefined (47132148)_140706998 | chr9 | 5064863 | 5065060 | 4233.50 | EX7 | 6457.1 |
| JAK2_Cds_17087860_UserDefined (47132148)_140706999 | chr9 | 5064863 | 5065060 | 831.00 | EX7 | 6457.1 |
| JAK2_Cds_17087904_UserDefined (47132154)_140707006 | chr9 | 5077433 | 5077600 | 4535.50 | EX13 | 6457.1 |
| JAK2_Cds_17087904_UserDefined (47132154)_140707007 | chr9 | 5077433 | 5077600 | 868.50 | EX13 | 6457.1 |
| JAK2_Cds_17088449_UserDefined (47132152)_140707004 | chr9 | 5072472 | 5072646 | 2390.50 | EX11 | 6457.1 |
| JAK2_Cds_17088454_UserDefined (47229390)_140707005 | chr9 | 5073678 | 5073805 | 4408.50 | EX12 | 6457.1 |
| JAK2_Cds_17088950_UserDefined (47229384)_140706992 | chr9 | 5044383 | 5044540 | 7850.00 | EX3 | 6457.1 |
| JAK2_Cds_17089173_UserDefined (47229391)_140707008 | chr9 | 5078286 | 5078464 | 2013.00 | EX14 | 6457.1 |
| JAK2_Cds_17089231_UserDefined (47229386)_140706995 | chr9 | 5054543 | 5054904 | 711.00 | EX5 | 6457.1 |
| JAK2_Cds_17089231_UserDefined (47229386)_140706996 | chr9 | 5054543 | 5054904 | 2713.00 | EX5 | 6457.1 |
| JAK2_Cds_17090261_UserDefined (47132150)_140707001 | chr9 | 5069002 | 5069228 | 5903.00 | EX9 | 6457.1 |
| JAK2_Cds_17090261_UserDefined (47132150)_140707002 | chr9 | 5069002 | 5069228 | 4175.50 | EX9 | 6457.1 |
| JAK2_Cds_17091284_UserDefined (47229387)_140706997 | chr9 | 5055649 | 5055808 | 1269.50 | EX6 | 6457.1 |
| JAK2_Cds_17091671_UserDefined (47229388)_140707000 | chr9 | 5066658 | 5066809 | 330.00 | EX8 | 6457.1 |
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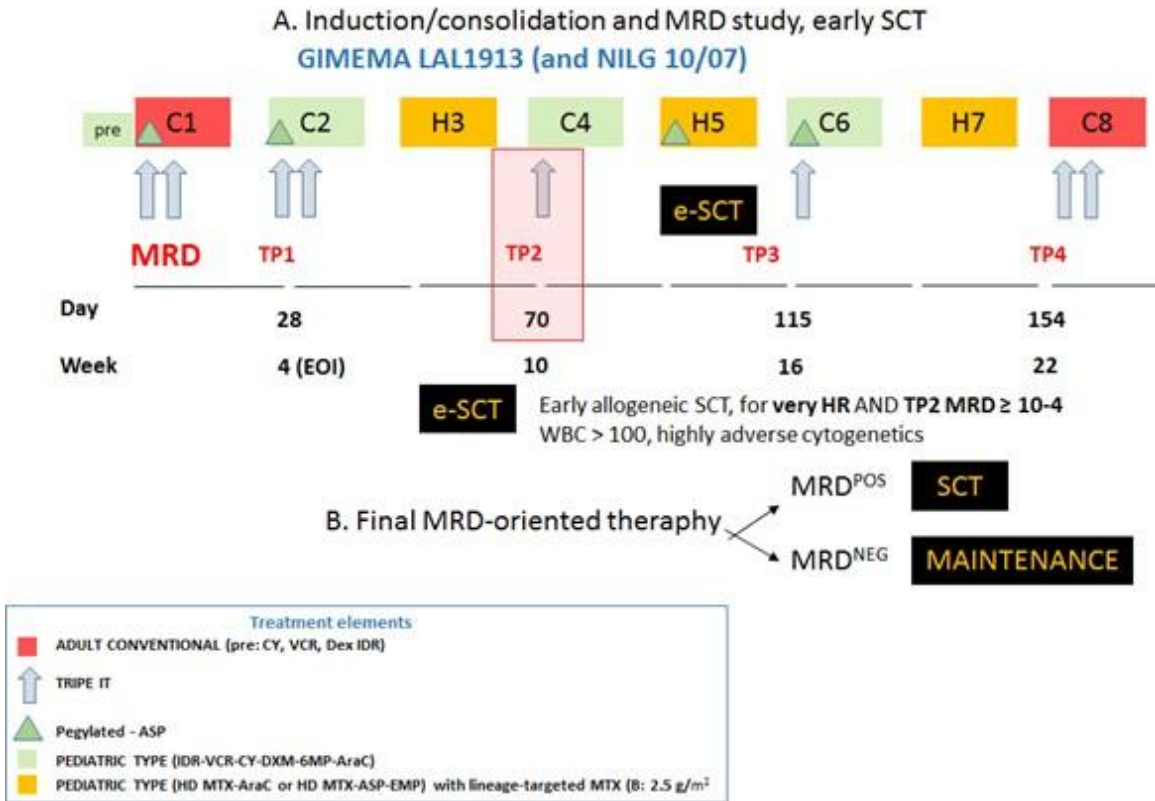
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|--|-------|-----------|-----------|---------|------------|---------|
| JAK3 + JAK3 + JAK3_UserDefined (47132137)_140706968 | chr19 | 17953816 | 17954729 | 1720.50 | EX2,3,4 | 12366.1 |
| JAK3 + JAK3 + JAK3_UserDefined (47132137)_140706969 | chr19 | 17953816 | 17954729 | 5329.00 | EX2,3,4 | 12366.1 |
