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ORIGINAL RESEARCH

Patient-Level Meta-analysis of Clofarabine in Acute Lymphoblastic Leukemia

Sima Jeha · Hiroaki Goto · André Baruchel · Emmanuelle Boëlle-Le Corfec · Christine Geffriaud-Ricouard · Rob Pieters · Hee Young Shin

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ABSTRACT

Introduction: Clofarabine monotherapy at a dose of 52 mg/m^2 per day was approved in the USA in 2004 for the treatment of relapsed or refractory acute lymphoblastic leukemia (R/R ALL) in patients aged 1–21 years after at least two prior regimens. To address a post-marketing requirement for additional evidence of the clinical benefit of clofarabine in its approved indication, a meta-analysis of patient-level data was conducted.

Methods: A systematic literature review was conducted, using the Dr.Evidence software platform, DOC Search, and Embase, to identify clinical trials with patients with R/R ALL who received clofarabine monotherapy at 52 mg/m^2. The primary endpoint was complete remission (CR). Secondary endpoints were overall remission (OR, defined by CR or CR with either incomplete platelet recovery or incomplete neutrophil and platelet recovery), duration of response, overall survival (OS), and safety.

Results: A total of 754 patients in 12 clinical studies were analyzed including 682 patients with R/R ALL treated with clofarabine monotherapy at 52 mg/m^2; of them, 374 were aged < 22 years (pediatric population). Rates of CR and OR were 16% (95% confidence interval

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S. Jeha (✉)
St Jude Children's Hospital Research, 262 Danny Thomas Place, Memphis, TN 38105-3678, USA
e-mail: sima.jeha@stjude.org

H. Goto
Division of Hematology/Oncology, Kanagawa Children's Medical Center, Minami-ku, Yokohama, Kanagawa, Japan

A. Baruchel
Department of Pediatric Hemato-Immunology, Hôpital Universitaire Robert Debré (APHP and Université Paris Cité), Paris, France

E. Boëlle-Le Corfec
Sanofi, Department of Statistics R&D, 1 avenue Pierre Brossolette, 91385 Chilly-Mazarin, France

C. Geffriaud-Ricouard
Sanofi, Global Medical Oncology, 46 avenue de la Grande Armée, 75017 Paris, France

R. Pieters
Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands

H. Y. Shin
Department of Pediatrics, Seoul National University Children's Hospital, Seoul National University College of Medicine, Seoul, Korea

[CI] 7, 26) and 28% (95% CI 20, 37), respectively, in the pediatric population and 12% (95% CI 5, 21) and 21% (95% CI 13, 31) in the overall population. Median OS (evaluable in three studies in pediatric patients) was 3.7 months (95% CI 0.1, 31.4), reaching 10.1 months (95% CI 0.3, 68.9) for those achieving OR. Sensitivity analyses supported these findings. The most frequent grade 3–4 adverse events were liver abnormalities, anemia, diarrhea, and febrile neutropenia.

Conclusion: In this meta-analysis, CR duration and median OS in pediatric patients with R/R ALL appeared to be slightly longer than in the phase II study. No new safety signals were identified. Results support the use of clofarabine monotherapy in its approved indication.

Keywords: Acute lymphoblastic leukemia; Clofarabine; Pediatric; Oncology; Hematological malignancies

Key Summary Points

A post-marketing requirement was requested by the US Food and Drug Administration to provide additional evidence supporting the use of clofarabine in monotherapy at a dose of 52 mg/m^2 per day after at least two prior regimens in patients with relapsed/refractory acute lymphoblastic leukemia (R/R ALL).

In this meta-analysis of 12 clinical studies that included 754 patients, 682 patients with R/R ALL received clofarabine in monotherapy at the registered dose of 52 mg/m^2, including 374 pediatric patients (< 22 years).

Complete remission (CR) was achieved in 16% and overall remission (OR) rate was 28%. The median duration of remission was almost 1 year for CR and 5 months for OR, allowing patients to receive hematopoietic stem cell transplantation (HSCT). Median OS was 3.7 months overall, reaching 10.1 months in patients with OR.

No new safety concerns were identified in this meta-analysis.

These results, which are in agreement with those of a phase 2 study conducted in the USA, support the use of clofarabine monotherapy for its approved indication of the treatment of relapsed or refractory ALL in pediatric patients aged < 22 years.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most prevalent cancer among children and adolescents, representing approximately 25% of cancer diagnoses in children younger than 15 years old [1, 2]. The risk for developing ALL is highest in children younger than 5 years [3, 4]. The outcome of pediatric patients with ALL on contemporary treatment regimens is excellent, with an overall survival (OS) rate reaching 90% [5, 6]. However, the prognosis of patients with relapsed or refractory (R/R) ALL remains a challenge. Historical series report a CR rate of 18% and a median overall survival (OS) of around 3 months after a second salvage therapy [7]. Recently, significant improvements in outcomes of pediatric patients with B cell R/R ALL have been achieved with chimeric antigen receptor (CAR) T cells but these novel therapies have an extremely high cost leading to notable disparities in their availability for the patients [8–10].

