Ponatinib alone or with chemo-immunotherapy in heavily pre treated Philadelphia-like acute lymphoblastic leukemia: a CAMPUS ALL real-life study

In early 2000s, different groups independently identified a new acute lymphoblastic leukemia (ALL) subset named Philadelphia-like (Ph-like ALL).[1-3] This entity was characterized by a peculiar gene expression profile (GEP) associated with tyrosine kinases activation, though lacking the true *BCR::ABL1* fusion transcript.[4] Ph-like ALL harbors cryptic translocations of tyrosine kinase genes or abnormal regulation of target receptors involved in B-cell development. Cytokine receptor-like factor 2 (*CRLF2*) overexpression accounts for approximately 50% of Ph-like cases, half of them being associated with *JAK-STAT* pathway mutations. Another mechanism is kinase deregulation, particularly *ABL1, ABL2, PDGFRA* and *PDFGRB*. Ph-like ALL accounts for 15-30% of B-lineage ALL and is associated with an inferior disease-free survival and 5-year overall survival (OS) in both children and adults.[5,6] Given the heterogeneity of Ph-like ALL, several diagnostic algorithms have been proposed. However, an internationally recognized tool for the recognition of these cases is to date not available. In Italy, the *BCR/ABL1*-like predictor is widely used.[7] In the World Health Organization (WHO 2022) classification, Ph-like ALL was classified as a definitive category.[8]

Several studies have addressed Ph-like *ABL*-class and *JAK* mutations as potential targets for tailored therapy with third generation tyrosine kinase inhibitors (TKI) and JAK2 inhibitors, with contrasting results.[9] In Italy, the third generation pan-TKI ponatinib is approved for adult Ph$^+$ ALL resistant or intolerant to dasatinib or if harboring a *T315I* mutation. *In vitro* data suggest that ponatinib could be effective for the management of Ph-like ALL regardless of the underlying molecular abnormalities and individual cases treated with ponatinib have been reported.[7,10,11,12] Specific targeted therapies for Ph-like ALL are still not available and therefore they represent a primary unmet medical need. To overcome this issue, between January 2019 and June 2023, *ad hoc* individual donation of ponatinib was put in place after request from treating physicians for Ph-like ALL patients; data were collected in the context of Campus ALL network in Italy.

The *BCR/ABL1*-like predictor tool was carried out as previously described.[7] Multiplex Ligation-dependent Probe Amplification (MLPA) was applied for copy number variation (CNV) assessment using the SALSA P335-C2 ALL-IKZF1 probemix (MRC Holland, Amsterdam, NL) and the 3500 Genetic Analyzer sequencer (Applied Biosystems, Life Technologies, CA). Targeted RNA sequencing was carried out using the TruSight RNA Pan-Cancer Panel Kit (Illumina, San Diego, CA) on the Illumina MiSeq Illumina Platform (Illumina, San Diego, CA).

Seventeen patients were included in this survey. The inclusion criteria were: i) Ph-like ALL diagnosed according to the *BCR/ABL1*-like predictor;[7] ii) patients with relapsed or refractory (R/R) disease or with evidence of minimal residual disease (MRD); iii) ponatinib treatment for at least 28 consecutive days. Sixteen of the 17 patients (1 did not complete the first cycle), were analyzed: median age was 29 years (range, 14-66), 11 were male and the median white blood cell count (WBC) count was 23.4x10^9/L (range, 2.7-317x10^9/L). Cytogenetic assessment at diagnosis showed a complex karyotype in two patients, one was hyperdiploid, three had different chromosomal abnormalities each and six had a normal karyotype; karyotyping failed in four cases. CNV was assessed in 12 patients: five were *IKZF1*^{plus}, six had an *IZKF1*^{loss}, and one was *IKZF1*$^{wild\text{-}type}$. Targeted RNA sequencing was performed in 11 cases: seven harbored a gene fusion (*ABL*-class mutations in 2, *JAK2* mutations in 2, and *CRLF2::P2RY8, IKZF1::DDC, RB1::RCBTB2* and *ZNF384::EP300* in 1 case each); two additional cases had a *CRLF2* rearrangement, documented by fluorescence *in situ* hybridization.

At diagnosis, only eight patients (50%) were classified as high or very-high risk based on clinico-biological features, published elsewhere.[13] At onset of disease, all patients were treated with pediatric-inspired regimens: seven in the context of national protocols that included blinatumomab in first-line and for the remaining nine patients a GIMEMA LAL1913 chemotherapy backbone was applied. Following front-line treatment, ten of the 16 patients (62.5%) had achieved a complete remission (CR), four patients were in CR but displayed MRD persistence. Six patients (37.5%) were primary refractory to chemotherapy. Seven patients (43.8%) had already undergone an allogenic stem cells transplant (SCT) before starting ponatinib. The median number of treatments prior to enrollment was two (excluding SCT), with 12 of the 16 patients (75%) previously treated with immunotherapy (blinatumomab and/or inotuzumab ozogamicin).

