### RESEARCH

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# Molecular characterization and predictors of relapse in patients with Ph + ALL after frontline ponatinib and blinatumomab



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#### Abstract

**Background** Several studies have suggested that chemotherapy-free regimens consisting of blinatumomab and a BCR::ABL1 tyrosine kinase inhibitor are highly effective in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph + ALL). However, the clinical and molecular characteristics that predict for relapse with these chemotherapy-free regimens are largely unknown.

**Methods** We conducted a prospective phase II clinical trial of the combination of blinatumomab and ponatinib in 76 patients with newly diagnosed Ph + ALL. Patients received 12–15 doses of intrathecal chemotherapy as central nervous systemic (CNS) prophylaxis. The patterns of relapse and the clinical and molecular predictors of relapse were analyzed.

**Results** With a median follow-up of 29 months, the estimated 3-year event-free survival rate was 78% and the 3-year overall survival rate was 88%. Ten patients (13%) relapsed, with a median time to relapse of 18 months (range, 8–24 months). Six relapses occurred only in extramedullary sites (CNS, n=5; peritoneum and lymph nodes, n=1). CD19 expression remained high at relapse in all patients. On univariate analysis, factors associated with an increased risk of relapse were: white blood cell (WBC)  $\ge 70 \times 10^9$ /L at diagnosis (sHR 8.86 [95% CI 2.33–33.70]; P=0.001), CNS involvement at diagnosis (sHR 6.87 [95% CI 1.54–30.68]; P=0.01), and *VPREB1* deletion (sHR 4.06 [95% CI 1.05–15.76]; P=0.04). WBC  $\ge 70 \times 10^9$ /L was present in 22% of the cohort and was associated with a 53% cumulative incidence of relapse (CIR), as compared with a CIR rate of 6% for patients with WBC  $< 70 \times 10^9$ /L. Neither *IKZF1*<sup>plus</sup> genotype, *BCR::ABL1* transcript type, nor measurable residual disease kinetics by next-generation sequencing for IG/TR rearrangements significantly impacted the risk of relapse. High WBC at diagnosis was the only variable significantly associated with relapse on multivariate analysis (sHR 16.29 [95% CI 2.35–113.00; P=0.005).

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**Conclusions** WBC  $\ge$  70 × 10<sup>9</sup>/L is a high-risk feature in patients with Ph + ALL receiving frontline blinatumomab and ponatinib and may supersede the prognostic importance of baseline molecular features. Alternative frontline treatment strategies may be needed for these patients to reduce the risk of relapse and improve long-term outcomes.

Trial registration ClinicalTrials.gov (NCT03263572).

Keywords Blinatumomab, Ponatinib, Philadelphia chromosome, Relapse

#### Introduction

The historical standard of care for adults with newly diagnosed Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) was chemotherapy plus a BCR::ABL1 tyrosine kinase inhibitor (TKI), followed by allogeneic stem cell transplant (alloSCT) in first remission [1]. Recently, chemotherapy-free regimens combining blinatumomab with a BCR::ABL1 TKI have shown high rates of deep molecular responses and encouraging survival in Ph+ALL, even without routine alloSCT consoildation [2, 3]. In the D-ALBA study, which evaluated the combination of blinatumomab and dasatinib in newly diagnosed Ph+ALL, the estimated 4-year disease-free survival (DFS) and overall survival (OS) rates were 76% and 81%, respectively [2]. Encouraging outcomes have also been observed with frontline use of blinatumomab and ponatinib [3, 4]. In a recent update, a 3-year OS rate of 91% was observed, despite < 5% of patients undergoing alloSCT in first remission [3]. However, despite promising outcomes in multiple studies of frontline blinatumomab + TKI combinations, some patients still relapse, and a relatively high proportion of these relapses occur in extramedullary spaces, particularly in the central nervous system (CNS).

In the D-ALBA study, lack of early molecular response or presence of the *IKZF1*<sup>plus</sup> genotype (defined as *IKZF1* deletion plus *CDKN2A/B* and/or *PAX5* deletion) were associated with worse DFS [2]. However, the clinical and molecular predictors of relapse with the blinatumomab and ponatinib regimen have not been well-described. Herein, we describe the molecular characteristics and predictors of relapse in patients treated prospectively with blinatumomab and ponatinib for newly diagnosed Ph+ALL.

