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Review Article

Treatment of Adult Patients with Relapsed/Refractory B-Cell Philadelphia-Negative Acute Lymphoblastic Leukemia

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ABSTRACT

The majority of adult patients affected by B-cell acute lymphoblastic leukemia (B-ALL) will relapse after an initial response, while approximately 20% will display primary resistant disease. Patients suffering from relapsed/refractory B-ALL have a very poor outcome. Allogeneic hematopoietic cell transplantation (HCT) still represents the only curative approach, but is not so frequently feasible, because of patient's fitness, donor availability, and the ability to achieve a remission prior to HCT. The estimated remission rates with conventional cytotoxic agents are around 30%, but they are short-lived. These disappointing results led to the introduction of new immunologic-based treatments—blinatumomab and inotuzumab. They produced a substantial improvement in terms of response rates, with the ability, in most cases, to induce a minimal residual disease (MRD)-negative status. Similarly, T cells engineered to express a CD19-specific chimeric antigen receptor (CAR-T) have yielded sensational results among patients with relapsed/refractory B-ALL, with unexpectedly high MRD-negative complete remissions rates. However, the first studies looking at long-term outcomes after CAR-T infusions told us that a significant fraction of such responses are not durable, and may benefit from a consolidation approach such as an allogeneic HCT.

1. INTRODUCTION

Over the last few years, several studies confirmed the usefulness of a pediatric-type therapy applied to adult patients with B-cell acute lymphoblastic leukemia (B-ALL). Current therapies for adults with newly diagnosed B-ALL are associated with complete remission (CR) rates of 60%–90%. For those with relapsed or refractory (R/R) B-ALL chemotherapy is able to induce 31%–44% CR in first and 18%–25% in second salvage therapy [1]. For patients who achieve a second CR after re-induction chemoimmunotherapy, allogeneic hematopoietic cell transplantation (HCT) is still considered the standard of care (SC). The most relevant, predictive factor of better clinical outcome in B-ALL is the absence of measurable residual disease (MRD) in the marrow after intensive multi-agent cytotoxic chemotherapy [2]. Persistent MRD at the time of allogeneic HCT is also associated with relevant relapse rates [3]. New immune-targeted therapies, including drugs targeting B-cell-associated antigens such as CD20, CD19, and CD22 may potentially circumvent B-ALL cells' chemo-refractoriness through novel mechanisms of action, and potentially eradicate MRD, enabling more patients to receive allogeneic HCT in a MRD-negative status, for better clinical outcomes.

2. CONVENTIONAL CHEMOTHERAPY

2.1. Cytarabine-Based Regimens

A retrospective analysis of 40 adults with R/R ALL receiving the MEC regimen—a combination of cytarabine 1 g/m^2/day, etoposide 100 mg/m^2/day, and mitoxantrone 8 mg/m^2/day for 5 days—showed a CR rate of 30%, with median event-free survival (EFS) of 11.2 months and 30-day mortality of 7.5% [4]. Another retrospective study on 46 adults with R/R ALL treated with cytarabine and mitoxantrone with or without etoposide showed an overall response rate (ORR) of 48%, with nearly half of the responders receiving an allogeneic HCT. The median overall survival (OS) was 6.2 months and the nonrelapse mortality (NRM) was 15% [5]. The unusual association of cytarabine 3 g/m^2/daily for 5 days with amsacrine at 200 mg/m^2/daily for 3 days was reported to be highly active among 36 R/R ALL in a previous report, with an overall remission rate of 75% [6].