| JAK3 + JAK3 + JAK3_UserDefined (47132137)_140706970 | chr19 | 17953816 | 17954729 | 1962.50 | EX2,3,4 | 12366.1 |
| JAK3 + JAK3 + JAK3_UserDefined (47132137)_140706971 | chr19 | 17953816 | 17954729 | 5370.00 | EX2,3,4 | 12366.1 |
| JAK3 + JAK3 + JAK3_UserDefined (47229377)_140706957 | chr19 | 17945360 | 17946044 | 3898.50 | EX14,15,16 | 12366.1 |
| JAK3 + JAK3 + JAK3_UserDefined (47229377)_140706958 | chr19 | 17945360 | 17946044 | 4453.00 | EX14,15,16 | 12366.1 |
| JAK3 + JAK3 + JAK3_UserDefined (47229377)_140706959 | chr19 | 17945360 | 17946044 | 6911.50 | EX14,15,16 | 12366.1 |
| JAK3 + JAK3 + JAK3_UserDefined (47229377)_140706960 | chr19 | 17945360 | 17946044 | 3776.00 | EX14,15,16 | 12366.1 |
| JAK3 + JAK3_UserDefined (47132131)_140706947 | chr19 | 17940897 | 17941449 | 88.50 | EX21,22 | 12366.1 |
| JAK3 + JAK3_UserDefined (47132131)_140706948 | chr19 | 17940897 | 17941449 | 31.00 | EX21,22 | 12366.1 |
| JAK3 + JAK3_UserDefined (47132131)_140706949 | chr19 | 17940897 | 17941449 | 1424.00 | EX21,22 | 12366.1 |
| JAK3 + JAK3_UserDefined (47132133)_140706954 | chr19 | 17943308 | 17943758 | 1471.00 | EX17,18 | 12366.1 |
| JAK3 + JAK3_UserDefined (47132133)_140706955 | chr19 | 17943308 | 17943758 | 355.50 | EX17,18 | 12366.1 |
| JAK3 + JAK3_UserDefined (47132133)_140706956 | chr19 | 17943308 | 17943758 | 1707.00 | EX17,18 | 12366.1 |
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| JAK3 + JAK3_UserDefined (47229376)_140706950 | chr19 | 17942017 | 17942627 | 3947.50 | EX19,20 | 12366.1 |
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| JAK3 + JAK3_UserDefined (47229376)_140706953 | chr19 | 17942017 | 17942627 | 2191.50 | EX19,20 | 12366.1 |
| JAK3 + JAK3_UserDefined (47229378)_140706964 | chr19 | 17952178 | 17952591 | 6383.00 | EX6,7 | 12366.1 |