#### Methods

#### Patients and study design

This was a single-center, phase 2 study to assess the efficacy and safety of the combination of ponatinib and blinatumomab in patients with newly diagnosed Ph+ALL (ClinicalTrials.gov identifier NCT03263572). Following initial ramp-up (9 mcg/day on days 1–4) in cycle 1, patients received blinatumomab at 28 mcg/day for up to 5 cycles on a standard 4-week on, 2-week off schedule. Ponatinib was given at a dose of 30 mg orally daily until complete molecular response (CMR) was achieved, after which the dose was reduced to 15 mg orally daily and continued for at least 5 years. Initially, patients received 12 doses of intrathecal chemotherapy with alternating doses of cytarabine and methotrexate. However, beginning with patient #64, the number of doses of IT chemotherapy was increased to 15 due to concern for an increased risk of CNS relapse with chemotherapyfree regimens in Ph+ALL. The study specifics, including patient eligibility, detailed treatment plan, response endpoints, and statistical analysis have been previously reported and are detailed in the **Supplemental File** [3, 4]. The study continues to enroll patients.

## Targeted mutation sequencing and single-nucleotide polymorphism (SNP) microarrays

Mutational analysis was prospectively performed at diagnosis using an 81-gene next-generation sequencing panel as previously described, with a sensitivity of 1% (Supplemental Table 1) [5, 6]. Genome-wide copy-number analysis was retrospectively performed on banked samples using SNP microarrays (Illumina Infinium Global Diversity Array-8 v1.0). The raw intensity data (\*.idat files) were analyzed using the Genotyping Module in the Illumina GenomeStudio software program version 2.0.3 (Illumina). After correcting for over-segmentation, statistically significant regions (false discovery rate [FDR] <0.05) with copy-number alterations were identified using the GISTIC 2.0 algorithm [7]. The actual copy-number alterations were manually inspected using conumee R package (version 1.9.0) [8].

#### **Response and outcomes definitions**

CMR was defined as absence of a detectable *BCR::ABL1* transcript by polymerase chain reaction at a sensitivity of 0.01%. Next-generation sequencing (NGS) MRD assessment of IG/TR gene rearrangements was performed on bone marrow specimens using the clonoSEQ assay (Adaptive Biotechnologies) with a sensitivity of 0.0001%. Cumulative incidence of relapse (CIR) was calculated considering death as a competing event. Event-free survival (EFS) was defined as the time from the start of therapy to time of no response, relapse, or death from any cause. Overall survival (OS) was defined as the time from the start of therapy to death from any cause.

