BH3 mimetics in relapsed and refractory adult acute lymphoblastic leukemia: a Campus ALL real-life study

The introduction of pediatric-inspired regimens has improved outcomes remarkably in both B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) and T-ALL, with 5-year overall survival rates approaching 60% in patients <55 years old[1] and between 35% and 55% in older patients.[2] Nevertheless, disease relapse and chemo-refractoriness (R/R) are still clinical problems, especially in T-ALL, for which very few novel drugs are available, and in BCP-ALL patients who fail to respond to or relapse after chemo-immunotherapy.[3]

Selective inhibition of BCL-2 with venetoclax and inhibition of BCL-2 and BCL-XL with navitoclax directly releases apoptosis activators from pro-apoptotic proteins, causing permeabilization of the mitochondrial outer membrane, leading to cell death.[4] BCL-2 and BCL-XL overexpression has been reported in ALL,[5,6] and the pre-clinical and clinical efficacy of BCL-2 family inhibitors (BH3-mimetics) alone[7,8] or in combination with targeted agents such as ponatinib[9] or inotuzumab ozogamicin[10] showed promising results. For these reasons, a compassionate-use program, based on treatment with BH3 mimetics, was started in Italy and was granted by Abbvie (Abbvie, Rome, Italy).

We performed a retrospective multicenter analysis, comprising 28 adult R/R ALL patients treated with venetoclax alone or in association with low-dose navitoclax in the context of the Campus ALL national network. All patients signed consent to participation in the compassionate-use program in agreement with the Helsinki Declaration. According to the national named-used treatment program, R/R ALL patients for whom no other treatment option was available, with bone marrow or extramedullary leukemia, were included, whereas cases with only molecular relapse were not eligible. Combined chemotherapy was allowed.

The venetoclax ramp-up schedule and final dose were at the clinician's discretion depending on the patient's concomitant medications and comorbidities, disease burden and previous lines of chemotherapy.

Navitoclax was administered at the recommended 25 mg or 50 mg daily dose, according to body weight (< or >45 kg). Minimal residual disease evaluation was performed by quantitative polymerase chain reaction for IG/*TR* gene rearrangements or specific fusion transcripts with marker sensitivity up to 10^{-4} or by flow cytometry. Patients with extramedullary involvement were evaluated by both bone marrow aspirate and total body computed tomography/positron emission tomography.[11] Safety analysis was conducted by grading all toxicities according to National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE, version 5.0).

Descriptive statistics were carried out; to compare differences between groups, the χ^2 test was used with *P* values <0.05 deemed statistically significant. Kaplan-Meier and log-rank tests were used to assess survival. For duration of remission, patients who were alive with no disease progression were censored at the last follow-up; likewise, patients alive at the last follow-up were censored for overall survival. The data cut-off was December 1st, 2022. Tests were performed using R (version 4.0, The R Foundation for Statistical Computing 2020).

We collected clinical data from 37 patients with R/R ALL treated at 20 Italian hematology institutions participating in the Campus ALL network from July, 2019 to December, 2022. Nine patients died prior to authorization of named-use treatmen, while seven started venetoclax and 21 venetoclax-navitoclax (*Online Supplementary Figure S1*). The median turnaround time for drug supply from application to the named-use program was 19 days (range, 10-42). In the venetoclax-navitoclax cohort, one patient died of progression before completing the first cycle. Within the intention-to-treat population (n=28), six patients (22%) had BCP-ALL and 22 (78%) had T-ALL, as detailed in Table 1, with 75% of patients being <55 years old. Five patients (18%), all with T-ALL, had isolated extramedullary relapse, while 12 patients (43%) had combined bone marrow and extramedullary disease. Patients had undergone a median number of three lines of therapy (range, 1-6) and four of them (14%) had primary refractory disease (all with T-ALL). Allogeneic stem cell transplant had been previously carried out in 11 patients (40%), and most of the BCP-ALL patients had already undergone salvage treatment with blinatumomab and inotuzumab (4 and 5 patients, respectively), with two having also received CD19-directed chimeric antigen receptor T cells. Venetoclax was ramped up from 100 mg to 400 mg daily in 12 patients; three patients started venetoclax 400 mg daily without dose escalation; and the remaining started venetoclax with personalized ramp-up based on the characteristics of the patient and disease and the clinician's choice. The final daily doses of <400 mg, 400 mg, 600 mg and 800 mg were reached in nine, 15, one and two patients, respectively. Navitoclax was administered at 50 mg and 25 mg daily in 17 patients and four patients, respectively; combination anthracycline- and/or asparaginase- and/or alkaloid-based chemotherapy was utilized in 13 patients (48%), three of seven in the venetoclax cohort and ten of 21 in the venetoclax-navitoclax cohort, respectively (*Online Supplementary Table S1*). The remaining patients were treated with BH3-mimetics without chemotherapy.