Clofarabine is a second-generation purine nucleoside analogue developed for its greater activity and reduced extramedullary toxicity as compared with fludarabine and cladribine [11]. Its antitumor activity involves three mechanisms: (1) inhibition of DNA synthesis and repair, (2) inhibition of ribonucleotide reductase, and (3) disruption of mitochondrial membrane integrity with the release of cytochrome *c* and other proapoptic factors leading to programmed cell death even in non-dividing lymphocytes [11]. Clofarabine has also been shown to inhibit cancer growth by

targeting ZRANB3, a recently identified DNA synthesis promoter and nuclear-localized interactor of ribonucleotide reductase α-subunit, which plays a major role in regulating tumor invasion and H-rasG12V-oncogenic signaling pathway [12]. In preclinical studies, clofarabine was effective against mixed lineage leukemia (MLL)-rearranged infant ALL cells with median lethal concentration (LC_{50}) values of approximately 25 nM [13]. Furthermore, clofarabine inhibits growth of a range of infant ALL cell lines, including primary leukemic cells, cell lines derived from patients with ALL, and cell lines with common molecular abnormalities found in ALL, with IC_{50} values ranging from 0.1 to 0.01 μM [14]. This activity of clofarabine in various leukemia subtypes and the lack of neurotoxicity commonly associated with other nucleoside analogues were confirmed by several phase I–II studies conducted in both adult and pediatric patients with solid and hematological cancers [15–18].

One of those studies, conducted in 25 heavily pretreated pediatric patients with leukemia (17 with ALL; 8 with acute myeloid leukemia [AML]) established that the clofarabine maximum tolerated dose was 52 mg/m^2 per day for 5 days; overall, five patients achieved a complete remission (CR) and three achieved a partial remission (PR), resulting in a 30% response rate with a manageable safety profile [15]. These encouraging results were followed by a phase II clinical trial in which 61 pediatric patients with R/R ALL were treated with clofarabine (52 mg/m^2 for 5 days) as a single agent. The overall remission (OR) rate was 20%, including seven patients with CR and five with CR without platelet recovery (CRp); six patients had a PR. Remissions were sufficiently long-lasting to allow patients to proceed to hematopoietic stem cell transplantation (HSCT) after clofarabine. Median CR duration in patients who did not receive HSCT was 6 weeks, with four patients maintaining CR or CRp for 8 weeks or more on clofarabine therapy alone. The most common grade ≥ 3 adverse events were febrile neutropenia, anorexia, hypotension, and nausea [18].

On the basis of the results mentioned above, clofarabine monotherapy (Clolar®; Sanofi, Paris, France) at a dose of 52 mg/m^2 for five consecutive days was approved in the USA on 28 December 2004 under the accelerated approval pathway for the treatment of pediatric patients aged 1–21 years with R/R ALL after at least two prior treatment regimens. Subsequently, clofarabine monotherapy (Evoltra®; Sanofi, Paris, France) was approved by the European Medicines Agency (EMA) on 29 May 2006 and by the Japanese Ministry of Health, Labor, and Welfare as Unapproved Drugs with High Medical Needs. Authorization in Europe was dependent on agreement to a risk management plan (RMP) requiring performance of required pharmacovigilance activities and interventions. In Japan, mandatory post-marketing surveillance registries evaluating the effectiveness and safety of clofarabine in daily conditions of use were requested.

As part of approval in the USA, the Food and Drug Administration (FDA) issued a post-marketing requirement (PMR) 1253-2 to provide additional evidence of the clinical benefit of clofarabine monotherapy in patients with ALL. While several proposals of randomized studies were discussed with the FDA, none of them were able to meet the requirements of PMR 1253-2 because of substantial advances in the management of ALL since the approval of clofarabine [19–21]. It was therefore agreed to use a "body of evidence" approach to support the use of clofarabine in patients with no alternative treatment options. As part of this approach, a meta-analysis was conducted to evaluate the efficacy and safety of clofarabine monotherapy at the registered dose of 52 mg/m^2 from studies conducted in the treatment of ALL in adult and pediatric patient populations, the results of which are reported here.

METHODS

Literature Search

A comprehensive systematic literature review (SLR) was first conducted to identify clinical studies evaluating the efficacy and safety of clofarabine in patients of all age groups with ALL. Searches were conducted in the Dr.Evidence® software platform, docsearch, and

Embase using terms "acute lymphoblastic leukemia" and "clofarabine". No restrictions were placed on date of publication or language, provided there was an English abstract. Retrieved items were further evaluated manually on the basis of pre-defined criteria on study design (clinical study) and outcomes (at least one of OS; efficacy or effectiveness; response or remission; safety or adverse events; relapse or refractory; treatment failure).