**Table 1** Baseline characteristics and response rates of the study population (n = 76)

| Baseline characteristics                           | n/N (%) /   |  |  |
|--|-------------|--|--|
|  | median      |  |  |
|  | [range]     |  |  |
| Age (years)  | 50 [18–83]  |  |  |
| $Age \ge 60$ years                                 | 28 (37)     |  |  |
| Baseline cardiovascular risk factor <sup>1</sup>   | 40 (53)     |  |  |
| WBC at diagnosis (x10 <sup>9</sup> /L)             | 15.4        |  |  |
|  | [0.6-322.1] |  |  |
| WBC at diagnosis $\geq$ 70 x10 <sup>°</sup> /L     | 17 (22)     |  |  |
| Central nervous system involvement at diagnosis    | 3 (4)       |  |  |
| CD19 expression (%)                                | 99.8        |  |  |
|  | [/4.9–100]  |  |  |
| Additional chromosomal abnormalities               | 37/52 (71)  |  |  |
| BCR::ABL1 transcript                               | ( )         |  |  |
| p190   | 60/75 (80)  |  |  |
| p210   | 15/75 (20)  |  |  |
| Gene mutations                                     |             |  |  |
| IKZF1  | 5/57 (9)    |  |  |
| ASXL1  | 3/57 (5)    |  |  |
| DNMT3A   | 3/57 (5)    |  |  |
| TET2   | 3/57 (5)    |  |  |
| BCORL1   | 1/57 (2)    |  |  |
| ETV6   | 1/57 (2)    |  |  |
| RUNX1  | 1/57 (2)    |  |  |
| SF3B1  | 1/57 (2)    |  |  |
| Gene deletions                                     |             |  |  |
| IKZF1  | 35/48 (73)  |  |  |
| CDKN2A/B   | 27/48 (56)  |  |  |
| PAX5   | 13/48 (27)  |  |  |
| XBP1   | 13/48 (27)  |  |  |
| RB1  | 11/48 (23)  |  |  |
| BTG1   | 7/48 (15)   |  |  |
| VPREB1   | 7/48 (15)   |  |  |
| CD200  | 5/48 (10)   |  |  |
| <i>IZKF1</i> <sup>plus</sup> genotype <sup>2</sup> | 25/48 (52)  |  |  |
| Response rates                                     | n/N (%)     |  |  |
| Hematologic response <sup>3</sup>                  |             |  |  |
| CR   | 51/53 (96)  |  |  |
| CRi  | 1/53 (2)    |  |  |
| Early death  | 1/53 (2)    |  |  |
| MRD response <sup>4</sup>                          |             |  |  |
| CMR after cycle 1                                  | 41/69 (59)  |  |  |
| CMR at any time                                    | 57/68 (83)  |  |  |
| NGS MRD negative after cycle 1                     | 17/36 (47)  |  |  |
| NGS MRD negative at any time                       | 55/57 (96)  |  |  |

Abbreviations: WBC, white blood cell count; CR, complete remission; CRi, complete remission with incomplete hematologic recovery; MRD, measurable residual disease; CMR, complete molecular response; NGS, next-generation sequencing

<sup>1</sup> Risk factors include: hypertension, hyperlipidemia, diabetes and/or coronary artery disease

<sup>2</sup> Includes patients with an *IKZF1* deletion or *IKZF1* frameshift mutation in combination with a *CDKN2A/B* and/or *PAX5* deletion

<sup>3</sup> Excludes patients who were in morphological remission at the time of enrollment

<sup>4</sup> Excludes patients who were MRD-negative at the time of enrollment

#### Statistical analysis

This is an interim analysis of the clinical data from this ongoing study, with the primary objective of identifying predictors of relapse based on retrospectively analyzed molecular data. The cutoff for WBC was determined using a time-dependent receiver operating characteristic analysis to determine the optimal WBC threshold for prediction of relapse risk. The Youden Index was applied at each time point to identify the best threshold. We also applied the bootstrap method to confirm the robustness of our findings. For univariate analysis, the variables were pre-selected based on biological considerations and previous publications. Each predictor was examined individually using the Fine-Gray competing risks model, and *P*-values were adjusted for multiple testing using the false discovery rate (FDR) method. P-values in cumulative incidence plots are derived from Gray's test, assessing overall differences in cumulative incidence, while *P*-values in the Forest plot are based on the Fine and Gray model for univariate risk analysis. For multivariate analysis (MVA), we used the Fine-Gray competing risks model, including all statistically significant variables (P < 0.05) from the univariate analysis, as well as *IKZF1*<sup>plus</sup> genotype, given its prior reported association with outcomes in some Ph + ALL studies. Results were presented as subdistribution hazard ratios (sHR) with 95% confidence intervals (CI) in forest plots. These analyses were performed using the cmprsk package (version 2.2.11) in R software (version 4.1.2).

#### Results

#### **Clinical patient characteristics**

Between June 2018 and May 2024, 76 patients were treated. The baseline characteristics of the study population are shown in Table 1. The median age of the study population was 50 years (range, 18–83 years), and 28 patients (27%) were  $\geq 60$  years. The median WBC at diagnosis was  $15.4 \times 10^9$ /L (range, 0.6-322.1 × 10<sup>9</sup>/L). Three patients (4%) had known CNS involvement prior to enrollment. 80% of patients had the p190 BCR::ABL1 transcript, and 20% had the p210 transcript.