The two cohorts were not significantly different, except for a higher daily dose of venetoclax in the venetoclax mono-

Table 1. Baseline patients' features.

Baseline parameters	All patients N=28	Patients treated with venetoclax-navitoclax N=21	Patients treated with venetoclax N=7	*P*
Age in years, median (range)	31 (20-79)	31 (21-79)	31 (20-65)	
Distribution				
<55 years, N (%)	21 (75)	15 (71)	6 (85)	0.449
>55 years, N (%)	7 (25)	6 (29	1 (15)	
Male sex, N (%)	21 (75)	16 (76)	5 (71)	0.801
ALL subtype, N (%)				
BCP-ALL	6 (22)	5 (24)	1 (15)	
Philadelphia-chromosome-positive	1 (3)	1 (4)	0	
KMT2A fusion	1 (3)	1 (4)	0	0.576
T-ALL	22 (78)	16 (76)	6 (85)	
ETP-ALL	8 (28)	5 (24)	3 (43)	
EM leukemia, N (%)	17 (61)	11 (52)	6 (86)	
Lymph nodes	9 (32)	5 (24)	3 (43)	
Breast	8 (28)	1 (4)	1 (15)	0.344
Mediastinum	5 (18)	1 (4)	1 (15)	
Other combinations*	5 (18)	3 (15)	2 (29)	
Isolated EM leukemia	5 (18)	5 (24)	0	
Salvage regimen, N (%)				
1st salvage	4 (14)	2 (9)	2 (29)	
2nd salvage	4 (14)	3 (15)	1 (15)	0.534
≥3rd salvage	20 (72)	16 (76)	4 (58)	
Primary refractory	4 (14)	4 (19)	0	
Previous treatment lines, median (range)	3 (1-6)	3 (1-6)	3 (1-6)	
Previous immunotherapy, N (%)				
Allogeneic SCT	11 (39)	8 (38)	4 (57)	
Blinatumomab	4/6 (66)	3/5 (60)	1 (15)	0.165
Inotuzumab	5/6 (83)	4/5 (80)	1 (15)	
CD19-CAR T cells	2/6 (33)	2/5 (40)	0	
Disease characteristics at the time of starting venetoclax				
Platelets x10^9/L, median (range)	130 (2-382)	130 (2-382)	125 (25-240)	0.051
WBC x10^9/L, median (range)	3.51 (0.9-48)	3.51 (0.9-48)	3.51 (2.1-9.6)	0.058
Distribution of ECOG PS >1, N (%)	8 (28)	7 (33)	1 (15)	0.334
BM blasts percentage, median (range)	15 (0-90)	21.5 (0-90)	10 (5-40)	0.827

*In the venetoclax single-agent cohort, one patient had multiple lymphoadenopathies, liver and bone localizations, one patient presented with peritoneal localization and multiple lymph nodes. In the venetoclax-navitoclax cohort three patients presented, one each, with bowel, testis and spleen localization. ALL: acute lymphoid leukemia; BCP: B-cell precursor; T-ALL: T-cell acute lymphoid leukemia; ETP-ALL: early T-cell precursor acute lymphoid leukemia; EM: extramedullary; SCT: stem cell transplant; CAR: chimeric antigen receptor; WBC: white blood cells; ECOG PS: Eastern Cooperative Oncology Group Performance Status; BM: bone marrow.

therapy cohort (*P*=0.001).