Ponatinib was started in either hematologic relapse or MRD-positive (MRD$^+$) setting at different time points; as for hematologic relapsed patients (N=7), five were in second or further hematologic relapse. Four had also a documented extramedullary disease (EMD): one central nervous system, one breast and one cutaneous localization, associated in all cases with a bone marrow involvement; a single case

experienced an isolated nodal relapse. A further patient was refractory to three lines of treatment, including immunotherapy. Eight patients were treated in a MRD$^+$ status (Table 1).[14]

Ponatinib dose was left to clinician' choice: the majority of patients (N=13) received 45 mg/daily whereas three and one, respectively, received 30 and 15 mg/daily. Ponatinib was administered alone or in combination with steroids and/or intrathecal chemotherapy in eight patients, while eight were treated with ponatinib and blinatumomab (N=3) or chemotherapy (N=5).

An overall response, consisting of either CR in R/R group or complete molecular response (CMR) in MRD$^+$ group, was observed in 56% of patients (9/16). CMR was overall achieved in 43.8% of patients (N=7). Among relapsed/refractory (R/R) patients (N=8), three did not respond (2 being *CRLF2* rearranged), two had a stable disease and two achieved a MRD$^+$ CR; notably, the refractory patient achieved a CMR. In the MRD$^+$ group (N=8), all but two patients (including a *CRLF2* rearranged case) achieved a CMR after ponatinib (75%).

Toxicity was acceptable: three patients experienced a grade 2 transaminitis, one developing a concomitant fungal pneumonia, and one a grade 2 increase in pancreatic enzymes. Of the nine ponatinib responsive patients, among the three R/R, one is in CMR in search of a suitable donor, whereas two relapsed after an initial response: one patient received inotuzumab followed by chimeric antigen receptor-CIK and a second SCT and is in CMR, and the other was treated with salvage chemotherapy and died of disease progression. Within the six MRD$^+$ cases, five underwent a SCT: four are in continuous CMR and one died of complications; the single not allografted patient is also in continuous CMR (Figure 1). Regardless of the previous hematologic status, at a median follow-up of 6 months (range, 3-43), ten of 16 patients are alive in CMR, including three patients who have been treated with additional therapy (Table 2).

Ph-like ALL represents a highly heterogeneous disease from a biological standpoint, whose recognition is still challenging. Likewise, from a clinical standpoint Ph-like ALL cases are often misallocated at diagnosis to a standard-risk category.[13] The underlying molecular mechanisms that characterize Ph-like ALL subtypes suggest a diverse drug-sensitivity profile to potential targeted therapy, that, while effective *in vitro*, often fails in the clinical setting. Indeed, JAK2 inhibitors were not successful in a clinical trial,[15] while sporadic cases treated with the third generation TKI ponatinib have shown efficacy.[10,11] Thus, within the Campus ALL consortium in Italy we sought to collect findings on Ph-like ALL patients treated with ponatinib in an individual donation program; to our knowledge, this survey represents the largest retrospective series collected so far. Our data confirm the poor prognosis of Ph-like ALL patients, despite the use of immunotherapy, even in a relatively young population treated with intensive regimens. Ponatinib appears as a promising opportunity especially in the MRD$^+$ setting, where a CMR was obtained in 75% patients and was a feasible strategy for bridging to SCT in the majority of cases. A single case, refractory to intensive chemotherapy and to two different monoclonal antibodies, was also rescued and achieved a sustained CMR. Ponatinib was overall less effective in R/R patients, in which a CR was recorded only in 37.5% of cases. EMD cases experienced a worse response (1/4 achieved CMR) than isolated medullary relapsed patients (8/12 responded). However, even in these heavily pretreated patients, two were eventually salvaged with the use of ponatinib and immunotherapy followed by various cellular approaches. While we cannot conclusively prove that ponatinib itself was truly effective in these cases, also considering the exiguous number of cases, it nonetheless delayed tumor progression, buying time for alternative treatments. Combination treatment with either blinatumomab or chemotherapy was administered in three of nine patients who achieved a CR and in five of seven who proved refractory, suggesting, with the caveat of the small number and the retrospective nature of the study, that the combination has slight to no impact on outcome.

Table 1. Patients' clinico-biologic features and previous treatments.