#### Response, disposition, and survival outcomes

Response rates for the study cohort are shown in Table 1. Among 53 patients with active disease at study enrollment, 52 patients (98%) achieved complete remission (CR) or CR with incomplete hematologic recovery (CRi). One patient experienced early death due to intracranial hemorrhage. The CMR rate after cycle 1 was 59%, and the overall CMR rate was 83%. The rate of NGS MRD negativity after cycle 1 was 47%, and the overall NGS MRD negativity rate was 96%. Among 8 patients who did not achieve CMR and were also assessed by NGS MRD, all were NGS MRD negative.

Patient disposition is shown in Supplemental Fig. 1. Among the 76 patients, 3 patients (4%) died in CR, 1 patient (1%) died in cycle 1 prior to response assessment, 10 patients (13%) relapsed, 2 patients (3%) underwent alloSCT in first remission (both due to lack of achievement of CMR), and 58 patients (76%) are in ongoing continuous remission without alloSCT. Three of these patients were taken off protocol to receive additional therapy for MRD-positive disease (inotuzumab ozogamicin, n = 1; CD19 CAR T-cells, n = 2).

With a median follow-up of 29 months (range, 5-75 months), the median EFS and OS has not been reached. Seven patients have died: 1 early death during induction, 3 deaths in CR, and 3 due to ALL relapse. The estimated 2-year and 3-year CIR are 15% and 17%, respectively, the 2-year and 3-year EFS rates are 80% and 78%, respectively, and the 2-year and 3-year OS rates are 93% and 88%, respectively (Supplemental Fig. 2). In a post hoc analysis of "MRD-based EFS" considering NGS MRD persistence or recurrence as an event (3 additional patients), the 2-year and 3-year EFS rates were 78% and 76%, respectively (Supplemental Fig. 3).

Overall, 57 patients (75%) underwent baseline targeted sequencing, and 48 patients (63%) underwent SNP microarray. There were no significant differences in clinical characteristics between patients who underwent SNP array versus those who did not, although patients with SNP data tended to be older (P=0.10) and have higher WBC at diagnosis (P=0.13) (Supplemental Table 2). The incidence of individual genomic alterations in the study population is shown in Table 1. Among patients who underwent targeted sequencing, the most common mutations were IKZF1 in 5 patients (9%), ASXL1, DNMT3A, and TET2 in 3 patients (5%) each, and BCORL1, ETV6, RUNX1, and SF3B1 in 1 patient (2%) each. Among the 5 IKZF1 mutations, 2 were frameshift and 3 were missense. Among patients who underwent SNP microarray, the most common alterations were an IKZF1 deletion (73%), CDK2NA/B deletion (56%), and PAX5 and XBP1 deletions (27% each). Fourteen of the IKZF1 deletions were dominant negative (40% of total IKZF1 deletions) [9]. Seven patients (15%) had a VPREB1 deletion. Twenty-five patients (52%) were classified as having the IKZF1<sup>plus</sup> genotype (defined as an IKZF1 deletion or frameshift mutation in combination with deletion of CDKN2A/B and/or PAX5). An Oncoplot of the 48 patients with complete molecular information, WBC count at diagnosis, transcript type, CNS involvement at

diagnosis, and relapse status is shown in Fig. 1.

35 IKZF1 del 73% IKZF1 plus 52% CDKN2A/B del 56% PAX5 del 27% XBP1 del 27% RB1 del 23% BTG1 del 15% VPREB1 del 15% CD200 del 10% IKZF1 mut ASXL1 mut DNMT3A mut TET2 mut BCORL1 mut ETV6 mut RUNX1 mut SF3B1 mut NS at diagnosis Deletion Missense WBC>70K Transcript CNS at diagnosis Relapse Frame shift Multi-hit High p190 No No IKZF1 del subtype Low p210 Yes Yes

