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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

by Sabina Chiaretti, Monica Messina, Irene Della Starza, Alfonso Piciocchi, Luciana Cafforio, Marzia Cavalli, Akram Taherinasab, Michela Ansuinelli, Loredana Elia, Guglielmo Albertini Petroni, Roberta La Starza, Martina Canichella, Alessia Lauretti, Maria Ctristina Puzzolo, Valentina Pierini, Alessandra Santoro, Orietta Spinelli, Valerio Apicella, Saveria Capria, Francesco Di Raimondo, Paolo De Fabritiis, Cristina Papayannidis, Anna Candoni, Roberto Cairoli, Marco Cerrano, Nicola Fracchiolla, Daniele Mattei, Chiara Cattaneo, Antonella Vitale, Enrico Crea, Paola Fazi, Cristina Mecucci, Alessandro Rambaldi, Anna Guarini, Renato Bassan, and Robin Foà

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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

Sabina Chiaretti[1]*, Monica Messina[1,2*], Irene Della Starza[1,2], Alfonso Piciocchi[2], Luciana Cafforio[1], Marzia Cavalli[1], Akram Taherinasab[1], Michela Ansuinelli[1], Loredana Elia[1], Guglielmo Albertini Petroni[1], Roberta La Starza[3], Martina Canichella[1], Alessia Lauretti[1], Maria Cristina Puzzolo[1], Valentina Pierini[3], Alessandra Santoro[4], Orietta Spinelli[5], Valerio Apicella[1], Saveria Capria[1], Francesco Di Raimondo[6], Paolo De Fabritiis[7], Cristina Papayannidis[8], Anna Candoni[9], Roberto Cairoli[10], Marco Cerrano[11], Nicola Fracchiolla[12], Daniele Mattei[13], Chiara Cattaneo[14], Antonella Vitale[1], Enrico Crea[2], Paola Fazi[2], Cristina Mecucci[3], Alessandro Rambaldi[5], Anna Guarini[15], Renato Bassan[16], Robin Foà[1]

[1]Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome; [2]GIMEMA Data Center, Fondazione GIMEMA Franco Mandelli Onlus, Rome; [3]Department of Medicine, Hematology and Bone Marrow Transplantation Unit, University of Perugia, Perugia; [4]Division of Hematology and Bone Marrow Transplantation, Ospedali Riuniti Villa Sofia-Cervello, Palermo; [5]Hematology and Bone Marrow Transplant Unit, Ospedale Papa Giovanni XXIII, Bergamo; [6]Section of Haematology, Department of General Surgery and Medical-Surgical Specialties, University of Catania, Catania; [7]Hematology Division, S. Eugenio Hospital, Rome; [8]Seragnoli Institute of Hematology, Bologna University School of Medicine, Bologna; [9]Clinica di Ematologia e Unità di terapie Cellulari 'Carlo Melzi'-Azienda Sanitaria-Universitaria, Integrata, Udine; [10]Division of Hematology, Niguarda Hospital, Milan; [11]Department of Oncology, Division of Hematology, Presidio Molinette, AOU Città della Salute e della Scienza di Torino, Torino; [12]UOC Oncoematologia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Università degli Studi di Milano; [13]Department of Hematology, Ospedale S. Croce, Cuneo; [14]Department of Hematology, ASST Spedali Civili, Brescia; [15]Department of Molecular Medicine, Sapienza University, Rome; [16]Hematology Unit, Ospedale dell'Angelo and Ospedale Ss Giovanni e Paolo, Mestre Venezia. *Equal contribution

Corresponding authors: Sabina Chiaretti and Robin Foà
E-mail: chiaretti@bce.uniroma1.it; rfoa@bce.uniroma1.it
Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome, Italy
Via Benevento 6, 00161 Rome, Italy
Phone: +39-06-85795753, Fax: +39-06-85795792

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Article summary:

1. Ph-like ALL correlates with low CR rate, MRD persistence and poor survival in adult B-ALL patients also when treated in pediatric-like, MRD-driven trial.

2. The design of *ad hoc* front-line clinical trials is warranted in order to improve the management and outcome of this difficult to treat population.

ABSTRACT

Early recognition of Ph-like acute lymphoblastic leukemia cases could impact on the management and outcome of this subset of B-lineage ALL. To assess the prognostic value of the Ph-like status in a pediatric-inspired, minimal residual disease (MRD)-driven trial, we screened 88 B-lineage ALL cases negative for the major fusion genes (*BCR-ABL1*, *ETV6-RUNX1*, *TCF3-PBX1* and *KTM2Ar*) enrolled in the GIMEMA LAL1913 front-line protocol for adult *BCR/ABL1*-negative ALL. The screening - performed using the "*BCR/ABL1*-like predictor" - identified 28 Ph-like cases (31.8%), characterized by *CRLF2* overexpression (35.7%), JAK/STAT pathway mutations (33.3%), *IKZF1* (63.6%), *BTG1* (50%) and *EBF1* (27.3%) deletions, and rearrangements targeting tyrosine kinases or *CRLF2* (40%). The correlation with outcome highlighted that: i) the complete remission (CR) rate was significantly lower in Ph-like compared to non-Ph-like cases (74.1% *vs* 91.5%, p=0.044); ii) at time point 2 (TP2), decisional for transplant allocation, 52.9% of Ph-like cases *vs* 20% of non-Ph-like were MRD-positive (p=0.025); iii) the Ph-like profile was the only parameter associated with a higher risk of being MRD-positive at TP2 (p=0.014); iv) at 24 months, Ph-like patients had a significantly inferior event-free and disease-free survival compared to non-Ph-like patients (33.5% *vs* 66.2%, p=0.005 and 45.5% *vs* 72.3%, p=0.062, respectively). This study documents that Ph-like patients have a lower CR rate, EFS and DFS, as well as a greater MRD persistence also in a pediatric-oriented and MRD-driven adult ALL protocol, thus reinforcing that the early recognition of Ph-like ALL patients at diagnosis is crucial to refine risk-stratification and to optimize therapeutic strategies.

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INTRODUCTION

Ph-like acute lymphoblastic leukemia (ALL) accounts for 15-30% of B-lineage ALL, with an increasing incidence starting from adolescence. The growing interest in this subgroup of ALL arises from the distinctive gene expression profile - that resembles that of the true Ph-positive cases - and by the unfavorable clinical outcome.[1,2] The in-depth and large-scale genetic characterization has shown that the majority of Ph-like ALL cases carry fusion genes involving tyrosine kinases (i.e. ABL-class and *JAK2* rearrangements), or cytokine receptor rearrangements (i.e. *P2RY8/CRLF2* and *IGH/CRLF2*), frequently associated with mutations of the JAK/STAT pathway genes.[3-5] Among the other cooperating events, a relevant role is played by *IKZF1* deletions present in about 70% of cases.[4-7] The possibility of recognizing these cases at diagnosis has important prognostic implications and would also pave the way to testing tyrosine kinase inhibitors (TKI) and other targeted therapeutic approaches that have proven successful in pre-clinical models and *in vivo* in a few relapsed patients.[3,8-12] So far, several strategies[13-15] have been reported in an attempt to identify *Ph*-like cases, but none of them is deemed as the gold standard for the diagnostic work-up of these patients. To this end, our group recently reported a predictive tool called "*BCR/ABL1*-like predictor" based on the levels of expression of 9 genes together with *CRLF2* transcript quantification.[7] From a clinical standpoint, Ph-like patients are characterized by a worse outcome which is due to an inferior response to induction therapy, a higher incidence of relapses and a lower survival.[1,2,4] Since minimal residual disease (MRD) is considered today the most important prognostic factor in ALL, the role of the Ph-like status has been investigated in the context of MRD-driven protocols, with contradicting results. Roberts and colleagues reported in a pediatric cohort that Ph-like patients, though displaying higher MRD levels at the end of induction, had a survival probability similar to that of non-Ph-like childhood ALL when treated with intensive therapies.[16] Opposite results were obtained by Heatley et al[14] who demonstrated that, despite a risk-adjusted treatment approach, a high rate of relapse was recorded among children who were retrospectively identified as Ph-like. In adolescents and young adults, the results of the CALGB10403 trial, based on a pediatric inspired regimen, have shown that parameters associated with inferior survival rates were indeed represented by the Ph-like signature and obesity.[17] In adult cohorts, all reported studies so far agree on a shorter survival likelihood for Ph-like ALL compared to non-Ph-like patients.[5-7,18,19] However, the data are still insufficient to elucidate whether intensive treatments are capable of abolishing the negative impact of the Ph-like status on prognosis: conflicting results have been reported in the studies by Jain et al[20] and Herold et al.[6]