The median follow-up was 8.3 months (range, 1.9-23.6); patients received a minimum of one cycle and a maximum of 22. The overall response rate at day 29 was 48%, with a complete response (CR) rate of 33% (9/27 patients), which was higher in the venetoclax-navitoclax cohort than in the venetoclax single-agent cohort (40% *vs.* 14%, *P*=0.214). It is noteworthy that five of eight CR patients tested (62%) achieved a state of measurable residual disease negativity (1 CR patient had extramedullary disease only and measurable residual disease monitoring could not therefore be done). Patients who received associated chemotherapy did not show superior response rates: five of 13 patients achieved a CR (*P*=0.586). Fourteen patients (52%) did not respond. At the last follow-up, among the nine patients who achieved a CR at the end of cycle 1, three were allografted: one died of acute graft-*versus*-host disease and two are in continuous CR 14 and 16 months after their transplants. Three patients are on ongoing treatment and in continuous CR after 5, 19 and 20 months; finally, three patients with early T-cell precursor ALL, T-ALL and *KMT2A*-rearranged BCP-ALL relapsed while on therapy after 2, 3 and 8 months, respectively (Table 2).

With the caveat of the small sample size, achievement of CR was documented regardless of previous lines of treatment, although no efficacy was observed among patients who had primary refractory disease before enrollment. As detailed in the *Online Supplementary Table S1*, responses were reported in most ALL subgroups enrolled, including three patients with BCP-ALL and six patients with T-ALL (including 2 with early T-cell precursor ALL), although without significant differences among the groups, given the small numbers of patients. Among five patients with isolated extramedullary leukemia, two were in continuous CR at their last follow up. The median overall survival of 5.05 months (95% confidence interval: 2.39-not reached) with 6- and 12-month overall survivals of 35% and 26.6%, respectively (Figure 1A, B). CR patients had a significantly better survival compared to non-CR cases, with a median overall survival not reached *versus* 2.3 months (P=0.004) and a 1-year overall survival of 62.5% *versus* 11%. The median duration of response of the whole cohort was 4.46 months (95% confidence interval: 2.92-not reached), with a significantly better median duration of response for patients achieving a CR compared to those in partial response at day 29: 7.6 months *versus* 2.62 (P=0.001) (Figure 1C, D).

No early toxic fatal events or unexpected and dose-limiting toxicities were observed: cytopenias were the most common side effects, with grade ≥3 thrombocytopenia documented in six patients (21%), especially in the venetoclax-navitoclax cohort (n=5). Patients in the venetoclax cohort displayed modest cytopenia as the only adverse event, whereas three patients in the venetoclax-navitoclax cohort (11%) developed a bloodstream infection (2/3 of patients treated with combined chemotherapy). Adverse events were recorded in seven of 11 patients treated with a chemotherapy-free venetoclax-navitoclax regimen. These adverse events, mainly cytopenia, included the case of one patient who experienced pneumonia in the context of progressive disease and one patient with transient increases of transaminase levels. Likewise, chemotherapy-attributable cytopenia was reported in five patients, and one patient had an increase in pancreatic enzymes, probably linked to asparaginase therapy. All adverse events were recorded within the first cycle of treatment and were managed without withdrawal of the study drug (*Online Supplementary Table S2*).

ALL recurrence radically changes patients' life expectations, especially those for whom no new therapeutic compounds are available. For the latter, CR rates decrease to 20% and 10% for subsequent salvage therapies after the first, with a 5-year overall survival close to 10%.[12] Promising results were found in 47 patients (including 12 children) with R/R ALL treated with the venetoclax-navitoclax combination and chemotherapy. The composite CR rate was 60%, the median duration of response and overall survival were 4.2 months and 7.8 months, respectively.[13]

Here, we report the outcome of adult patients with R/R ALL of B- and T-lineage treated with one or two BH3-mimetics. Our population was heavily pre-treated, with a significant fraction of patients who had already received anti-CD19 or anti-CD22 targeted therapy. The 1-year overall survival in this cohort was 26.6%, a result better than the reported 1-year overall survival with conventional chemotherapy, which is around 4-10%.[14] It is worth noting that patients achieving a CR at the end of the first cycle displayed an improved

Table 2. Outcome measures from therapy initiation.