**Fig. 1** Oncoplot of patients with complete molecular profiling (n = 48)



#### **Relapse characteristics**

The clinical and molecular characteristics of the 10 patients who relapsed are shown in Table 2. One patient who relapsed did not have an available baseline sample for SNP array. The median age at relapse was 46 years (range, 18-70 years), and the median WBC at diagnosis of the relapsed patients was  $124.1 \times 10^9$ /L (range,  $12.9-322.1 \times 10^{9}$ /L). The median time to relapse was 18 months (range, 8-24 months). Four relapses occurred in the bone marrow, with a median time to relapse of 14 months (range, 8-24 months). At relapse, one patient had an ABL1 kinase domain (KD) E255V mutation, one had a T3151 mutation (in a patient who was on dasatinib at the time of relapse due to the development of worsening coronary atherosclerosis), and two did not have an ABL1 KD mutation detected. One patient with bone marrow relapse had concomitant leukemic involvement of the vitreous fluid. Five relapses occurred in the CNS only, with a median time to relapse of 21 months (range, 9-23 months). One patient experienced a non-CNS extramedullary relapse. This patient had an IGH::CRLF2 fusion at diagnosis and relapsed in the peritoneum and lymph nodes with Ph-negative disease. All tested patients retained high levels of CD19 expression at relapse (median 99.9% [range, 82.3-100%]).

All relapsed patients received salvage therapy. The salvage therapies administered and responses are shown in Supplemental Table 3. Nine patients (90%) achieved complete remission after first salvage therapy, and one patient died during reinduction due to complications from CNS

relapse. Five of the responding patients received subsequent CD19 chimeric antigen receptor T-cell as consolidation, none of whom has subsequently relapsed. No patient underwent alloSCT following relapse.

#### **Predictors of relapse**

On univariate analysis, factors associated with an increased risk of relapse on univariate analysis included: WBC $\geq$ 70×10<sup>9</sup>/L at diagnosis (sHR 8.86 [95% CI 2.33-33.70]; P=0.001), presence of CNS involvement at diagnosis (sHR 6.87 [95% CI 1.54-30.68]; P=0.01), and VPREB1 deletion (sHR 4.06 [95% CI 1.05-15.76]; P = 0.04) (Fig. 2A). Among 69 patients who were evaluable for complete molecular response (CMR) after cycle 1, there were 9 relapses; paradoxically, all these relapses occurred in patients who achieved early CMR. The 3-year CIR for patients with IKZF1<sup>plus</sup> was 29% and for patients without the  $\mathit{IKZF1}^{\rm plus}$  genotype was 14% (sHR 2.02 [95% CI 0.51-7.9]; P=0.31) (Supplemental Fig. 4). Similarly, neither transcript type (p190 vs. p210: sHR 2.84 [95% CI 0.38-21.19; P=0.31) nor NGS MRD positivity after cycle 1 (sHR 1.89 [95% CI 0.38-9.26; P=0.43) significantly impacted the risk of relapse (Supplemental Figs. 5-6).

Overall, 17 patients (22% of the entire cohort) had WBC  $\geq$  70 × 10<sup>9</sup>/L at the time of diagnosis. The CIR for patients with WBC  $\geq$  70 versus < 70 × 10<sup>9</sup>/L was 53% and 6%, respectively (Fig. 3A). The CIR for patients with *VPREB1* deletion versus no deletion was 52% and 17%, respectively (Fig. 3B). Notably, only 3 of the 58 patients with WBC < 70 × 10<sup>9</sup>/L relapsed; 2 of these patients