Likewise, the role of the Ph-like status in the context of MRD-driven clinical trials is still unclear, since the data produced by the German study group were derived from a small cohort of patients.[6] In order to clarify these aspects, we hereby evaluated the incidence and clinico-biologic features of Ph-like cases - identified using the *BCR/ABL1*-like predictor[7] - and the prognostic role of the Ph-like profile in terms of CR achievement, MRD persistence and survival in a cohort of adult ALL patients homogeneously and intensively treated in the pediatric-oriented, MRD-driven LAL1913 GIMEMA front-line protocol for adult Ph-negative ALL.

METHODS

Study population and experimental strategy

This study included B-lineage ALL patients negative for major molecular aberrations (*BCR/ABL1*, *KT2MA* and *TCF3/PBX1,* B-NEG) enrolled in the GIMEMA LAL1913 front-line clinical trial (NCT02067143, Supplemental Figure 1) - designed for Ph-negative ALL patients aged 18-65 years - based on a pediatric-oriented backbone, in which Peg-Asparaginase was administered instead of Asparaginase, and on a MRD-driven transplant allocation[20]; MRD time-points and MRD analysis are detailed in Supplementary Materials and Methods. The EC study number approval is 5629.

Diagnostic bone marrow samples were available from 105 patients (median age 38.7 years, range 18.2-64.7). Baseline patients' characteristics are summarized in Supplementary Table 1; there were no differences in clinico-biologic features between our cohort and the remaining population enrolled in the protocol (Supplementary Table 2). All cases underwent a centralized molecular screening: i) the "*BCR/ABL1*-like predictor" assay, ii) sequencing of the JAK/STAT and RAS cascades by NGS, iii) Multiplex Ligation-dependent Probe Amplification (MLPA), iv) targeted RNA sequencing. In 17 cases, the *BCR/ABL1*-like predictor was not feasible due to lack of RNA (Supplementary Table 3 and Supplementary Figure 2).

BCR/ABL1-like predictor

To detect the Ph-like cases, we applied the "*BCR/ABL1*-like predictor"[7] to 88 patients (Supplementary Materials and Methods).

Screening of recurrent mutations and deletions

The members of the JAK/STAT (*JAK1*, *JAK2*, *JAK3*, *IL7R* and *CRLF2*) and RAS (*FLT3*, *NRAS*, *KRAS* and *PTPN11*) pathways (181 amplicons) were sequenced by NGS (Supplementary Materials and Methods).

NGS experiments were performed in 91 cases (74 in common with the *BCR/ABL1*-like predictor analysis - 24 Ph-like and 50 non-Ph-like ALL cases -, Supplementary Materials and Methods and Table 3). Variants recognized as single nucleotide polymorphisms (SNPs) were excluded, unless of prognostic value or previously reported in Ph-like ALL.[21]

Recurrent deletions (*IKZF1*, *CDKN2A/2B*, *PAX5*, *EBF1*, *BTG1*, *RB1*, *ETV6* and *CRLF2)* were screened in 87 samples (70 in common with the *BCR/ABL1*-like predictor analysis - 22 Ph-like and 48 non-Ph-like ALL cases -, Supplemental Table 3), by the Salsa MLPA P335 ALL-IKZF1 kit (MRC-Holland, Amsterdam, The Netherlands) and analyzed according to Coffalyser manual.[22] *P2RY8/CRLF2* was inferred when a deletion within the PAR1 region was documented. Samples were defined *IKZF1+ CDKN2A/2B and/or PAX5* when *IKZF1* deletion co-occurred with *CDKN2A/2B* and/or *PAX5* deletions.[23]

Targeted RNA-sequencing and FISH analysis

To detect fusion genes, libraries were prepared using the TruSight RNA Pan-Cancer Panel (Illumina, San Diego, CA) kit, targeting 1385 cancer- genes (Supplementary Materials and Methods).

Double-color FISH studies were performed in 20 B-ALL, 13 Ph-like and 7 non-Ph-like with high levels of *CRLF2* expression (Supplementary Materials and Methods).

Overall, 85 cases were screened (25 Ph-like and 60 non- Ph-like ALL cases, Supplemental Table 3).

Statistical analyses

Patients' characteristics were compared by chi-squared or Fisher's exact test for categorical variables and Wilcoxon test for continuous data. OS, DFS and EFS were estimated by the Kaplan-Meier product-limit and compared by log-rank test. OS was defined as the time between the date of diagnosis and death for any cause; patients still alive were censored at the time of the last follow-up. DFS was defined as the time between the evaluation of CR - after the induction phase - and relapse or death in CR; patients still alive in first CR, were censored at the time of the last follow-up. Finally, EFS was defined as the time between diagnosis and non-achievement of CR in the induction phase, relapse or death in CR, whichever occurred first; patients still alive, in first CR, were censored at the time of the last follow-up.

Multivariate analysis was performed with the Cox proportional hazards regression model to adjust the effect of *BCR/ABL1*-like predictor for clinically relevant parameters (age, WBC, Hb level, platelet count, gender and allogeneic transplant (HSCT) and for genetic aberrations impacting on prognosis (*IKZF1+ CDKN2A/2B and/or PAX5*, *K/NRAS* clonal mutations, JAK/STAT clonal mutations).[21,22] All tests were 2-sided, accepting $p<0.05$ as statistically significant. All analyses relied on the SAS v9.4 software. Study data were collected and managed using REDCap[24] electronic data capture tools hosted at the GIMEMA Foundation.

RESULTS

Incidence and clinical features of Ph-like ALL

We identified 28 (31.8%) Ph-like cases with a median score of 0.85 (range: -0.18 - 6.37); the remaining 60 cases had a median score equal to -1.24 (range -1.7 - -0.33). Overall, the clinical features (age, gender, WBC and platelet counts) at diagnosis of Ph-like and of non-Ph-like cases were similar. Ph-like patients had lower hemoglobin levels ($p=0.016$), as detailed in Table 1. The incidence of Ph-like ALL cases was slightly higher in adults (≥36 years) than in young adults (18-35 years), being 36.2% (17/47) and 26.8% (11/41), respectively. As per clinical protocol guidelines, only 45% of Ph-like cases were assigned to the high-risk category.