Study Selection

Study inclusion criteria for the meta-analysis included company-sponsored and investigator-sponsored clinical trials (published or not) and published clinical trials on patients aged < 22 years (pediatric population) or ≥ 22 years (adult population) with R/R ALL after at least two prior treatment regimens and who were receiving clofarabine monotherapy at the recommended phase 2 dose of 52 mg/m^2. Studies with at least one of the following efficacy endpoints were included: CR, OR (defined as CR, CRp or CR without neutrophil and platelet recovery [CRi]), duration of response (DOR) for CR and OR, and overall survival (OS). Response criteria used were those of the National Comprehensive Cancer Network (NCCN) guideline 2020 or those used at the time of study implementation. Studies supporting the benefit of clofarabine in other patient populations (e.g., very high risk patients), other hematological cancers, and/or different treatment regimens, i.e., not reflecting the currently approved conditions of use, were excluded. This analysis is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

Endpoints

The primary efficacy endpoint was CR, defined as meeting all of the following criteria: no evidence of circulating blasts or extramedullary disease, M1 marrow ($\leq$ 5% bone marrow blasts), and recovery of peripheral counts (platelets $\geq 100 \times 10^9$/L and absolute neutrophil count $\geq 1.0 \times 10^9$/L), in line with previous research [18]. Secondary endpoints included OR (defined as CR, CRp or CRi), DOR (defined from date of remission to date of last assessment before objective disease progression or death, includes DOR after transplantation), OS (calculated from date of clofarabine initiation to date of death due to any cause), and safety.

Statistical Analysis

The analysis was based on results of individual studies, which were available from study reports or publications, or calculated from the reported data. Results not available from data sources were indicated in statistical outputs. Data collected from pediatric and adult patients were analyzed separately by age group (< 22 or ≥ 22 years) and for the overall population. Demographic and baseline characteristics were described by study and included age, sex, ALL subtype, prior treatment regimens, prior HSCT, and duration of study.

The combined CR rate across studies was estimated as follows: an arcsine square root transformation was utilized on each individual study CR rate to stabilize the variance and the transformed CR rates were approximated by the normal distribution. For combining, the transformed proportions and corresponding standard errors were used in the standard inverse variance method [22]. The transformed proportions were used for statistical modelling. The heterogeneity due to study-to-study differences was assessed by providing forest plots on CR rates of component studies. A random-effects model was used to account for heterogeneity across studies and the calculated CR and corresponding 95% confidence interval (CI) from the meta-analysis were back-transformed and presented in their original unit of percent. OR rates were analyzed similarly. A sensitivity analysis on the primary endpoint was performed using Freeman–Tukey double arcsine transformation [23] on individual study CR rates. To assess publication bias, a funnel plot and the Egger's test were produced for CR. Safety analysis was based on available summaries of adverse events, deaths, and laboratory toxicities of interest.

RESULTS

Study Characteristics

The SLR retrieved a total of 639 unique records after exclusion of duplicates (Fig. 1). After title/abstract screening, 528 publications were excluded, mostly because of incompatible study design. Full texts of the remaining 111 publications were reviewed and 63 were further excluded, mainly because of lack of outcomes available. Of the remaining 48 publications, 36 were further excluded because they enrolled patients with other types of hematological cancers (e.g., AML, lymphoma, myelodysplastic syndrome) and/or who received off-label clofarabine regimens (clofarabine combined with other agents or clofarabine given at doses different from 52 mg/m^2). Thus, only 12 clinical studies including 754 patients satisfied meta-