Parameter	Venetoclax	Venetoclax-Navitoclax	All patients
Pts in the Tx group, N	7	20	27
Efficacy parameters			
Overall response, N (%)	2 (28)	11 (55)	13 (48)
Complete response, N (%)	1 (14)	8 (40)	9 (33)
Partial response, N (%)	1 (14)	3 (15)	4 (15)
No response, N (%)	5 (71)	9 (45)	14 (52)
MRD negativity*, N (%)	0/1	5/7 (71)	5/8 (62)
Subsequent alloSCT among CR pts, N (%)	1/1 (100)	2/8 (25)	3/9 (33)
Ongoing treatment among CR pts, N (%)	0/1	3/8 (37)	3/9 (33)
Duration of Tx in months, median (range)	1.4 (0.9-2.2)	1.5 (1-20)	1.5 (0.9-20)

*Among responders, one patient with isolated extramedullary leukemia achieved a complete response as assessed by positron emission tomography: measurable residual disease could not be assessed in the subject. Pts: patients; Tx: treatment; MRD: measurable residual disease; alloSCT: allogeneic stem cell transplant; CR: complete response.

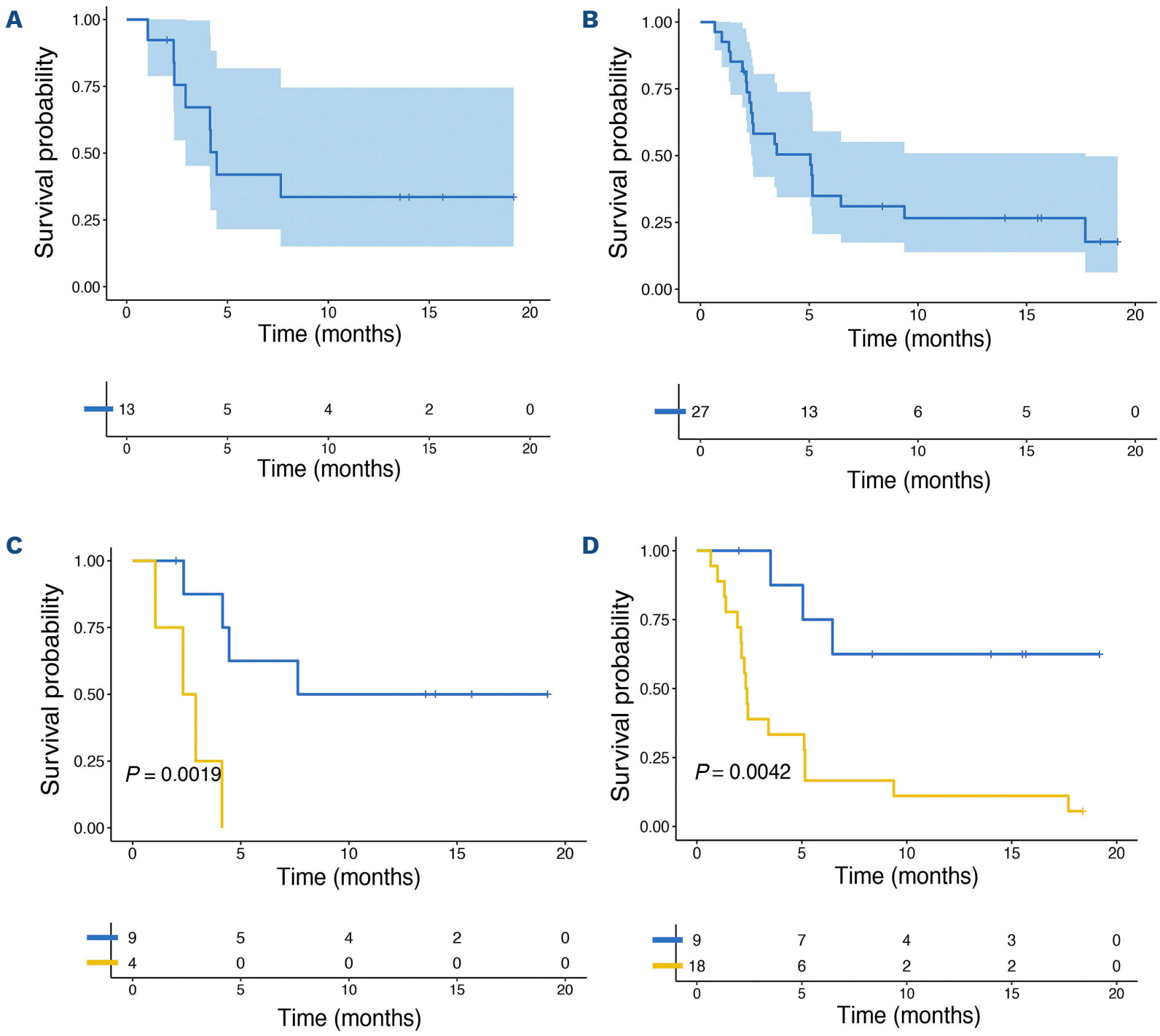

Figure 1. Survival in the whole cohort. (A) Duration of response. (B) Overall survival. Comparison between patients who had obtained complete response and those not in complete response at the day 29 evaluation after starting therapy. (C) Duration of response. (D) Overall survival.

1-year overall survival of 62%, whereas for those achieving only a partial or no response, survival remained dismal. Additional chemotherapy did not improve outcomes. Although not statistically significant, we observed a higher number of CR in the venetoclax-navitoclax cohort, supporting the dual inhibition of BCL-2 and BCL-XL as a synergistic strategy to prevent mechanisms of escape from venetoclax.[15] These data are in constrast to those recently reported in a retrospective study of 17 R/R ALL patients, with one out of 13 patients treated with a combination of a BH3 mimetic and chemotherapy achieving CR, although five of them had already been exposed to venetoclax and the therapy schedule differed from ours.[16] Moreover, the day 29 (end of first cycle) response represented a crucial timepoint in our survey: indeed, CR - as well as measurable residual disease negativity - was achieved within the first cycle. We registered responses regardless of the prior heavy treatment load. Toxicity was manageable in our cohort, and occurred during the first cycle, in line with previous reports.[13]