Genetic features of Ph-*like ALL cases*

The identified Ph-like cases were evaluated for the following genetic features*: CRLF2 e*xpression levels (n=28), JAK/STAT and RAS pathways mutations (n=24), CNA aberrations (n=22) and fusion genes (n=23), the latter either by RNA-sequencing and/or FISH. A *CRLF2* overexpression, defined as ΔCt <8,[25] was found in 10/28 Ph-like cases (35.7%). Among the *CRLF2*-high cases with a ΔCt value <4.5, we observed that 3 harbored a *CRLF2* rearrangement, with 1 displaying a concomitant F232C *CRLF*2 mutation. Of the remaining 7 *CRLF2*-high cases, 3 had a concomitant rearrangement (2 ABL-class and 1 *DDX3X/USP9X*), 1 displayed a JAK1 and RAS mutation, and in 2 cases the mutational screening could not be performed due to lack of genomic material; finally, in 1 case no additional lesions were detected. Among the 24 Ph-like cases analyzed for the mutational status, we detected a total of 13 JAK/STAT pathway mutations - 9 clonal and 4 subclonal - in 8 cases (33.3%). Despite a high heterogeneity among samples, the most frequently mutated genes were *JAK1* - affected by 5 mutations mainly targeting the hotspot V658 - and *JAK2* - affected by 3 mutations focused in the hotspot R683. *IL7R* and *CRLF2* were mutated in 2 samples, while *JAK3*

only in 1. Furthermore, 6 of the 8 mutated samples (75%) displayed a concomitant *CRLF2* overexpression. Nine RAS pathway mutations - only 1 being clonal - were found in 6 patients (25%). The most frequent mutations (n=5) involved the hotspot G12-13 of *KRAS* and *NRAS.* CNA analysis in Ph-like cases revealed *IKZF1*, *BTG1, CDKN2A/2B, PAX5* and *EBF1* deletions in 14 (63.6%), 11 (50%), 7 (31.8%), 7 (31.8%) and 6 (27.3%) cases, respectively. Furthermore, *IKZF1 + CDKN2A/2B* and *PAX5* deletions, known to confer a very poor outcome, were identified in 10 cases (45.5%). Finally, RNA-sequencing and/or FISH experiments of the Ph-like ALL cases revealed 11 TK activating lesions (47.8%): 5 ABL-class fusion genes (3 *NUP214/ABL1*, 1 *ZC3HAV1/ABL2* and 1 *EBF1/PDGFRB*), 2 *BCR/JAK2,* 3 *CRLF2*-r and 1 *DDX3X/USP9X*, the latter known to be associated with *CRLF2* deregulation.[26]

Overall, Ph-like associated lesions were identified in 70.8% (17/24) of cases and are summarized in Table 2.

When the genetic landscape of Ph-like ALL was compared to that of the non-Ph-like cases, significant differences emerged. As shown in Table 3, *CRLF2*-high was significantly more frequent in Ph-like ALL (35.7% vs 13.3%, p=0.018). Similarly, clonal JAK/STAT mutations were specific of the Ph-like subset (33.3% vs 4%, p=0.001), while RAS pathway clonal mutations were more frequent in non-Ph-like than in Ph-like ALL cases (46% vs 4.2%, p=0.001). CNAs analysis documented that *IKZF1*, *EBF1* and *BTG1* deletions were significantly more common of the Ph-like than in the non-Ph-like subset (63.6%, 50% and 27.3% vs 25%, 7.8% and 2.1%, respectively; p=0.002, p<0.001 and p=0.007); *CDKN2A/2B* and *PAX5* deletions were equally distributed among Ph-like and non-Ph-like cases (31.8% *vs* 47.9% and 31.8% *vs* 22.9%, respectively).

The analysis of fusion genes, performed on a total of 85 patients, showed that rearrangements involving TKs or cytokine receptors were significantly higher in the Ph-like cases with 10 fusion genes involving either *CRLF2* or a TK compared to only 1 *CRLF2*-r case in the non-*BCR/ABL1*-like cases (43.5% *vs* 1.6%, p<0.001).

The genetic lesions documented in both the Ph-like and non-Ph-like subgroups are detailed in the Supplemental Table 3 and their distribution is provided in Figure 1; further details on non-Ph-like ALL cases, as well as on NGS coverage, are provided in Supplemental Results and Supplemental Table 5, respectively.

Response to treatment, MRD evaluation and transplant allocation

The Ph-like status was significantly associated with response to treatment: in fact, Ph-like patients had a significantly inferior CR rate at TP1 compared to non-Ph-like cases (74.1% *vs* 91.5%, p=0.044,

Table 4) and this translated into a lower probability of CR achievement (p=0.038, OR=0.265, CI 95% 0.071-0.921, Supplemental Table 6). The latter data retained statistical significance also in a multivariate model adjusted for clinically relevant parameters, as well as for genetic lesions with a prognostic relevance.

MRD evaluation - feasible in 64 patients at TP1, 62 at TP2 and 49 at TP3 - showed that at TP1, 77.8% of Ph-like cases and 41.3% of non-Ph-like were MRD-positive (p=0.012); at TP2, 52.9% of Ph-like cases and 20% of non-Ph-like were MRD-positive (p=0.025); similarly, at TP3, 41.7% of Ph-like cases and 13.5% of non-Ph-like cases were MRD-positive (p=0.05). These data, summarized in Table 4, indicate that in the Ph-like patients there is a significantly higher MRD persistence at all TPs evaluated compared to non-Ph-like cases. Consistently, the univariate analyses for MRD results showed that - when considering both clinically relevant parameters and genetic prognostic markers - only the Ph-like status was a risk factor for being MRD-positive at TP2 (p=0.014, OR=4.5, CI 95% 1.373-15.508) (Table 5).

As a consequence, HSCT rate in first CR was significantly higher (p=0.015) in Ph-like vs non-Ph-like cases (8/20 *vs* 6/54, 40% *vs* 11%, respectively), in line with the guidelines of the trial, in which MRD persistence was a criterion for HSCT allocation. Importantly, among 5 MRD+ Ph-like patients who did not undergo a transplant, 4 relapsed at a median period a 7.8 months from CR, whereas no relapses occurred in the 3 MRD+ Ph-like patients undergoing HSCT.

Survival analyses

Survival analyses at 24 months showed that Ph-like ALL patients had a significantly inferior EFS than non-Ph-like patients (33.5% *vs* 66.2%, p=0.005); this difference was also evident with regard to DFS (45.5% *vs* 72.3%, p=0.062), though to a lesser extent, as illustrated in Figure 2; OS was also investigated, and although not significant, it was inferior in Ph-like ALL cases than in non-Ph-like patients (48.5% vs 72.9%, p=0.16, Supplemental Figure 3). The lack of significance is most likely due to the fact that a higher number of Ph-like patients, because of persistent MRD positivity underwent, as per protocol guidelines, HSCT (40% *vs* 11% in Ph-*like* vs non-Ph-like cases, respectively, p=0.015).

In a multivariate model for EFS, adjusting for relevant clinical parameters - including HSCT, evaluated as a time dependent covariate - and genetic prognostic markers, the Ph-like profile, age and Hb levels were the only risk factors that retained statistical significance (Table 6). Notably, however, Ph-like patients undergoing an allogeneic transplant showed a trend towards better EFS (p=0.078).