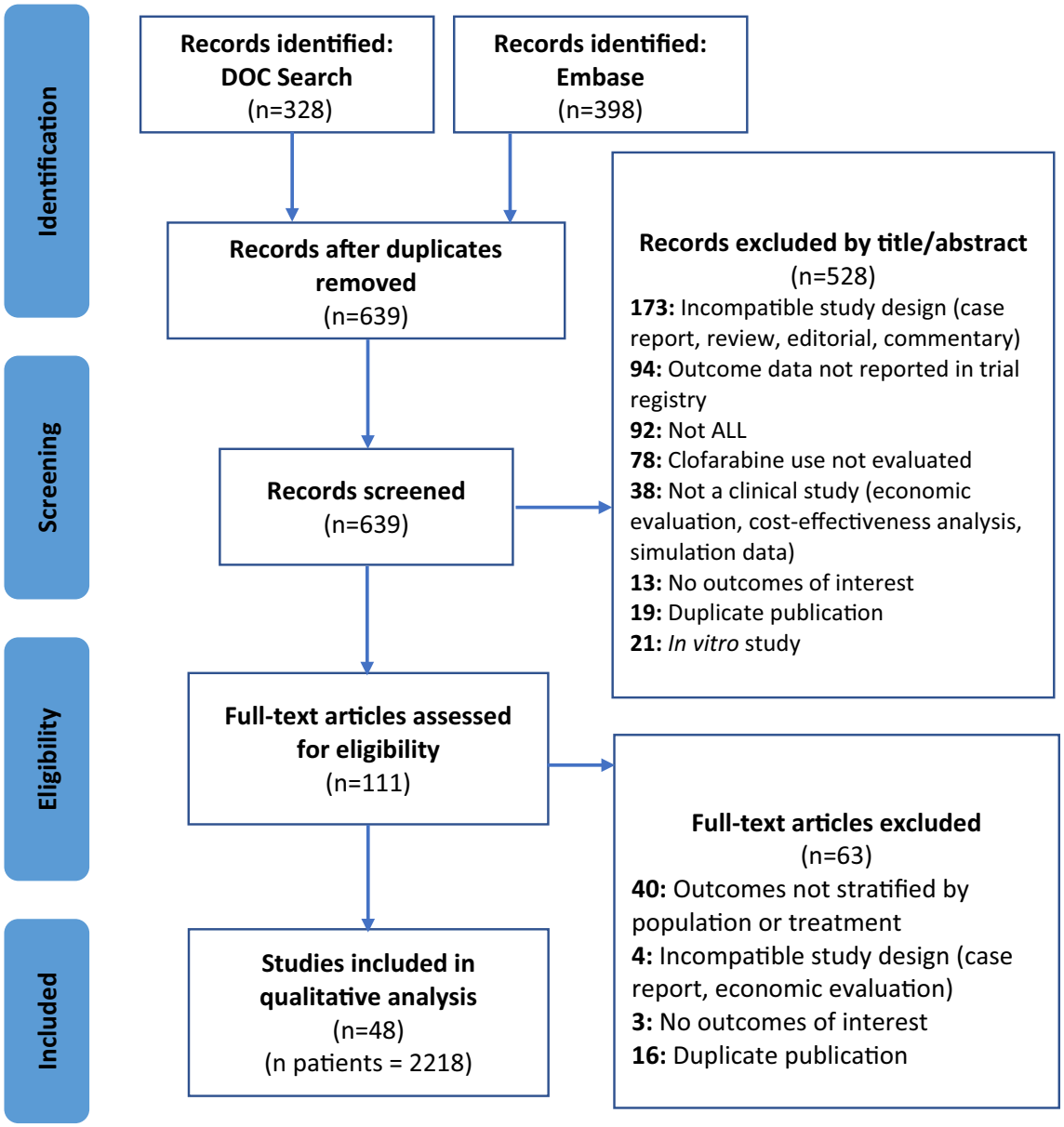

Fig. 1 Selection of studies through the systematic literature review

Table 1 List of studies included in the meta-analysis

Study ID title	Sponsorship/ study phase	Therapy dose and regimen (center)	Number of patients		
			Enrolled; exposed to clofarabine	R/R ALL/ other ALL type	R/R ALL treated with clofarabine monotherapy at 52 mg/m^2 after ≥ 2 prior regimens (pediatrics[a]/adults)
Total number of patients	NA	NA	754 (754)	682/ 72	474 (374/100)
CLO-212 [18] Phase II study of clofarabine in pediatric patients with R/R ALL	Company-sponsored clinical study Uncontrolled phase I	Clofarabine monotherapy 52 mg/m^2 IV over 2 h for 5 days Multicenter	61 (61)	61/0	61 (61/0)
BIOV-111 [36] Phase II, open-label study of clofarabine in pediatric patients with R/R ALL	Company-sponsored clinical study Uncontrolled phase I	Clofarabine monotherapy 52 mg/m^2 IV over 2 h for 5 days Multicenter	71 (71)	71/0	65 (65/0)
CLO05908 [24] Phase I study of clofarabine in pediatric patients with R/R ALL in Japan	Company-sponsored clinical study Uncontrolled phase I	Clofarabine monotherapy Cohort 1: 30 mg/m^2 IV over 2 h for cycle 1, then 52 mg/m^2 IV over 2 h for 5 days for subsequent cycles Cohort 2: 52 mg/m^2 IV over 2 h for 5 days Multicenter	7 (7)	7/0	4 (4/0)
CLO08708 (OBS12879) [41] The Evoltra® European Registry Programme: Pediatric Leukemia	Company-sponsored prospective observational study (European registry) Uncontrolled	Clofarabine monotherapy 52 mg/m^2 IV over 2 h for 5 days Clofarabine in combination (varying doses ranging from 20 to 40 mg/m^2 for 5 days) Multicenter	112 (112)	93/19	2 (2/0)

Table 1 continued

Study ID title	Sponsorship/ study phase	Therapy dose and regimen (center)	Number of patients		
			Enrolled; exposed to clofarabine	R/R ALL/ other ALL type	R/R ALL treated with clofarabine monotherapy at 52 mg/m^2 after ≥ 2 prior regimens (pediatrics[a]/adults)
CLOFAL06790 [37] A pragmatic, non-interventional study to evaluate effectiveness and safety of clofarabine (Evoltra) in Korean children with ALL who failed to respond or relapsed after 2 or more regimen (BACH)	Company-sponsored prospective observational study (Korean registry) Uncontrolled	Clofarabine monotherapy 52 mg/m^2 IV over 2 h for 5 days Clofarabine in combination with cyclophosphamide and etoposide Multicenter	60 (60)	60/0	2 (2/0)
CLOFAL06952 [39] Drug use investigation of Evoltra® for acute lymphoblastic leukemia (ALL) patients	Company-sponsored prospective observational study (Japanese post-marketing survey) Uncontrolled	Clofarabine monotherapy 52 mg/m^2 IV over 2 h for 5 days Multicenter	260 (260)	226/ 34	222 (138/84)
CLOFAL07263 [40] Specified drug use surveillance for effectiveness evaluation of Evoltra monotherapy in Japanese patients with R/R ALL	Company-sponsored prospective observational study (Japanese post-marketing survey) Uncontrolled	Clofarabine monotherapy 52 mg/m^2 IV over 2 h for 5 days Multicenter	27 (27)	25/2	20 (4/16)