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| JAK3_Cds_16875134_UserDefined (47229397)_140707016 | chr19 | 17953105 | 17953439 | 4232.50 | EX5 | 12366.1 |
| JAK3_Cds_16875134_UserDefined (47229397)_140707017 | chr19 | 17953105 | 17953439 | 1160.50 | EX5 | 12366.1 |
| JAK3_Cds_16876878_UserDefined (47131220)_140707014 | chr19 | 17947918 | 17948042 | 251.50 | EX12 | 12366.1 |
| JAK3_Cds_16877020_UserDefined (47229395)_140707013 | chr19 | 17946713 | 17946880 | 397.00 | EX13 | 12366.1 |
| JAK3_Cds_16877924_UserDefined (47132127)_140706939 | chr19 | 17950274 | 17950639 | 1674.50 | EX9 | 12366.1 |
| JAK3_Cds_16877924_UserDefined (47132127)_140706940 | chr19 | 17950274 | 17950639 | 5601.50 | EX9 | 12366.1 |
| JAK3_Cds_16877924_UserDefined (47132127)_140706941 | chr19 | 17950274 | 17950639 | 1461.50 | EX9 | 12366.1 |
| JAK3_Cds_16878504_UserDefined (47229398)_140707018 | chr19 | 17955023 | 17955246 | 1238.50 | EX1 | 12366.1 |
| JAK3_Cds_16878504_UserDefined (47229398)_140707019 | chr19 | 17955023 | 17955246 | 1417.00 | EX1 | 12366.1 |
| JAK3_Cds_16879644_UserDefined (47229373)_140706938 | chr19 | 17937543 | 17937924 | 3472.50 | EX23 | 12366.1 |
| JAK3_Cds_16880177_UserDefined (47229396)_140707015 | chr19 | 17951019 | 17951170 | 6106.50 | EX8 | 12366.1 |
| KRAS_Cds_16746132_UserDefined (47229450)_140707104 | chr12 | 25362712 | 25362865 | 859.00 | Ex4 | 8702.1 |
| KRAS_Cds_16746135_UserDefined (47132216)_140707105 | chr12 | 25368358 | 25368514 | 8414.00 | EX4 | 8703.1 |
| KRAS_Cds_16746575_UserDefined (47229400)_140707021 | chr12 | 25380148 | 25380366 | 7348.00 | Ex2 | 8702.1 |
| KRAS_Cds_16746575_UserDefined (47229400)_140707022 | chr12 | 25380148 | 25380366 | 6820.00 | EX2 | 8702.1 |
| KRAS_Cds_16746855_UserDefined (47229399)_140707020 | chr12 | 25378528 | 25378727 | 1316.00 | EX3 | 8702.1 |
| KRAS_Cds_16749892_UserDefined (47229381)_140706986 | chr12 | 25398188 | 25398338 | 2348.00 | EX1 | 8702.1 |