DISCUSSION

The possibility of an early recognition of Ph-like ALL patients offers the unprecedented opportunity to refine the prognostic categories of Ph-negative ALL, and to better understand the reasons for the poor outcome. In the present study, we investigated a cohort of adult B-NEG ALL patients enrolled in the front-line GIMEMA LAL1913 protocol,[20] based on a pediatric-inspired backbone and in which MRD quantification at week 10 is pivotal for transplant allocation, in order to assess the prognostic impact of the Ph-like status. In particular, we aimed at understanding the interplay between the Ph-like status and MRD response. Furthermore, we sought to analyze the clinical and genetic features, the hematologic responses to treatment and the outcome of the identified Ph-like ALL patients.

The screening carried out using the *BCR/ABL1*-like predictor[7] led to the identification of 28 Ph-like cases - representing 31.8% of the B-NEG cohort - with a slightly higher incidence in adults than in young adults. This finding is in agreement with the recently reported data in other adult cohorts and resembles the epidemiologic behavior of "true Ph-positive" ALL.[5,6,19] The comparison of the clinico-biologic features of Ph-like and non-Ph-like cases revealed a substantial homogeneity in terms of WBC count and gender distribution, as in the GMALL and the MDACC clinical trials,[6,19] and at variance from Roberts and colleagues[5] who reported that adult *BCR/ABL1*-like patients have a higher WBC and are prevalently of male gender. In children, an association with hyperleukocytosis has been described by Den Boer *et al*[1] and Reshmi *et al*[27], the latter based on the COG AALL1131 high-risk cohort. The association with male gender was documented in the Total Therapy XV cohort.[16] On the contrary, Roberts and colleagues[28] did not find significant differences in the WBC count and gender in the standard-risk subset of childhood B-ALL patients enrolled in the COG AALL0331. In addition to the WBC count and gender, it is worth underlying that in our study the population of Ph-like patients was allocated to both the standard- (56%) and high-risk (44%) categories: this finding has important clinical implications since the prompt identification of these cases might lead to a better therapeutic stratification that ultimately would avoid undertreating these high-risk patients. In adults, a similar distribution was reported also by Herold *et al*[6], while in the pediatric setting this issue is still controversial. Indeed, most Ph-like cases were associated to a high risk in both the COALL and DCOG cohorts[1], while in the Total Therapy XV trial[16] Ph-like cases were equally distributed in the standard and high NCI risk groups. Of note, in the report on 139

children classified as standard-risk, Roberts and colleagues[28] showed that the Ph-like status did not affect outcome, suggesting that in children risk stratification is clinically more significant than the genomic features.

From a genetic standpoint, the present study further corroborates the notion that *CRLF2* overexpression, JAK/STAT mutations and deletions of *IKZF1*, *BTG1* and *EBF1* are significantly more frequent in Ph-like ALL cases. In addition, we observed that clonal JAK/STAT mutations were almost exclusively found in Ph-like ALL, while clonal RAS mutations were specific of non-Ph-like cases, thus suggesting that they play a different role in the two molecular subtypes. Moreover, when focusing on *CRLF2* overexpression, it emerges that it is not sufficient to induce a Ph-like profile: indeed, of the 8 Ph-like cases that were fully characterized, 7 had at least another lesion. Furthermore, the results on the incidence of rearrangements targeting TKs and cytokine receptors indicate that they prevail in the Ph-like subgroup, with ABL-class gene rearrangements outnumbering the other lesions. Thus, we could identify at least 1 underlying genetic lesion in 70.8% of Ph-like patients. Not for all cases it was possible to perform an extensive biologic screening due to the lack of genomic material (4 cases) and RNA-sequencing was carried out using targeted approaches and not genome-wide tools. This may help to explain why no further genetic lesions could be found in the remaining cases (29.2%) that proved positive with the BCR/ABL1 predictor. The validity and reproducibility of the *BCR-ABL1-like* predictor has been externally validated by other institutions and from external samples in Europe, showing an overall concordance with other tools (FISH and NGS) of 88%.[29]

Concerning the relationship between the Ph-like status, MRD response and outcome, we showed that Ph-like ALL patients have a higher risk of CR failure: in fact, 74.1% of Ph-like ALL and 91.4% non-Ph-like achieved a CR. This difference was not detected in the intensive GMALL trials 06/99 and 07/03 - where all patients achieved a CR, though with a short duration -,[6] nor in the hyper-CVAD-based protocols or the augmented BFM regimen administered at MDACC.[19]

More importantly, our study allowed to correlate the Ph-like status with MRD, that is presently regarded as the most important prognostic marker in ALL management. In fact, this analysis showed that in the GIMEMA LAL1913 protocol, at all TPs analyzed, the percentage of MRD-positive patients was significantly higher in the Ph-like ALL subset than in non-Ph-like cases. This difference was particularly evident at TP2 (HSCT decisional point), when 52.9% of Ph-like and only 20% of non-Ph-like cases were MRD-positive. Indeed, when both clinically relevant parameters and genetic prognostic markers were taken into account the Ph-like profile proved the only risk

factor for MRD positivity at TP2. Thus, considering both response to induction treatment and MRD monitoring, the Ph-like status, if identified early, permits not only to recognize patients who are likely to be refractory to induction treatment, but also to identify - within cases who achieve a CR - those who are likely to remain MRD-positive. This strong association may allow to anticipate therapeutic changes.

To our knowledge, this is the first study that analyzes the interaction between the Ph-like status and MRD - assessed by quantitative polymerase chain reaction of the *IG* and *TR* gene rearrangements - in a broad cohort of uniformly and prospectively treated adult ALL patients within a clinical trial. Similar results were provided by Herold and colleagues[6] who found that Ph-like patients were less likely to achieve a MRD-negative status in a small cohort of 31 patients with overlapping MRD and Ph-like status information. In the pediatric setting, contradicting results have been reported.[14,16]

Furthermore, the comparison of survival curves highlighted that Ph-like patients experienced a significantly worse EFS at 24 months compared to that of non-Ph-like cases (33.5% and 66.2%, respectively). Along the same line, also in cases achieving a CR, the Ph-like profile had a negative prognostic impact, as shown by the worse DFS of Ph-like patients. Although limited by the small sample size, our study demonstrates that transplant is beneficial in these cases and should be pursued at the earliest opportunity, as shown by the high rate of relapses within non-transplanted Ph-like patients (4/5 MRD positive patients relapsed).

Lastly, in all outcome parameters evaluated - CR achievement, MRD at TP2 and EFS - the Ph-like status emerged as an independent prognostic marker.

In addition to confirming the inferior outcome of Ph-like ALL patients, these data indicate that the differences between Ph-like and non–Ph-like cases are not abolished by pediatric-like intensive therapeutic schemes, in agreement with the results of the MDACC group.[18] Based on the MRD findings hereby reported, this is primarily contributed to the significantly lower rates of complete molecular responses observed in Ph-like patients.