Table 1 continued

Study ID title	Sponsorship/ study phase	Therapy dose and regimen (center)	Number of patients		
			Enrolled; exposed to clofarabine	R/R ALL/ other ALL type	R/R ALL treated with clofarabine monotherapy at 52 mg/m^2 after ≥ 2 prior regimens (pediatrics[a]/adults)
ID99-383 [15] Clofarabine, a novel nucleoside analog, is active in pediatric patients with advanced leukemia	Company-sponsored dose escalation clinical study/ publication Uncontrolled phase	Clofarabine monotherapy 11.25–70 mg/m^2 over 2 h for 5 days Single center	25 (25)	17/8	17 (17/0)
Lu et al. [25] Efficacy, safety and pharmacokinetics of clofarabine in Chinese pediatric patients with advanced leukemia	Publication Uncontrolled phase I	Clofarabine monotherapy 52 mg/m^2 IV over 2 h for 5 days Multicenter	44 (44)	43/1	43 (43/0)
O'Connor [38] Early UK experience in the use of clofarabine in the treatment of relapsed and refractory pediatric acute lymphoblastic leukemia	Publication Retrospective study Uncontrolled	Clofarabine monotherapy 52 mg/m^2 IV over 2 h for 5 days Clofarabine in combination with cyclophosphamide and etoposide Multicenter	23 (23)	23/0	5 (5/0)
Suo et al. [26] Therapeutic effect of clofarabine in children with relapsed or refractory acute lymphoblastic leukemia	Publication Uncontrolled phase I	Clofarabine monotherapy 52 mg/m^2 IV over 2 h for 5 days Single center	26 (26)	26/0	26 (26/0)

Table 1 continued

Study ID title	Sponsorship/ study phase	Therapy dose and regimen (center)	Number of patients		
			Enrolled; exposed to clofarabine	R/R ALL/ other ALL type	R/R ALL treated with clofarabine monotherapy at 52 mg/m^2 after ≥ 2 prior regimens (pediatrics[a]/adults)
Trioche [39]	Publication	Clofarabine monotherapy	38 (38)	30/8	7 (7/0)
French "real life" experience of clofarabine in children with refractory or relapsed acute lymphoblastic leukemia	Retrospective study Uncontrolled	52 mg/m^2 IV over 2 h for 5 days Combination: mostly clofarabine 40 mg/m^2 + cyclophosphamide and etoposide Multicenter			

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *IV* intravenous, *R/R* refractory or relapsed
[a]Pediatrics: aged < 22 years

analysis inclusion criteria, nine studies of which reported the use of clofarabine as monotherapy only and three as either monotherapy or combination therapy (Table 1). Most (10/12) studies enrolled exclusively pediatric patients (aged < 22 years) and two studies (CLO-06952 and CLO-07263) included both pediatric and adult patients.

Patient Characteristics

Of the 754 patients exposed to clofarabine in the 12 included studies, 682 patients had R/R ALL and 474 patients had R/R ALL treated with clofarabine in monotherapy at the approved dose of 52 mg/m^2 (374 of whom were aged < 22 years). Of the 597 patients with available immunophenotype, most (79.1%) had a B precursor or B cell ALL, 13.9% had T cell ALL, and 1.5% patients had a biphenotypic ALL. Ten studies reported prior chemotherapy use in 648 patients and most of them (63.7%) received two or more chemotherapies prior to initiation of clofarabine therapy. Overall, 32.1% of patients from nine studies received at least one prior HSCT, 34.9% had no prior HSCT, and information was lacking for the remaining 191 patients. The number of clofarabine monotherapy treatment cycles ranged from one to six. Detailed patient characteristics are reported in Table 2.

Efficacy

A total of 474 patients with R/R ALL (374 aged < 22 years; 100 aged ≥ 22 years) who received clofarabine at the approved dose of 52 mg/m^2 after at least two prior regimen were evaluable for efficacy. CR (primary endpoint) and OR (defined as CR plus CRp plus CRi) were documented for all patients (Supplementary Table 1).

In the pediatric population, using the random-effects model, the combined CR rate was 16% (95% CI 7, 26) and the combined OR was 28% (95% CI 20, 37) (Fig. 2). Median duration of CR, reported in one pediatric study (CLO-212), was 11 months (95% CI 1.4, NR). Median duration of OR, reported in three pediatric studies (CLO-212, BIOV-111, Lu-2016), was 5 months (95% CI 0.2, 31.3). Median OS reported in three pediatric studies (CLO-212, BIOV-

Table 2 Patient characteristics

Characteristic	*n*/Total (%)
Gender	
Female	290/754 (38.5)
Male	463/754 (61.4)
Gender information missing	1/754 (0.1)
Other characteristics	
R/R ALL	682/754 (90.5)
Pediatric patients with R/R ALL receiving clofarabine monotherapy at 52 mg/m^2	374/754 (49.6)
Overall R/R ALL population (pediatric and adult) receiving clofarabine monotherapy at 52 mg/m^2	474/754 (62.9)
Baseline disease status per FAB classification[a] (five studies)	
L1	164/384 (42.7)
L2	94/384 (24.5)
L3	10/384 (2.6)
Unknown	109/384 (28.4)
Other	7/384 (1.8)
Tumor immunophenotype (ten studies)	
B precursor or B cell ALL	472/597 (79.1)
T cell ALL	83/597 (13.9)
Biphenotypic	9/597 (1.5)
Unknown/other tumor immunophenotype	33/597 (5.5)
Number of prior chemotherapies (ten studies)	
1	194/648 (29.9)
2	222/648 (34.3)
3	121/648 (18.7)
4	50/648 (7.7)
> 4	20/648 (3.1)
Unknown	41/658 (6.3)
Number of prior HSCTs (nine studies)	
0	202/579 (34.9)
1	176/579 (30.4)
2	9/579 (1.6)
3	1/579 (0.2)