The limitations of our study are its retrospective nature, the fact that no drug profiling analyses were carried out on primary samples, and the relatively small number of patients; this hampered subgroup analyses, which haven not been reported in any of the most recent clinical reports. At present, venetoclax and navitoclax are being investigated in association with either chemotherapy and monoclonal antibodies in both R/R (NCT05268003, NCT05016947) and frontline settings (NCT05386576, NCT03319901), but the clinical trials are still ongoing and the results have yet to be published. Hopefully, an earlier use of these agents might represent a strategy to improve CR rates and subsequently as a bridge to allogeneic stem cell transplantation for patients predicted to have poor response to standard salvage therapy, such as B-ALL patients relapsing after im-

munotherapy or R/R T-ALL patients, especially those with early T-cell precursor disease, for whom improvements are also needed in the frontline setting. From this standpoint, *ex vivo* drug sensitivity profiling in patients affected by ALL subtypes with typically poor prognosis is currently being investigated to predict response to a set of investigational compounds (NCT04582487). Furthermore, BH3 profiling might be a useful laboratory tool to establish anti-apoptotic protein dependency in individual patients in order to address BH3-mimetic therapy better. In conclusion, BH3 mimetics appear as a promising, orally available and relatively safe new therapeutic strategy in ALL, useful in several disease settings and capable of rescuing patients otherwise considered incurable.

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No conflicts of interest to disclose.

Contributions

FM took care of patients, analyzed data and wrote the manuscript. IT took care of patients, and analyzed data, MP, CP, VF, VM, ER, ET, SM, GC, PC, MG, VG, FG, MM, AM, FS, CV, and FZ took care of patients, LVC analyzed the data, GP, RF, MB, and SC designed the research and critically 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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