| NRAS_Cds_16673855_UserDefined (47229424)_140707057 | chr1 | 115258651 | 115258801 | 5145.50 | Ex1 | 877.1 |
| NRAS_Cds_16676341_UserDefined (47229423)_140707055 | chr1 | 115256401 | 115256619 | 758.00 | Ex2 | 877.1 |
| NRAS_Cds_16676341_UserDefined (47229423)_140707056 | chr1 | 115256401 | 115256619 | 392.50 | Ex2 | 877.1 |
| NRAS_Cds_16677908_UserDefined (47229422)_140707054 | chr1 | 115252170 | 115252369 | 778.00 | Ex3 | 877.1 |
| NRAS_Cds_16678533_UserDefined (47229452)_140707107 | chr1 | 115251139 | 115251295 | 4885.00 | Ex4 | 877.1 |
| PTPN11 + PTPN11_UserDefined (47132141)_140706983 | chr12 | 112915435 | 112915839 | 5164.50 | EX8,9 | 9163.1 |
| PTPN11 + PTPN11_UserDefined (47132141)_140706984 | chr12 | 112915435 | 112915839 | 4770.00 | EX8,9 | 9163.1 |
| PTPN11 + PTPN11_UserDefined (47132141)_140706985 | chr12 | 112915435 | 112915839 | 9136.00 | EX8,9 | 9163.1 |
| PTPN11 + PTPN11_UserDefined (47132214)_140707103 | chr12 | 112924259 | 112924454 | 4029.50 | EX11 | 9163.1 |
| PTPN11_Cds_16763256_UserDefined (47229418)_140707049 | chr12 | 112910728 | 112910864 | 2483.50 | EX7 | 9163.1 |
| PTPN11_Cds_16764105_UserDefined (47229416)_140707043 | chr12 | 112888102 | 112888336 | 318.00 | EX3 | 9163.1 |
| PTPN11_Cds_16764105_UserDefined (47229416)_140707044 | chr12 | 112888102 | 112888336 | 5040.50 | EX3 | 9163.1 |

| | | | | | | |
|--|-------|-----------|-----------|----------|------|--------|
| PTPN11_Cds_16764883_UserDefined (47229419)_140707051 | chr12 | 112926227 | 112926334 | 5671.50 | EX12 | 9163.1 |
| PTPN11_Cds_16765751_UserDefined (47131242)_140707048 | chr12 | 112893734 | 112893887 | 5584.50 | EX6 | 9163.1 |
| PTPN11_Cds_16765752_UserDefined (47131244)_140707050 | chr12 | 112919858 | 112920029 | 5056.00 | EX10 | 9163.1 |
| PTPN11_Cds_16766151_UserDefined (47229417)_140707047 | chr12 | 112892348 | 112892504 | 3610.50 | EX5 | 9163.1 |