Patients	N=16
Male/female, N	11/5
Median age in years (range)	29 (14-66)
WBC x10^9/L, median (range)	23.4 (2.7-317)
Karyotype, N (%)	
Complex karyotype	2 (12.5)
Normal	6 (37.5)
IKZF1 status, N (%)	12/16
IKZF1^{plus}	5 (31.3)
IKZF1^{loss}	6 (37.5)
IKZF1$^{wild\text{-}type}$	1 (6.2)
Risk stratification,[13] N (%)	
Very high risk/high risk	8 (50)
Standard risk	8 (50)
Treatment prior to ponatinib	
Lines of treatment before ponatinib, median (range)	2 (1-4)
Monoclonal antibodies before ponatinib, N (%)	12 (75)
Blinatumomab	5 (31.3)
Inotuzumab	2 (12.5)
Both	5 (31.3)
SCT, N (%)	7 (43.8)
Response to first-line therapy, N (%)	10 (62.5)
Disease status before ponatinib, N (%)	
R/R	7/1 (50)
CR, MRD$^+$	8 (50)

WBC: white blood cell count; SCT: stem cell transplantation; R/R: relapsed/refractory; CR: complete remission; MRD: minimal residual disease; [13]VHR: very high risk, if white blood cell count >100×10^9/L and/or and/or poor-risk cytogenetics/genetics (t4;11/*KMT2A* rearrangement, 11q23, +8, –7, del6q, t(8;14) abnormalities, low hypodiploidy, near triploidy or complex karyotype with ≥5 unrelated anomalies); HR: high risk, if WBC count >30×10^9/L to 100×10^9/L, pro-B phenotype; SR: none of the risk factors previously listed.

In light of these results and of the observed low toxic profile, the GIMEMA LAL 2922 protocol is currently enrolling newly diagnosed adult Ph-like ALL patients (<65 years). Treatment is based on a combination of a pediatric inspired regimen together with ponatinib, with the removal of asparaginase for toxicity concerns. In this cohort, cardiovascular toxicity was not observed; this could be due to the relatively young age of patients. Nevertheless, caution is required in the