| Patient | Age | WBC at<br>diag-<br>nosis (x<br>10 <sup>9</sup> /L) | Tran-<br>script<br>type | Mutations<br>(targeted<br>sequencing) | Gene deletions (SNP array)                    | NGS MRD response<br>after C1 | PCR<br>MRD<br>response<br>after C1 | Duration<br>of CR1<br>(months) | Type of<br>relapse                             |
|---------|-----|--|-------------------------|---------------------------------------|---|------------------------------|------------------------------------|--------------------------------|--|
| #1      | 57  | 2.0  | p190                    | IKZF1                                 | CDKN2A/B,<br>PAX5,<br>VPREB1, BTG1, RB1, XBP1 | Negative                     | CMR                                | 8.6                            | Peritoneum and<br>lymph nodes<br>(Ph-negative) |
| #2      | 60  | 322.1  | p190                    | IKZF1                                 | CDKN2A/B, PAX5                                | Not done                     | CMR                                | 24.5                           | Bone marrow                                    |
| #3      | 44  | 152.6  | p190                    | None                                  | CDKN2A/B                                      | Positive (1/million)         | CMR                                | 7.6                            | Bone marrow                                    |
| #4      | 18  | 4.5  | p190                    | None                                  | Not done                                      | Positive<br>(below LOD)      | CMR                                | 11.3                           | Bone marrow                                    |
| #5      | 48  | 95.5   | p190                    | None                                  | IKZF1, CDKN2A/B, PAX5, BTG1                   | Not done                     | Not done                           | 17.0                           | Bone mar-<br>row+vitreous<br>fluid             |
| #6      | 28  | 270.5  | p190                    | Not done                              | IKZF1, CDKN2A/B, PAX5,<br>VPREB1              | Not done                     | CMR                                | 22.0                           | CNS  |
| #7      | 43  | 12.9   | p190                    | BCORL1                                | IKZF1, VPREB1                                 | Negative                     | CMR                                | 19.8                           | CNS  |
| #8      | 49  | 84.9   | p190                    | None                                  | IKZF1, CDKN2A/B, RB1, XBP1                    | Not done                     | CMR                                | 23.2                           | CNS  |
| #9      | 44  | 236.7  | p190                    | None                                  | IKZF1, CDKN2A/B, XBP1                         | Positive (57/million)        | CMR                                | 8.5                            | CNS  |
| #10     | 70  | 181.2  | p210                    | DNMT3A, SF3B1,<br>TFT2                | IKZF1   | Positive (below LOD)         | CMR                                | 20.7                           | CNS  |

 Table 2 – Clinical and molecular characteristics of relapsed patients

Abbreviations: WBC, white blood cell; SNP, single nucleotide polymorphism; NGS, next-generation sequencing; MRD, measurable residual disease; C1, cycle 1; LOD, level of detection; PCR, polymerase chain reaction; CMR, complete molecular response; CR1, first remission; Ph, Philadelphia chromosome; CNS, central nervous system





Fig. 2 Predictors for relapse with blinatumomab and ponatinib in newly diagnosed Ph + ALL. (A) Forest plot for univariate analysis and (B) Forest plot for multivariate analysis

had a *VPREB1* deletion and the other patient did not have a sample available for testing (this patient was also non-compliant and relapsed while off all therapy for 11 months). Thus, among 9 patients with full clinical and molecular data who relapsed, all relapses could be explained by either high WBC at presentation or by the presence of a *VPREB1* deletion. Neither Pearson nor Spearman correlations indicated a statistically significant association among WBC  $\geq 70 \times 10^9$ /L, *IKZF1<sup>plus</sup>* genotype, and *VPREB1* deletion. On MVA, only WBC  $\geq 70 \times 10^9$ /L at diagnosis was significantly associated with relapse risk (sHR 16.29 [95% CI 2.35–113.00]; *P* = 0.005 (Fig. 2B).

#### Discussion

Various prognostic factors have been previously described in Ph+ALL, including high-risk additional chromosomal abnormalities, *IKZF1*<sup>plus</sup> genotype, and MRD response [10, 11, 12]. However, most studies were performed in the context of regimens combining intensive chemotherapy with a first- or second-generation BCR::ABL1 TKI. In this analysis of patients treated uniformly with a chemotherapy-free regimen of blinatumomab and ponatinib, only WBC  $\geq$  70 × 10<sup>9</sup>/L was independently associated with an increased risk of relapse, superseding the prognostic value of early MRD clearance and baseline genomic alterations such as *IKZF-1*<sup>plus</sup> genotype.