ALL acute lymphoblastic leukemia, *FAB* French–American British, *HSCT* hematopoietic stem cell transplant, *R/R* relapsed/refractory

[a]FAB classification—L1, small cells with homogenous chromatin, regular nuclear shape, small or absent nucleolus, and scanty cytoplasm; L2, large and heterogenous cells, heterogenous chromatin, irregular nuclear shape, and nucleolus often large; L3, large and homogenous cells with multiple nucleoli, moderate deep blue cytoplasm, and cytoplasmic vacuolization that often overlies the nucleus

111, and Lu-2016) was 3.7 months (95% CI 0.1, 31.4) and reached a median of 10.1 months (95% CI 0.3, 68.9) in patients who achieved a response (CR or CRp or CRi)..

In the overall population (pediatric plus adult patients), using the random-effects model, the combined CR estimated was CR 12% (95% CI 5, 21) and the combined OR was 21% (95% CI 13, 31) (Fig. 2). Other parameters were collected exclusively for pediatric patients.

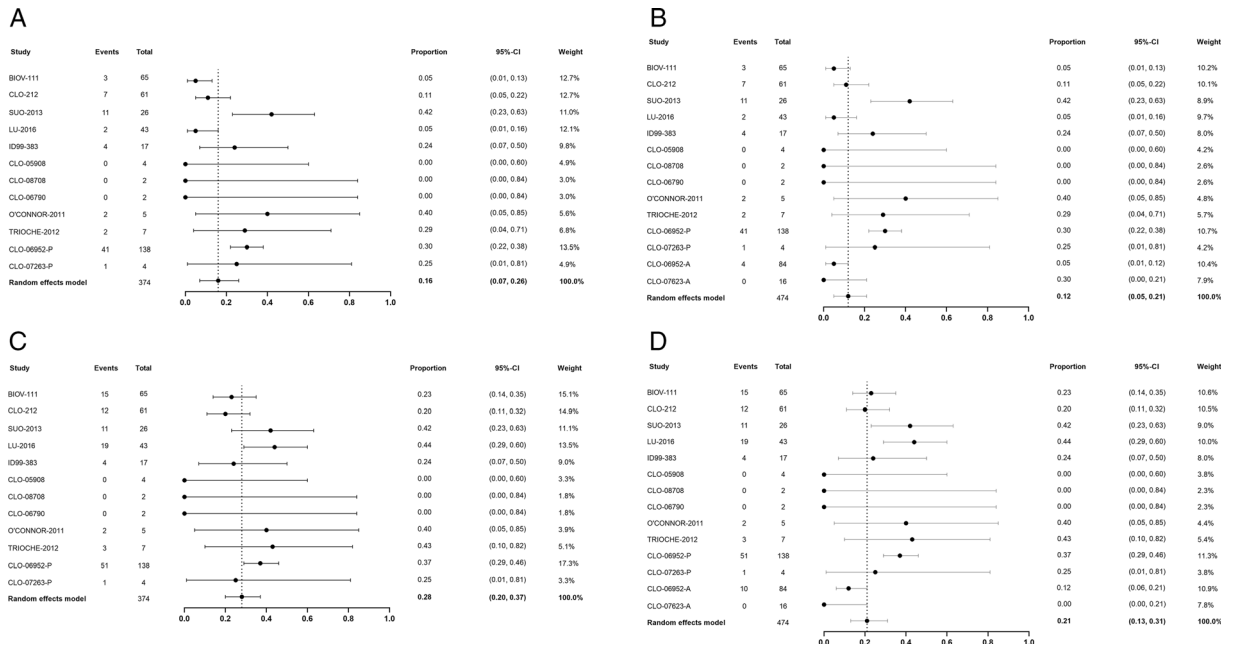

Fig. 2 **A** Complete response rates in patients with acute lymphoblastic leukemia treated with clofarabine monotherapy (52 mg/m^2) in pediatric patients aged < 22 years, **B** in pediatric patients aged < 22 years and adult patients aged > 22 years, **C** overall response rates in patients with acute lymphoblastic leukemia treated with clofarabine monotherapy (52 mg/m^2) in pediatric patients aged < 22 years, and **D** in pediatric patients aged < 22 years and adult patients aged > 22 years

Sensitivity Analysis

All results from the sensitivity analyses for CR and OR were consistent with those from the primary analysis. In the pediatric population, the CR estimate from the random-effects model for all 12 studies was 14% (95% CI 5, 26). In the overall population, the CR estimate for all 12 studies was 11% (95% CI 3, 21). The OR estimate was 28% (95% CI 20, 37) in the pediatric population and 21% (95% CI 12, 31) in the overall population.

Publication Bias

The funnel plot showed approximate symmetry and the Egger's test p value was 0.58, suggesting that there was minimal publication bias in the assessment of CR (Supplementary Fig. 1).