In light of the poor outcome of Ph-like ALL and of the possibility of using targeted approaches[30], different clinical trials specifically designed for Ph+ ALL and Ph-like ALL cases are testing the efficacy of dasatinib (NCT02420717, NCT02883049, NCT03564470, NCT02143414) or of dasatinib in combination with blinatumomab (SWOG-S1318, NCT02143414). Other studies are investigating the impact of blinatumomab in combination with chemotherapy in Ph-negative B-lineage ALL (GIMEMA LAL2317- NCT03367299 and NCT02003222). In these latter studies, it is being

investigated if the addition of blinatumomab can increase the rates of CR and MRD-negativity in Ph-like patients, as already observed in Ph+ ALL.[32] In support of the fact that Ph-like patients may benefit from targeted treatment, a recent study from Tanasi and colleagues has reported that the introduction of TKIs front-line was associated with a 3-years OS of 77%.[31] Other compounds, such as ruxolitinib (NCT02420717, NCT03571321, NCT02723994) and the histone deacetylase inhibitor chidamide (NCT03564470) are under investigation.

Taken together, the results of this study carried out on adult B-NEG ALL cases enrolled in the front-line GIMEMA LAL1913 clinical protocol confirm that the *BCR/ABL1*-like predictor[7] is a valid tool to rapidly recognize Ph-like cases that account for about 30% of adult B-NEG ALL. In addition, we could show that also in a pediatric-oriented and MRD-driven clinical trial Ph-like patients have a lower probability of achieving a CR, are more likely to remain MRD-positive and have a significantly shorter EFS. The Ph-like profile is an independent risk factor for CR failure and MRD-persistence, thus further underlying the need that Ph-like cases - a primary unmet clinical need in ALL - are rapidly recognized at diagnosis in order to refine the risk stratification of Ph-negative ALL and optimize patients' management. Further investigations are currently ongoing to unravel if within Ph-like ALL there are subgroups of patients with a different outcome likelihood.

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AUTHORSHIP CONTRIBUTION

SC designed research, analyzed data, provided clinical samples and clinical data,and wrote the manuscript; MM performed experiments, analyzed data and wrote the manuscript; AP performed statistical analyses; IDS, LC, AT, MC, LE, GAP, RLS, MCAL, MCP, VP, AS, OS, VA performed experiments; SC, FDR, PDF, CP, AC, RC, MC, NF, DM, CC, AV, provided samples and clinical data; EC and PF contributed to protocol management; AG and CM critically revised the manuscript; AR and

RB designed the trial and critically revised the manuscript; RF designed the research and the trial, and critically revised the manuscript.

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Table 1. Comparison between Ph-like and non-Ph-like clinical features.

	Ph-like	non-Ph-like	*p*-value
N	28	60	
Age (median [range])	42.24 [18.18-64.53]	34.52 [18.23-64.59]	ns
Wbc x10^9/L (median [range])	3.34 [0.23-347]	5.74 [1-75.5]	ns
Hb g/dL (median [range])	8.70 [3.70-13.00]	9.75 [5.00-15.70]	0.034
Plts x10^9/L (median [range])	40 [1.23-399]	66.5 [7.5- 630]	ns
Gender			
M	19 (67.9%)	34 (56.7%)	ns
F	9 (32.1%)	26 (43.3%)	
Risk category			
Standard risk	14 (56%)	34 (63%)	ns
No Standard risk	11 (44%)	20 (37%)	

Record ID	BCR/ABL1-like prediction	Score	CRLF2 expression	RAS pathway status	RAS pathway mutations (VAF)	JAK/STAT pathway status	JAK/STAT pathway mutations (VAF)	*IKZF1*	*CDKN2A/2B*	*PAX5*	*IKZF1 +CDKN2A* and/or *PAX5*	*BTG1*	*EBF1*	*CDKN2A/2B* and/or *RB1*	Gene rearrangements (RNAseq and/or FISH analysis)
B-ALL_1	BCR/ABL1-like	3.073	Low	WT		WT		no-Δ	no-Δ	no-Δ		no-Δ	Δ	no-Δ	*EBF1-PDGFRB*
B-ALL_3	BCR/ABL1-like	0.928	Low	M	FLT3_ITD (5.4%)	WT		Δ	Δ	Δ	Yes	no-Δ	no-Δ	Δ	No
B-ALL_4	BCR/ABL1-like	0.347	Low	WT		WT		no-Δ	no-Δ	no-Δ		Δ	no-Δ	Δ	No
B-ALL_7	BCR/ABL1-like	1.216	High	WT		M clonal	JAK1 DI630-631V (44.5%), JAK1V658I (35.5%)	Δ	Δ	no-Δ	Yes	no-Δ	no-Δ	Δ	*DDX3X/USP9X*
B-ALL_16	BCR/ABL1-like	0.788	Low	WT		WT		Δ	Δ	Δ	Yes	Δ	no-Δ	Δ	*BCR/JAK2*
B-ALL_22	BCR/ABL1-like	0.157	Low	M	FLT3_V491L (11.2%)	WT		Δ	no-Δ	no-Δ		Δ	no-Δ	Δ	*NUP214/ABL1*
B-ALL_26	BCR/ABL1-like	3.128	High	M	NRAS_G13D (4.1%)	M clonal	JAK1_V658I (35.5%)	Δ	no-Δ	Δ	Yes	no-Δ	Δ	no–Δ	No
B-ALL_31	BCR/ABL1-like	2.382	High	WT		M clonal	CRLF2_F232C (46.8%)	Δ	no-Δ	Δ	Yes	no-Δ	Δ	no-Δ	*IGH/CRLF2*
B-ALL_32	BCR/ABL1-like	5.720	Low	WT		WT		no-Δ	no-Δ	no-Δ		no-Δ	no-Δ	no-Δ	NA
B-ALL_34	BCR/ABL1-like	0.725	Low	M	PTPN11_Y279S (1.9%); NRAS_G12D (2.6%); KRAS_G12GG (5.2%)	WT		Δ	no-Δ	no-Δ		Δ	no-Δ	no-Δ	*NUP214/ABL1*
B-ALL_36	BCR/ABL1-like	0.205	High	WT		M clonal	JAK2_R683G (43.9%)	Δ	Δ	no-Δ	Yes	no-Δ	no-Δ	Δ	*P2RY8/CRLF2*
B-ALL_37	BCR/ABL1-like	0.386	Low	WT		WT		no-Δ	no-Δ	no-Δ		Δ	Δ	no-Δ	No
B-ALL_41	BCR/ABL1-like	0.726	Low	M	KRAS_G12A (4.4%); PTPN11 V194L (4.5%)	M clonal	IL7R_INDEL (38.4%); JAK2_C618F (3.3%)	Δ	Δ	no-Δ	Yes	no-Δ	no-Δ	Δ	No
B-ALL_44	BCR/ABL1-like	1.587	High	WT		WT		Δ	no-Δ	Δ	Yes	Δ	no-Δ	Δ	*ZC3HAV1/ABL2*
B-ALL_45	BCR/ABL1-like	0.262	Low	WT		M clonal	JAK3_T21M (19.1%); JAK1_T688I (5.7%)	NA	NA	NA		NA	NA	NA	No
B-ALL_46	BCR/ABL1-like	2.449	Low	WT		WT		no-Δ	no-Δ	no-Δ		no-Δ	no-Δ	no-Δ	No
B-ALL_52	BCR/ABL1-like	1.013	Low	WT		WT		no-Δ	Δ	Δ		Δ	no-Δ	Δ	No
B-ALL_55	BCR/ABL1-like	0.544	Low	WT		WT		no-Δ	no-Δ	no-Δ		Δ	no-Δ	no-Δ	No
B-ALL_61	BCR/ABL1-like	2.722	Low	NA		NA		NA	NA	NA		NA	NA	NA	No
B-ALL_62	BCR/ABL1-like	0.335	High	NA		NA		NA	NA	NA		NA	NA	NA	No
B-ALL_64	BCR/ABL1-like	-0.043	Low	WT		WT		NA	NA	NA		NA	NA	NA	NA
B-ALL_73	BCR/ABL1-like	0.048	Low	M clonal	KRAS_G12D (35.9%)	WT		Δ	no-Δ	no-Δ		no-Δ	Δ	no-Δ	*BCR/JAK2*
B-ALL_76	BCR/ABL1-like	1.971	Low	NA		NA		NA	NA	NA		NA	NA	NA	NA
B-ALL_81	BCR/ABL1-like	1.150	High	WT		WT		Δ	Δ	no-Δ	Yes	Δ	no-Δ	Δ	No
B-ALL_92	BCR/ABL1-like	-0.112	High	NA		NA		NA	NA	NA		NA	NA	NA	No
B-ALL_96	BCR/ABL1-like	6.371	High	WT		M clonal	CRLF2_V136M (60%)	Δ	no-Δ	Δ	Yes	Δ	Δ	no-Δ	*NUP214/ABL1*
B-ALL_97	BCR/ABL1-like	3.432	High	WT		M clonal	JAK2_R683G (10.2%); IL7R_S185C (18.1%); JAK1_V658F (13.8%)	Δ	no-Δ	no-Δ		Δ	no–Δ	no-Δ	*IGH/CRLF2*
B-ALL_100	BCR/ABL1-like	-0.180	Low	WT		WT		no-Δ	no-Δ	no-Δ		no-Δ	no-Δ	no-Δ	No