The strong prognostic impact of high WBC in this cohort (CIR of 53%) may be at least partially explained



Fig. 3 Cumulative incidence of relapse by subgroup. (A) WBC count at diagnosis and (B) VPREB1 deletion status. The 95% confidence interval is represented by the shaded areas. The unadjusted P value is shown

by the chemotherapy-free nature of the regimen, which omits high-dose systemic methotrexate and cytarabine and therefore could predispose to a higher relative risk of CNS relapses, which accounted for half of the relapses in our study [13]. A similar proportion and rate of CNS relapses was also observed in the D-ALBA study [2]. In contrast, in a study of intensive chemotherapy with hyper-CVAD plus ponatinib in 86 patients with newly diagnosed Ph+ALL, 15 relapses were observed, none of which occurred in the CNS [14]. Other recent studies have suggested that omission of high-dose cytarabine in consolidation may increase the risk of relapse in Ph+ALL, which is consistent with our findings [15]. Additional strategies to prevent CNS relapses are therefore needed for patients with Ph+ALL receiving frontline chemotherapy-free regimens. To attempt to mitigate this risk of CNS relapse, we have amended the blinatumomab and ponatinib study to recommend 2 cycles of high-dose methotrexate and cytarabine to patients with WBC  $\ge$  70  $\times$  10<sup>9</sup>/L at presentation. All patients now also receive 15 doses of intrathecal chemotherapy, which was increased from 12 in the initial version of the protocol. A similar strategy of more intensive intrathecal prophylaxis has also been adopted by other groups evaluating chemotherapy-free blinatumomab and TKI combinations in newly diagnosed Ph + ALL [16].

Several previous analyses have identified the IKZF1<sup>plus</sup> genotype as a high-risk feature in Ph + ALL, including in studies of hyper-CVAD plus ponatinib and with blinatumomab plus dasatinib [2, 17]. However, in our analysis, *IKZF1*<sup>plus</sup> genotype was not significantly associated with relapse risk. A limitation of our study is the relatively small number of relapses (n = 10). Given that our study was underpowered to detect predictors of relapse risk with small to moderate effect sizes, it is possible that an association could be observed with more patients and longer follow-up. If the lack of prognostic impact of IKZF1<sup>plus</sup> is observed in other studies evaluating blinatumomab and ponatinib (e.g. the ongoing GIMEMA ALL2820 study) [16], this would suggest that this combination can overcome the poor-risk biology of this ALL subtype. However, at present, our findings are only hypothesis-generating.

The lack of association of MRD clearance and clinical outcomes is unexpected, given the strong association of MRD with relapse risk in many other ALL studies, including in Ph+ALL [2, 12, 18, 19]. The rates of both CMR and NGS MRD negativity were very high in our study (83% and 96%, respectively), which makes it challenging to compare outcomes according to best MRD response. Furthermore, recent data suggest the PCR for *BCR::ABL1* may be a suboptimal method of MRD assessment in many patients with Ph+ALL [19, 20]. Interestingly, early MRD dynamics (e.g. MRD response after

cycle 1) also did not predict relapse risk. Rather, we found that only baseline clinical characteristics—particularly high WBC at presentation—were independently associated with risk of relapse.

We observed a high risk of relapse in patients with a *VPREB1* deletion (CIR of 52%), although this association was not statistically significant on MVA. *VPREB1* is involved in normal B-cell development and encodes a surrogate light chain in the pre-B-cell receptor complex [21]. *VPREB1* deletion has been associated with worse outcomes in ALL, although most of these analyses did not did specifically evaluate its role in Ph+ALL [22, 23]. Given the small number of patients with *VPREB1* deletion and the lack of association of *VPREB1* on multivariate analysis, these results should be interpreted with caution. Future studies are needed to clarify the possible prognostic impact of *VPREB1* in Ph+ALL with different treatment approaches, including both chemotherapy-containing and chemotherapy-free regimens.

#### Conclusions

In conclusion, we observed that patients with Ph+ALL who have WBC  $\geq 70 \times 10^9$ /L at diagnosis have a very high relative risk of relapse with frontline blinatumomab and ponatinib. Contrary to other studies, neither IKZF1<sup>plus</sup> genotype nor MRD dynamics impacted relapse risk. High WBC at presentation appears to be the strongest prognostic factor for patients with Ph+ALL receiving frontline chemotherapy-free regimens, although this finding-as well as the lack of prognostic significance with IKZF1<sup>plus</sup> genotype and MRD response-will need to be validated in a larger independent cohort. An increase in the number of doses of intrathecal chemotherapy prophylaxis and incorporation of high-dose methotrexate and cytarabine are being evaluated prospectively in an attempt to reduce the risk of relapse in this subgroup.