Safety

All 12 studies included in the meta-analysis reported safety outcomes (Table 3). Of these, seven studies reported serious adverse events (SAEs) and five reported treatment emergent adverse events (TEAEs). The most frequently reported grade 3 or 4 TEAEs were increase in alanine aminotransferase (ALAT) levels (1.6–42.9%), febrile neutropenia (11.6–78.9%), anemia (4.2–57.1%), diarrhea (0–21.1%), hypokalemia (3.3–18.4%), nausea (0.0–16.4%), pyrexia (3.6–14.8%), thrombocytopenia (1.6–42.9%), vomiting (0.0–18.4%), and mucosal inflammation/mucositis (1.6–26.1%) (Table 4). The most frequently reported clofarabine-associated SAEs were febrile neutropenia, neutropenia, palmar-plantar erythrodysesthesia syndrome, increase in ALAT levels, increase in aspartate aminotransferase levels, sepsis, decrease in platelet count, and anemia.

Table 3 Summary of adverse events in pediatric and adult patients treated with clofarabine monotherapy from studies included in the meta-analysis

Study ID	*N*	Any TEAE	Serious TEAE	Related TEAE	NCI-CTC grade 3 TEAE	NCI-CTC grade 4 TEAE
CLO-212	61	61 (100.0%)	49 (80.3%)	59 (96.7%)	32 (52.5%)	13 (21.3%)
BIOV-111	71	71 (100.0%)	59 (83.1%)	67 (94.4%)	27 (38.0%)	16 (22.5%)
SUO-2013	26	26 (100.0%)	NA	NA	NA	NA
LU-2016	44	44 (100.0%)	4 (9.1%)	NA	38 (86.4%)	NA
ID99-383	17[a]	13 (76.5%)	NA	NA	NA	NA
CLO-05908	7	7 (100.0%)	0 (0.0%)	7 (100.0%)	2 (28.6%)	5 (71.4%)
CLO-08708	112	91 (81.2%)	59 (52.7%)	76 (67.9%)	39 (34.8%)	17 (15.2%)
CLO-06790	60	60 (100.0%)	37 (61.7%)	50 (83.3%)	NA	NA
O'CONNOR-2011	23	16 (69.9%)	NA	NA	NA	NA
TRIOCHE-2012[b]	38	> 30 (> 79%)	NA	NA	NA	NA
CLO-06952[c]	260	217 (83.5%)	170 (65.4%)	NA	NA	NA
CLO-07263[c]	27	19 (70.4%)	16 (59.3%)	NA	NA	NA

NA not available from data sources, *NCI-CTC* National Cancer Institute-Common Toxicity Criteria, *TEAE* treatment-emergent adverse events

[a]Patients with acute myeloid leukemia (n = 8) were not analyzed

[b]TRIOCHE-2012 did not provide overall TEAEs in the publication but provided the most frequently reported NCI-CTC grade $\geq$ 3 adverse events

[c]CLO-06592 and CLO-07263 studies had data from overall (adult and pediatric patients) and remaining studies only had pediatric patients

Of 12 studies included in the meta-analysis, three, namely CLO-05908 [24], LU-2016 [25], and SUO-2013 [26], did not report deaths and one Japanese product registry (CLOFAL07263 [25]) did not provide information related to cause of death. The number and causes of death are summarized in Supplementary Table 2. Main causes of death included disease progression and disease-related AEs.

DISCUSSION

This meta-analysis, which included 474 patients (374 aged < 22 years) with R/R ALL treated with clofarabine monotherapy at a daily dose of 52 mg/m^2 for five consecutive days, provides a robust estimation of the efficacy and safety of clofarabine in its approved indication. In this heavily pretreated population characterized by a particularly poor prognosis, (1) 16% of pediatric patients aged 1–21 years achieved a CR (primary endpoint) and 28% achieved an OR (consisting of CR plus CRp plus CRi); (2) the duration of remission (median duration of almost 1 year for CR and 5 months for OR) was sufficiently long-lasting to allow patients to receive HSCT; (3) median OS was 3.7 months overall but reached 10.1 months in pediatric patients with a response; and (4) there was no new safety signal.

The results of this meta-analysis are in agreement with those of the phase 2 study (CLO-212) conducted in the USA, with

Table 4 Summary of the most frequently reported adverse events (in three or more studies) with NCI-CTC grade 3 or 4

Study ID	*N*	ALAT increase[a]	Anemia[a]	Diarrhea	Febrile neutropenia	Hypokalemia[a]	Mucositis/Mucosal inflammation	Nausea	Pyrexia	Thrombocytopenia[a]	Vomiting
CLO-212	61	1 (1.6%)	NA	8 (13.1%)	30 (49.2%)	2 (3.3%)	1 (1.6%)	10 (16.4%)	9 (14.8%)	1 (1.6%)	4 (6.6%)
BIOV-111	71	4 (5.6%)	3 (4.2%)	5 (7%)	36 (50.7%)	3 (4.2%)	4 (5.6%)	5 (7%)	9 (12.7%)	6 (8.5%)	3 (4.2%)
SUO-2013	26	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
LU-2016	44	15 (34.1%)	NA	NA	NA	NA	NA	NA	NA	NA	NA
ID99-383	17[b]	1 (5.9%)	NA	NA	NA	NA	NA	2 (11.8%)	NA	NA	2 (11.8%)
CLO-05908	7	3 (42.9%)	4 (57.1%)	0 (0.0%)	3 (42.9%)	1 (14.3)	NA	0 (0.0%)	1 (14.3%)	3 (42.9%)	0 (0.0%)
CLO-08708	112	7 (6.2%)	10 (8.9%)	0 (0.0%)	13 (11.6%)	NA	9 (8.0%)	5 (4.5%)	4 (3.6%)	11 (9.8%)	1 (0.9%)
CLO-06790	60	NA	NA	NA	46 (76.7%)	NA	NA	NA	NA	NA	NA
O'CONNOR-2011	23	NA	NA	4 (17.4%)	15 (65.2%)	NA	6 (26.1%)	NA	NA	NA	NA
TRIOCHE-2012	38	NA	NA	8 (21.1%)	30 (78.9%)	7 (18.4%)	4 (10.5%)	NA	NA	NA	7 (18.4%)
CLO-06952	260	NA	NA	NA	66 (25.4%)	NA	NA	NA	NA	NA	NA
CLO-07263	27	NA	NA	NA	5 (18.5%)	NA	NA	NA	NA	NA	NA