Table 2. Genetic features of Ph-like cases.

Table 3. Comparison between Ph-like and non-Ph-like genetic features.

	***BCR/ABL1*-like**	**non-*BCR/ABL1*-**	***p*-value**
***CRLF2* expression level**			
***CRLF2* overexpressing samples**	10/28 (35.7%)	8/60 (13.3%)	0.018
Mutational status			
RAS pathway mutated samples	6/24 (25%)	26/50 (52%)	0.025
Clonal RAS mutated	1/24 (4.16%)	23/50 (46%)	0.001
JAK/STAT pathway mutated samples	8/24 (33.3%)	7/50 (14%)	0.054
Clonal JAK/STAT mutated	8/24 (33.3%)	2/50 (4%)	0.001
Copy number aberrations			
***IKZF1* deleted**	14/22 (63.6%)	12/48 (25%)	0.002
IKZF1+ CDKN2A/2B* and/or *PAX5	10/22 (45.5%)	7/48 (14.6%)	0.007
***BTG1* deleted**	11/22 (50%)	4/48 (8.3%)	<0.001
***EBF1* deleted**	6/22 (27.3%)	1/48 (2.1%)	0.003
***CDKN2A/2B* deleted**	7/22 (31.8%)	23/48 (47.9%)	ns
***PAX5* deleted**	7/22 (31.8%)	11/48 (22.9%)	ns
TK or cytokine receptor fusion genes	10/23 (43.5%)	1/37 (2.7%)	<0.001

Table 4. CR achievement and MRD evaluation in Ph-like and non-Ph-like cases.

	Ph-like	non-Ph-like	*p*-value
CR achievement			
	20 (74.1%)	54 (91.5%)	0.044
TP1 (week 4)			
MRD-positive patients	14/18 (77.8%)	19/46 (41.3%)	0.012
TP2 (week 10)			
MRD-positive patients	9/17 (52.9%)	9/45 (20%)	0.025
TP3 (week 16)			
MRD-positive patients	5/12 (41.7%)	5/37 (13.5%)	0.05

Table 5. Univariate analyses for MRD at TP2, considering clinically relevant variables and molecular prognostic markers.

	Univariate analysis for MRD_TP2	
	OR (95%CI)	***p*-value**
Ph-like *vs* non-Ph-like	4.5 (1.373-15.508)	0.014
Age	1.012 (0.98-1.045)	0.475
WBC	1.013 (1-1.033)	0.133
Plts	0.987 (0.974-0.998)	0.0365
Hb	0.832 (0.638-1.06)	0.152
F *vs* M	0.459 (0.145-1.315)	0.1602
No SR *vs* SR	0.304 (0.065-1.048)	0.083
IKZF1+ CDKN2A/2B and/or PAX5 vs IKZF1-only/WT	1.869 (0.49-6.674)	0.339
Cell cycle genes deletion *vs* WT	0.88 (0.279-2.773)	0.8253
RAS clonal *vs* WT/M subclonal	0.8 (0.239-2.51)	0.706
JAK/STAT clonal *vs* WT/M subclonal	2.596 (0.463-13.293)	0.2482

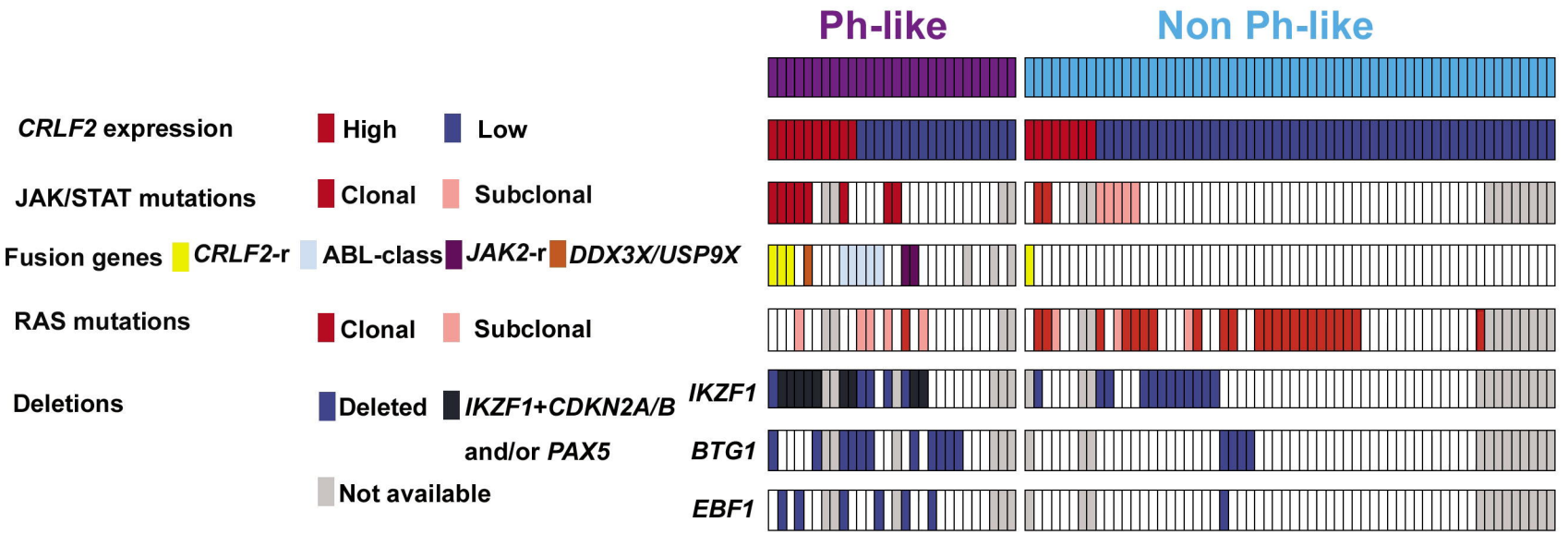
Table 6. Summary of univariate and multivariate analyses for EFS, considering clinically relevant variables and molecular prognostic markers.