2.2. Clofarabine-Based Regimens

Clofarabine (CLO), a second-generation nucleoside purine analogue, is currently approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for patients up to the age of 21 with R/R ALL after at least two prior regimens. However, experience among adult patients is scarce. The first reports on the use of CLO as a single agent at 40 mg/m^2 showed ORR of 17%

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[7,8]. Later on, the Spanish group PETHEMA and the Southwest Oncology Group (SWOG) reported on their experiences using CLO in R/R ALL adult patients. In the former study, of 31 heavily treated patients, one-third achieved a CR, although the treatment-related deaths were high at 23% [9]. In the SWOG S0530 trial, *Advani et al.* described the outcomes of 37 patients, 59% harbouring poor risk cytogenetics, treated with the combination of CLO and cytarabine at 1 g/m^2 daily, for 5 days. CR rates were 17%, with an OS of only 3 months [10]. The subsequent S0910 trial, based on the previously tested association of CLO and cytarabine, evaluated the introduction of the anti-CD22 monoclonal antibody epratuzumab in 31 adults with R/R ALL. The ORR was 52%, with an OS of 5 months [11]. A retrospective analysis of the Group for Research on Adult Acute Lymphoblastic Leukemia reported on results in 55 R/R ALL patients treated with the combination of CLO and conventional drugs, in an intensive schedule treatment programme with two different regimens (ENDEVOL, n = 18 and VANDEVOL, n = 37). CR was achieved by 50% of the patients and up to 35% could proceed to allogeneic HCT [12]. The association of CLO with cyclophosphamide +/- etoposide has also been evaluated in a number of trials, with variable efficacy [13], some of them still ongoing (*NCT01462253, NCT03136146*).

2.3. Liposomal Vincristine

Efficacy has been tested as a single-agent drug in 65 R/R ALL Ph-negative adult patients in a multicenter, single arm phase II trial. The ORR was 20%, with a median response duration of 5.8 months. Grade 3 neuropathy was seen in 23% of cases. A total of 19% of responders went on to receive an allogeneic HCT [14]. The FDA approval for adults with Ph-negative ALL in second or greater relapse was based on these results (Table 1).

3. IMMUNOLOGIC APPROACH

3.1. Inotuzumab

A humanized anti-CD22 monoclonal antibody conjugated to the cytotoxic antibiotic agent calicheamicin was demonstrated as highly efficacious in B-ALL patients with R/R disease. CD22 is a 135-kDa B-cell-specific adhesion molecule which is preferentially expressed on mature B lymphocytes. The normal function of CD22 is to regulate signal transduction from the surface immunoglobulin receptors on B-cells. CD22 is expressed on most blasts from 60% to 90% of B-cell malignancies [15]. Preclinical and phase 1 studies evaluated the safety, antitumor activity, pharmacokinetics, and pharmacodynamics of Inotuzumab (InO) for CD22-positive R/R ALL [16]. Findings from INO-VATE, a phase 3, open-label, randomized study, showed that patients who received InO had significantly higher CR rates (81% *versus* 29%; P < .001), a lower disease burden during remission (78% *versus* 28% had bone marrow blasts below the threshold for MRD), and more durable remission (median duration of remission, 4.6 *versus* 3.1 months), compared to patients who received the investigator's choice of standard chemotherapy (Table 2) [17]. Preliminary data have shown that InO may also be used in combination with chemotherapy in order to improve the rate of remission in relapsed B-cell ALL. In a recent paper by *Jabbour et al.*, the combination of InO with low-intensity chemotherapy (mini-hyper-CVD) for Ph-negative ALL patients in first relapse showed a 78% ORR, with a CR rate of 59% (82% MRD-negativity among responders). Of interest, 44% of the patients underwent allogeneic HCT, with a 15% incidence of veno-occlusive disease (VOD) of any grade [18] In fact, VOD is a major adverse event (AE) associated with InO therapy, being observed in 11% of the cases examined [19]. Prophylactic pharmacologic agents are recommended for VOD prevention, and in patients for whom HCT is planned, the number of InO cycles should be limited to two, leaving at least four to six weeks interval between drug infusion and the start of the conditioning regimen for allogeneic HCT. Other important or serious InO-related AEs include both haematological and nonhaematological toxicities: febrile neutropenia, thrombocytopenia, infusion-related reactions, tumor lysis syndrome, and prolonged QT syndrome [20]. A report from an expert panel of haematologists and transplant physicians has summarized the recommendations for evaluation and management of the important AEs associated with InO, with a focus on diagnosis, prevention, monitoring, and management of VOD [21]. The possible interventions included prophylaxis medications, patient monitoring and assessment, and InO dose adjustment or discontinuation. The application of these recommendations in our daily clinical practice, as well as the consolidated use of InO in ALL have significantly reduced the frequency of severe and mild AEs related to InO administration (Table 3). A recent study has also evaluated the quality of life (QoL) in ALL patients receiving InO. All subjects completed the European Organization for Research and

Table 1 | Conventional chemotherapy trials.