N number of patients included in safety reporting, *NA* not available from data sources, *NCI-CTC* National Cancer Institute-Common Toxicity Criteria, *ALAT* alanine aminostransferase

[a]Results for these parameters were based on adverse events data from the clinical study reports or publications and not from laboratory toxicity data

[b]Patients with acute myeloid leukemia ($n = 8$) were not analyzed

clofarabine monotherapy at a dosage of 52 mg/m^2 in 61 pediatric patients with relapsed or refractory ALL [18]. CR rate in CLO-212 was 11% versus 17% in the 11 other studies; OR rate in CLO-212 was 20% versus 28% in other studies. In those pediatric patients achieving a response, median OS was almost of 10 months. Main grade 3–4 AEs were related to myelosuppression and generally consistent with the known toxicities of clofarabine and/or other chemotherapeutic agents prescribed concomitantly. Deaths reported in the meta-analysis were mainly related to disease progression. These findings support the use of clofarabine monotherapy at the dose of 52 mg/m^2 in children aged < 22 years with R/R ALL per label, in this difficult-to-treat population when no other option including CAR T cells is available.

In this meta-analysis, many patients received clofarabine outside the approved indication. Of 754 patients enrolled in 12 clinical studies, only 374 (49.6%) of those aged < 22 years had R/R ALL and received clofarabine in monotherapy at the approved dose of 52 mg/m^2. This reflects daily practice where clofarabine is often prescribed in combination with other agents (mainly cyclophosphamide and etoposide), for the treatment of R/R ALL but also in patients with other types of leukemia. For example, a retrospective analysis of Chinese children with R/R ALL treated with a clofarabine/cyclophosphamide/etoposide regimen reported CR in 38%, CRp in 15%, and PR in 15% of patients after one course of treatment [27]. Similarly, an OR of 44% was reported in a phase 2 trial using the same combination in children with R/R ALL [28]. In the study by Gruber et al., clofarabine was incorporated into a frontline ALL regimen [5, 29]. For infants with rearrangements in KMT2A, event-free survival and OS were 44.4% and 55.6%, respectively [29]; event-free survival in these patients is typically around 34–37% on conventional chemotherapeutic regimens [30–32]. Several phase 3 trials have also been conducted in patients with AML but the clinical benefit of clofarabine in this setting has not yet been established, although it may contribute to reduce the risk of cardiotoxicity and secondary malignancy in children with AML [33–35].

The meta-analysis has several limitations, including inclusion of studies from different sources and the consequent inherent heterogeneity of the data. Although a random effects model was used to account for heterogeneity, some smaller studies that were included are likely to have made the estimates unstable as the transformed response rates may not follow the normal distribution effectively. There was also a risk of selection bias due to the nature of such meta-analyses.

CONCLUSIONS

Results of this meta-analysis suggest that clofarabine given as monotherapy at a dose of 52 mg/m^2 is active in pediatric patients with relapsed or refractory ALL for whom other therapies, such as CAR T cell therapy, are not an option. On the basis of the study-level data reviewed in this meta-analysis, the safety profile of clofarabine in patients with relapsed or refractory ALL is consistent with data that were previously reported, and no new safety concerns other than those included in the product labelling have been identified. Overall, the benefit–risk balance of clofarabine monotherapy remains positive for use in its approved indication for the treatment of pediatric patients with relapsed or refractory ALL following at least two prior therapy regimens, including CAR-T cell therapy. Additional research is needed to better define the place of clofarabine in combination with other therapies in such patients.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. Qualified researchers can request access to patient-level data and related study documents, including the clinical study report, study protocol with any amendments, blank case report forms, statistical analysis plan, and dataset specifications for Sanofi-sponsored trials. Patient-level data will be anonymized, and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi's data sharing criteria, eligible studies, and process for requesting access can be found at https://vivli.org/. Data were obtained from published trials where indicated.

Declarations

Conflict of Interest. Christine Geffriaud-Ricouard and Emmanuelle Boëlle-Le Corfec are employees of Sanofi, who funded the publication, and may hold share and/or stock option in the company. Hiroaki Goto, Hee Young Shin, Rob Pieters, Sima Chafic Jeha and André Baruchel report no conflicts of interest.

Ethical Approval. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

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