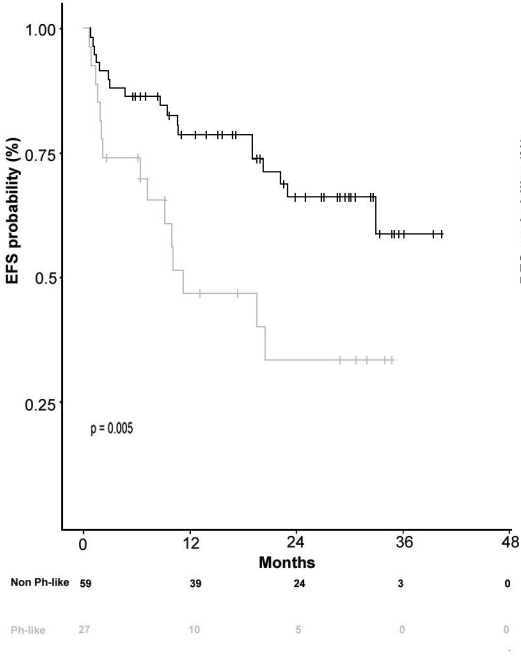
	Univariate analysis for EFS		**Multivariate analysis for EFS**	
	HR (95%CI)	***p*-value**	**HR (95%CI)**	***p*-value**
Ph-like vs non-Ph-like	2.6 (1.3-5.19)	0.007	2.3 (1.124-4.92)	0.023
Age	1.03 (1.01-1.05)	0.004	1.04 (1.015-1.067)	0.002
WBC	1.005 (0.999-1.010)	0.074		
Plts	0.993 (0.986-0.999)	0.023		
Hb	0.81 (0.69-0.94)	0.006	0.782 (0.649-0.943)	0.01
F *vs* M	0.78 (0.41-1.5)	0.455		
No SR *vs* SR	1.89 (0.97-3.67)	0.062		
HSCT vs. No HSCT as a time dependendent covariate	1.04 (0.35-3.10)	0.939		
IKZF1+ CDKN2A/2B and/or PAX5 vs IKZF1-only/WT	1.73 (0.76-3.98)	0.193		
Cell cycle genes deletion *vs* WT	0.967 (0.451-2.069)	0.93		
RAS clonal *vs* WT/M subclonal	0.604 (0.269-1,358)	0.222		
JAK/STAT clonal *vs* WT/M	0.85 (0.26-2.82)	0.796		

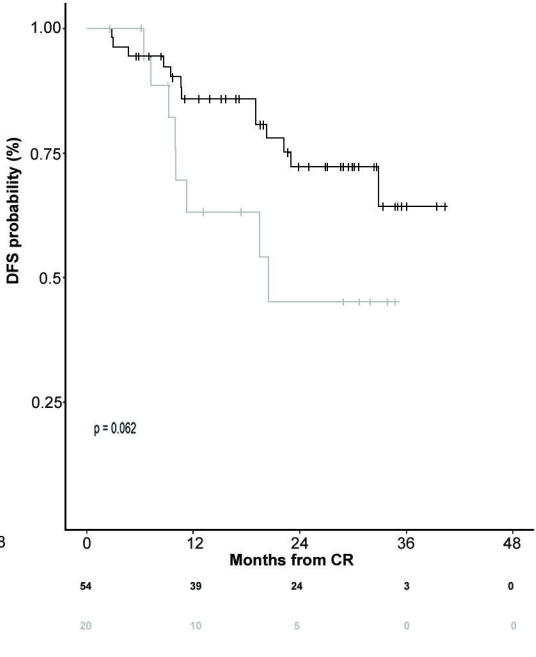
Figures legend

Figure 1. Distribution of the genetic lesions in the Ph-like and non-Ph-like cases study; only the samples evaluated for the *BCR/ABL1*-like predictor and mutational status are depicted.

Figure 2. Survival curves of Ph-like and non-Ph-like patients. EFS and DFS.


Ph-like
Non Ph-like
CRLF2 expression
High
Low
JAK/STAT mutations
Clonal
Subclonal
Fusion genes
CRLF2-r
ABL-class
JAK2-r
DDX3X/USP9X
RAS mutations
Clonal
Subclonal
Deletions
Deleted
IKZF1+CDKN2A/B
and/or PAX5
Not available
IKZF1
BTG1
EBF1


1.00
0.75
0.5
0.25
EFS probability (%)
p = 0.005
0
12
24
36
48
Months
Non Ph-like 59 39 24 3 0
Ph-like 27 10 5 0 0


1.00
0.75
0.5
0.25
DFS probability (%)
p = 0.062
0
12
24
36
48
Months from CR
54 39 24 3 0
20 10 5 0 0

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 ≥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] Biclonal 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, Montpelier, 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^(-ΔCt). 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 Zyto*Light*® 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 10^9/L (median [range])	5.1 [0.23-347]
Hb g/dL (median [range])	9.4 [3.7-15.7]
Plts x 10^9/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 *BCR/ABL1*-like predictor (n=88)**	***p*-value**
Age (median [range])	39.08 [18.18-64.71]	37.5 [18.18-64.59]	Ns
WBC x 10^9/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 10^9/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.

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

CSF1R	5q32	RP11-100O5		RP11-432O16
FGFR1	8p11	RP11-359P11		RP11-513D5
		RP11-495O10		RP11-265K5
JAK2	9p24	RP11-39K24		RP11-125K10
ABL1	9q34	RP11-57C19		RP11-83J21
Partners				
TNIP1	5q33.1	RP11-915J10		RP11-632E9
EBF1	5q33.3	RP11-1019K12		RP11-583A20
NUP214	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
JAK3 + JAK3 + JAK3_UserDefined (47132137)_140706967	chr19	17953816	17954729	607.50	EX2,3,4	12366.1