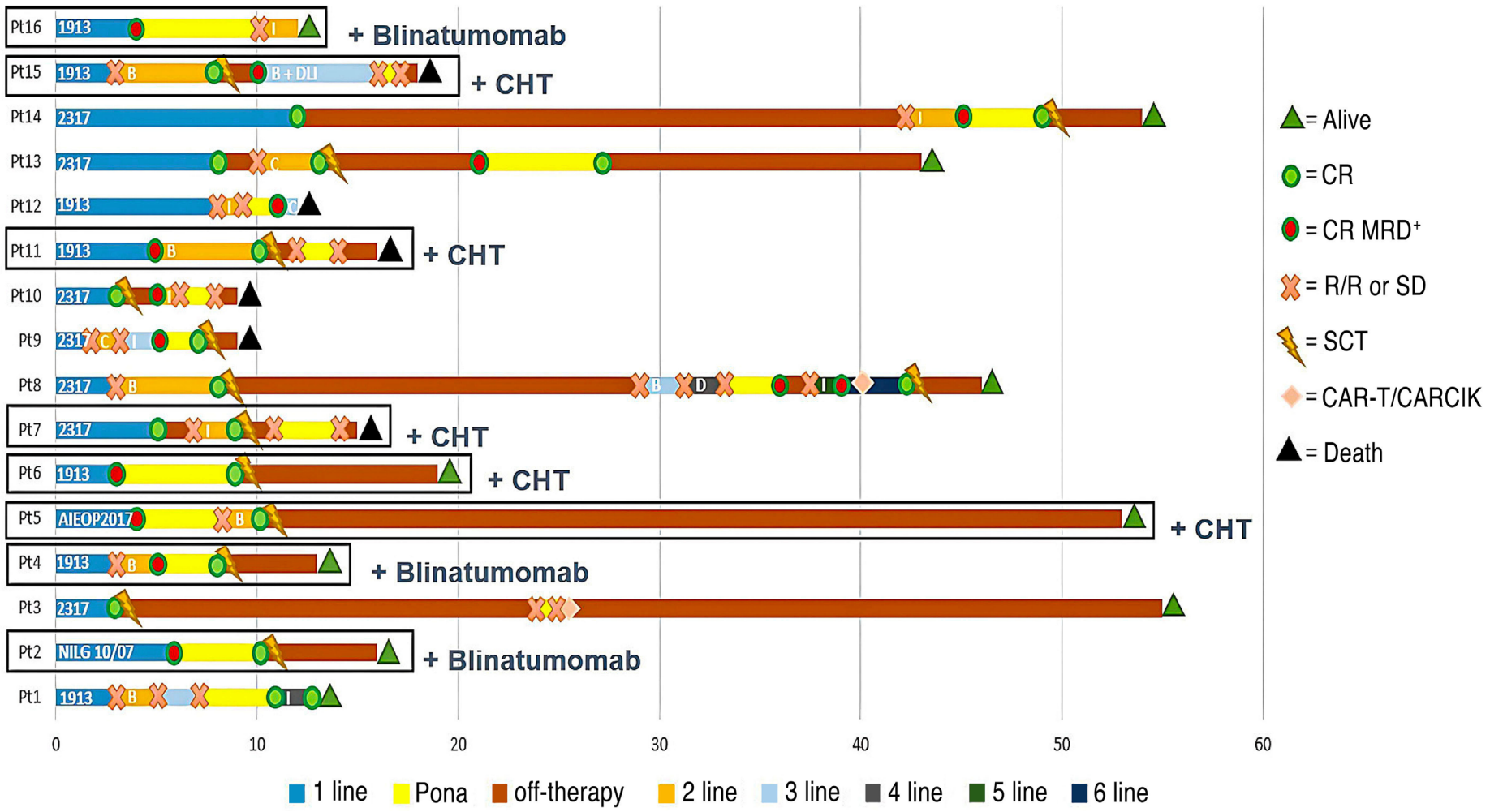

Figure 1. Patients' history. Black boxes indicate patients treated with ponatinib in combination with chemo- and/or immunotherapy. Pt: patient; CAR T: chimeric antigen receptor T-cell therapy; CARCIK: CAR cytokine-induced killer; CHT: chemotherapy; CR: complete remission; MRD: minimal residual disease; Pona: ponatinib; SD: stable disease; SCT: stem cell transplantation.

Table 2. Response to ponatinib.

Response to ponatinib	Responders R/R N=3	Responders MRD+ N=6	Non responders N=7
Time to response in months, median (range)	1 (1-5)		-
IKZF1 status, N	1 *IKZF1*^{plus} 1 *IKZF1*^{loss} 1 Unknown	2 *IKZF1*^{plus} 2 *IKZF1*^{loss} 1 *IKZF1*$^{Wild-type}$ 1 Unknown	3 *IKZF1*^{plus} 2 *IKZF1*^{loss} 2 Unknown
RNA-seq/FISH, N	1 *CRLF2-r* 1 *EBF1::PDGFRB* 1 unknown	1 *JAK2-r* 1 *ABL2-r* 1 *IKZF1::DDC* 1 *RB1::RCBTB2* 1 no fusions 1 unknown	2 *CRLF2-r* 1 *JAK2-r* 1 no fusions 3 unknown
Subsequent relapse, N	2	0	-
Follow-up in months, median (range)	6 months (3-43)		
Alive in CR, N	2	5	3

R/R: relapsed/refractory; MRD: minimal residual disease; RNA-seq: targeted RNA sequencing; FISH: fluorescence *in situ* hybridization; CR: complete remission.

elderly setting. In the setting of this Campus ALL survey, it emerged that the administration of ponatinib was mainly intended as a bridging therapy towards a SCT or other cellular therapies, given the nature of the patient population. Likewise, also in the GIMEMA LAL 2922 protocol SCT is foreseen in all eligible patients.

From a biological standpoint, targeted RNA sequencing was performed in 11 cases, and even though the limited number of patients does not allow definitive conclusions on response, it is worth noting that the presence of a *CRLF2* rearrangement seems to be less sensible to ponatinib, suggesting that this subset requires further *ad hoc* biologically-driven strategies.[16,17] In our cohort, two patients carried a *JAK2* rearrangement: one achieved CMR and one was resistant to ponatinib. Larger cohorts are needed to establish if other specific gene fusions (i.e. *ABL*-class) could be associated with a better outcome.

In conclusion, these findings confirm and extend on a larger series of patients the observation that ponatinib may be a cost-effective, easily accessible compound with a sustainable toxicity profile for Ph-like ALL, especially in the setting of MRD positivity, with ten of 16 patients (62.5%) being alive and in molecular response at the last follow-up. Additional information needs to be collected for a better characterization of Ph-like ALL, in order to redirect therapeutic strategies in the different identified patients' subgroups.

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Contributions

FK and ML analyzed data and wrote the manuscript. DC, VB, MB, IDS and MDT performed experiments. FM, CMB, MD, SM, DL, PS, MP, VC, AP, NSF, FDB and MA provided clinical and followed patients. RF and SC designed research, analyzed data, wrote and clinically revised the manuscript.

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Data-sharing statement

Datasets are maintained in an electronic database at the Department of Translational and Precision Medicine, Sapienza University of Rome; data are available from the corresponding author upon request.

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