| PTPN11_Cds_16766381_UserDefined (47229414)_140707041 | chr12 | 112856896 | 112856949 | 208.00 | EX1 | 9163.1 |
| PTPN11_Cds_16766939_UserDefined (47229451)_140707106 | chr12 | 112942479 | 112942585 | 3349.50 | EX15 | 9163.1 |
| PTPN11_Cds_16766945_UserDefined (47131248)_140707045 | chr12 | 112890979 | 112891211 | 10530.50 | EX4 | 9163.1 |
| PTPN11_Cds_16766945_UserDefined (47131248)_140707046 | chr12 | 112890979 | 112891211 | 637.00 | EX4 | 9163.1 |
| PTPN11_Cds_16767137_UserDefined (47229420)_140707052 | chr12 | 112926808 | 112926999 | 3461.00 | EX13 | 9163.1 |
| PTPN11_Cds_16767501_UserDefined (47229421)_140707053 | chr12 | 112939928 | 112940080 | 4683.50 | EX14 | 9163.1 |
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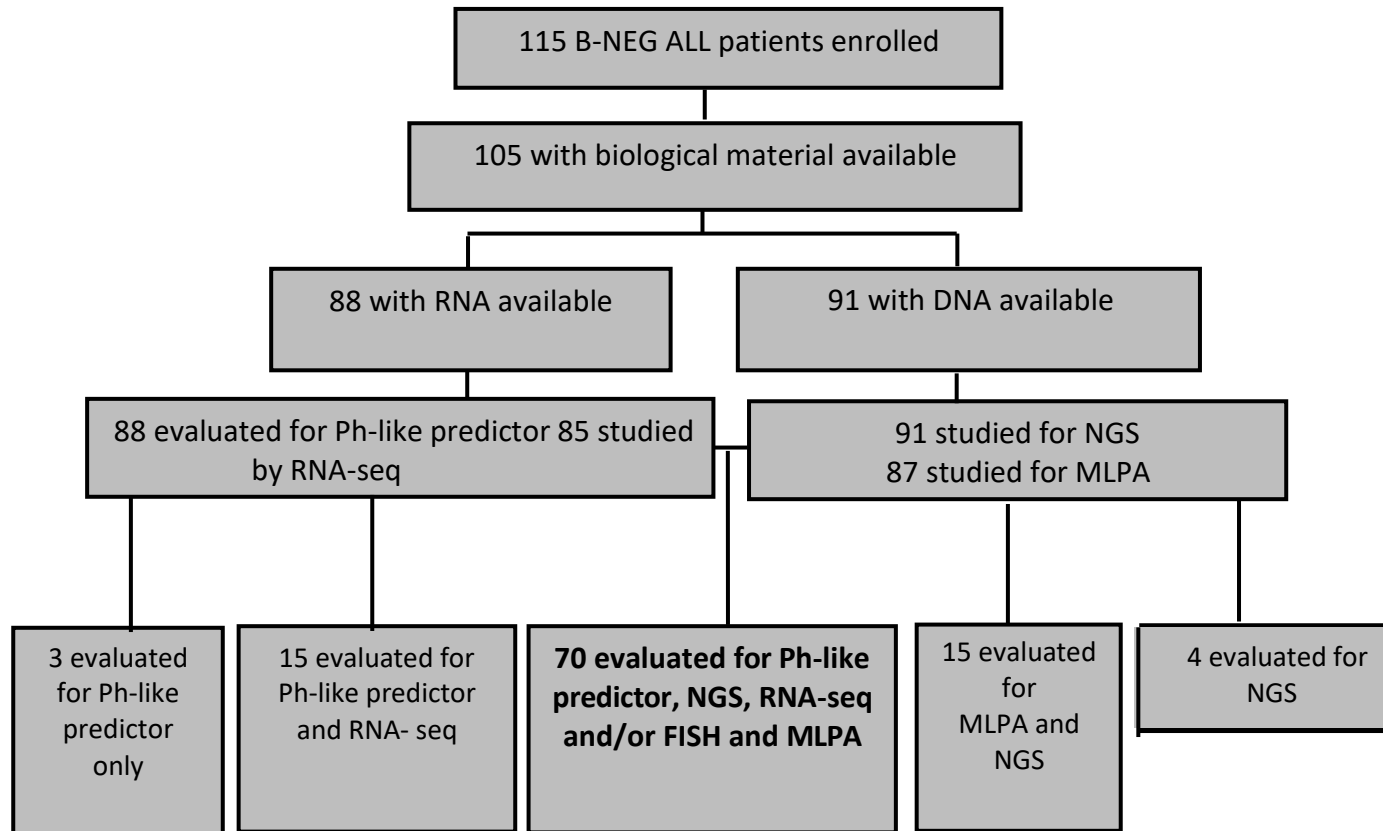
Supplemental Table 6. Univariate analyses for CR achievement, considering clinically relevant variables and molecular prognostic markers.

| | Univariate analysis for CR | |
|--|----------------------------|---------|
| | OR (95%CI) | p-value |
| Ph-like vs non-Ph-like | 0.265 (0.071-0.921) | 0.038 |
| Age | 0.995 (0.958-1.033) | 0.788 |
| WBC | 0.989 (0.977-1) | 0.062 |
| Plts | 1 (0.994-1.008) | 0.924 |
| Hb | 1.36 (1.011-1.89) | 0.051 |
| F vs M | 1.898 (0.589-7.313) | 0.306 |
| No SR vs SR | 0.311 (0.085-1.059) | 0.063 |
| <i>IKZF1+ CDKN2A/2B and/or PAX5 vs IKZF1-only/WT</i> | 0.362 (0.101-1.37) | 0.119 |
| Cell cycle genes deletion vs WT | 1.895 (0.547-7.605) | 0.329 |
| RAS clonal vs WT/M subclonal | 3.125 (0.757-21.247) | 0.158 |
| JAK/STAT clonal vs WT/M subclonal | 0.571 (0.12-4.139) | 0.515 |

Supplemental Figure 1. Scheme of GIMEMA LAL1913 clinical trial.



Supplemental Figure 2: Consort diagram summarizing the biological analyses carried out.



Supplemental Figure 3: OS of Ph-like (red line, n=27) vs non-Ph-like (n=59).