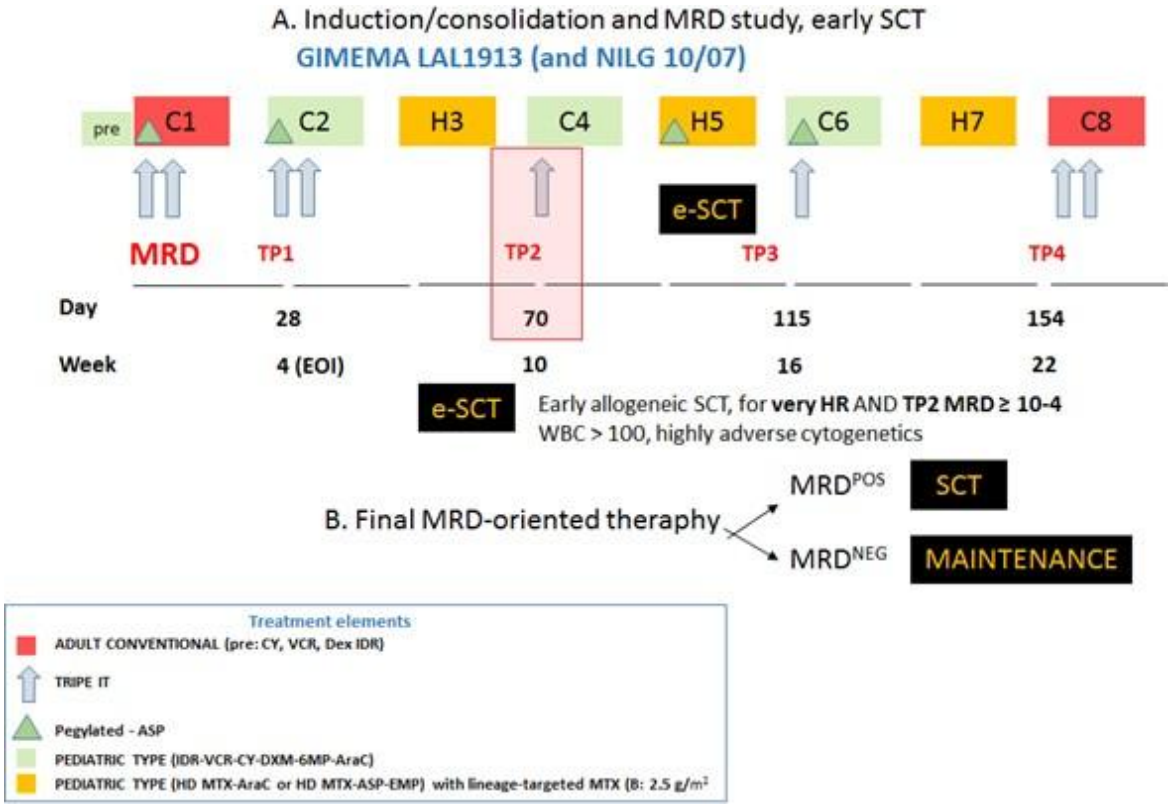
JAK3 + JAK3 + JAK3_UserDefined (47132137)_140706968	chr19	17953816	17954729	1720.50	EX2,3,4	12366.1
JAK3 + JAK3 + JAK3_UserDefined (47132137)_140706969	chr19	17953816	17954729	5329.00	EX2,3,4	12366.1
JAK3 + JAK3 + JAK3_UserDefined (47132137)_140706970	chr19	17953816	17954729	1962.50	EX2,3,4	12366.1
JAK3 + 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
JAK3 + JAK3_UserDefined (47132135)_140706961	chr19	17948721	17949219	7354.50	EX10,11	12366.1
JAK3 + JAK3_UserDefined (47132135)_140706962	chr19	17948721	17949219	4540.50	EX10,11	12366.1
JAK3 + JAK3_UserDefined (47132135)_140706963	chr19	17948721	17949219	5699.00	EX10,11	12366.1
JAK3 + JAK3_UserDefined (47229376)_140706950	chr19	17942017	17942627	3947.50	EX19,20	12366.1
JAK3 + JAK3_UserDefined (47229376)_140706951	chr19	17942017	17942627	626.00	EX19,20	12366.1
JAK3 + JAK3_UserDefined (47229376)_140706952	chr19	17942017	17942627	64.00	EX19,20	12366.1
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
JAK3 + JAK3_UserDefined (47229378)_140706965	chr19	17952178	17952591	711.50	EX6,7	12366.1
JAK3 + JAK3_UserDefined (47229378)_140706966	chr19	17952178	17952591	572.50	EX6,7	12366.1
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
PTPN11_Cds_16767914_UserDefined (47229415)_140707042	chr12	112884060	112884222	3707.50	EX2	9163.1

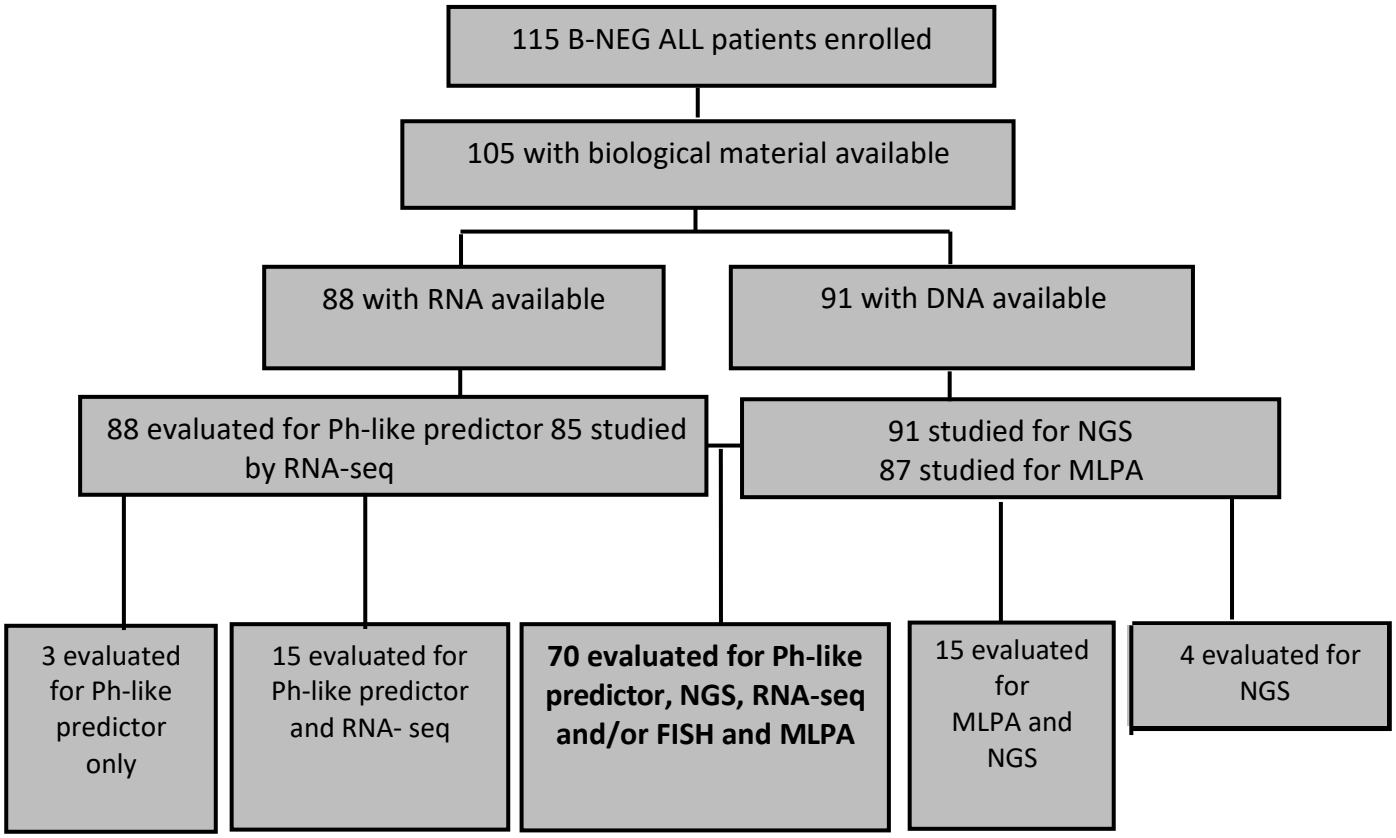
Supplemental Table 6. Univariate analyses for CR achievement, considering clinically relevant variables and molecular prognostic markers.

	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
IKZF1+ CDKN2A/2B and/or PAX5 vs IKZF1-only/WT	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).