#### Abbreviations

| ALL     | Acute lymphoblastic leukemia            |
|---------|---|
| TKI     | Tyrosine kinase inhibitor               |
| alloSCT | Allogeneic stem cell transplant         |
| DFS     | Disease-free survival                   |
| OS      | Overall survival                        |
| CNS     | Central nervous system                  |
| SNP     | Single-nucleotide polymorphism          |
| CMR     | Complete molecular response             |
| FDR     | False discovery rate                    |
| CIR     | Cumulative incidence of relapse         |
| EFS     | Event-free survival                     |
| MVA     | Multivariate analysis                   |
| sHR     | Subdistribution hazard ratios           |
| CR      | Complete remission                      |
| CRi     | CR with incomplete hematologic recovery |
| KD      | Kinase domain                           |
|         |   |

#### **Supplementary Information**

The online version contains supplementary material available at https://doi.or g/10.1186/s13045-025-01709-y.

Supplementary Material 1

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Not applicable.

#### Author contributions

N.J.S. designed the study, enrolled patients, analyzed the data and wrote the first draft of the manuscript; H.K. and E.J. designed the study and enrolled patients; K.F. performed bioinformatic and statistical analyses; N.J., F.R., F.G.H., and J.S. enrolled patients; S.L. performed pathological interpretation; O.K. and R.B. collected and analyzed the data; K.T. interpreted the genomic data.

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#### Data availability

The study data is not publicly available to respect participant confidentiality. Requests for sharing of deidentified data should be directed to the corresponding author.

#### Declarations

#### Ethics approval and consent to participate

The study was performed in accordance with the Declaration of Helsinki and in compliance with good clinical practice guidelines and applicable laws. The protocol was approved by the institutional review boards or ethics committee at study sites. Written informed consent was obtained before study entry from each patient or from the patient's legally authorized representative if the patient was unable to provide consent.

#### **Consent for publication**

Not applicable.

#### Competing interests

N.J.S. has received consulting fees from Pfizer Inc., GlaxoSmithKline, NKARTA, Autolus and Sanofi, research funding from Takeda Oncology, Astellas Pharma Inc., Xencor, Stemline Therapeutics and NextCure and honoraria from Adaptive Biotechnologies, Novartis, Amgen, Takeda Oncology, Pfizer Inc., Astellas Pharma Inc., Sanofi and BeiGene, N.J. has received consulting fees from Pharmacyclics, Janssen, AbbVie, Genentech, AstraZeneca, BMS, Adaptive Biotechnologies, Kite/Gilead, Precision Biosciences, Beigene, Cellectis, MEI Pharma, Ipsen, CareDX, MingSight, Autolus, Novalgen, and research funding from Pharmacyclics, AbbVie, Genentech, AstraZeneca, BMS, Pfizer, ADC Therapeutics, Cellectis, Adaptive Biotechnologies, Precision Biosciences, Fate Therapeutics, Kite/Gilead, Mingsight, Takeda, Medisix, Loxo Oncology, Novalgen, Dialectic Therapeutics, Newave, Novartis, Carna Biosciences, Sana Biotechnology, Kisoji Biotechnology. H.K. has received research funding from AbbVie, Amgen, Ascentage, BMS, Daiichi-Sankyo, Immunogen, and Novartis and honoraria from AbbVie, Amgen, Ascentage, Ipsen Biopharmaceuticals, KAHR Medical, Novartis, Pfizer, Shenzhen Target Rx, Stemline and Takeda Oncology. E.J. has received consulting fees and research funding from Abbvie, Adaptive biotechnologies, Amgen, Ascentage, ASTX, Astra-Zeneca, Autolus, BMS, Genentech, Hikma, Kite, Novartis, Pfizer, Takeda Oncology and Jazz. S.L. has received consulting fees Servier, Daiichi Sankyo, Syndax, Tempus AI, Kura oncology, Abbvie, and research funding from Amgen and Astellas. The other authors have no conflicts of interest to disclose.

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