Drug	Study	No. of Patients	ORR (%)	Median OS (Months)
Cytarabine (5 g/m^2) + Mitoxantrone (40 mg/m^2) + Etoposide (500 mg/m^2)	Liedtke *et al.* [4]	40	30	6.5
Cytarabine (5 g/m^2) + Mitoxantrone (40 mg/m^2) +/- Etoposide (500 mg/m^2)	Ahn *et al.* [5]	46	48	6.2
Cytarabine (15 g/m^2) + Amsacrine (600 mg/m^2)	Arlin *et al.* [6]	40	72	n.a.
Clofarabine-based regimens (n = 5 CLO-single agent)	Barba *et al.* [9]	31	31	5.0
Clofarabine (200 mg/m^2) + Cytarabine (5 g/m^2)	Advani *et al.* [10]	37 (phase II trial)	17	3.0
Clofarabine (200 mg/m^2) + Cytarabine (5 g/m^2) + Epratuzumab (360 mg/m^2 × 4 doses)	Advani *et al.* [11]	31 (phase II trial)	52	5.0
Clofarabine (120 mg/m^2) + Cyclophosphamide (1.2 g/m^2)	Faderl *et al.* [13]	50 (phase I trial)	14	3.0
Liposomal Vincristine (2.25 mg/m^2 weekly)	O'Brien *et al.* [14]	65 (phase II trial)	35	4.6

ORR: Overall response rate; OS: Overall survival.

Table 2 | Monoclonal antibody trials.

Drug	Study	No. of Patients	ORR (%)	MRD Rate (%)	Median OS (Months)	HCT Rate (%)
Inotuzumab	Kantarjian *et al.* [17]	218	81	78	7.7	41
Inotuzumab	Jabbour *et al.* [18]	59	78	82	11.0	44
Blinatumomab	Kantarjian *et al.* [33]	405	44	76	7.7	24
Blinatumomab	Topp *et al.* [32]	189	43	82	6.1	40
Blinatumomab	Gokbuget *et al.* [40]	116	n.a.	78	36.5	67

ORR: Overall response rate; MRD: Measurable residual disease; OS: Overall survival; HCT: Hematopoietic cell transplantation.

Table 3 | General recommendation for prevention or monitoring of veno-occlusive disease (VOD) in patients receiving Inotuzumab.

VOD Prevention	VOD Monitoring
Avoid HCT conditioning regimens containing dual alkylating agents, thiotepa, or both	In patients who have had severe or ongoing VOD, follow recommendations in country-specific prescribing information to determine appropriate use of InO.
Use prophylactic agents (ursodeoxycholic acid, defibrotide—experimental)	In patients proceeding to HCT, closely monitor LFTs during the first month post-HCT, then less frequently thereafter based on standard practice.
Among patients proceeding to HCT, limit treatment with InO to maximum two cycles	Daily weight monitoring for fluid retention evaluation.
	Monitor ALT, AST, total bilirubin, and alkaline phosphatase levels before and after each InO infusion and adjust InO dose accordingly.

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HCT: Hematopoietic cell transplantation; LFT: Liver function test; ULN: Upper limit of normal; InO: Inotuzumab

Treatment of Cancer (EORTC)-QoL Questionnaire and the EuroQoL 5 Dimensions Questionnaires, at baseline, on day 1 of each cycle, and at the end of treatment [22]. The current patient-reported outcome data support the favourable benefit/risk ratio of InO for the treatment of R/R ALL, with superior clinical efficacy and better QoL, as compared with SC.

The pattern of CD22 expression on ALL blasts can be of importance in predicting disease response to InO [23]. In the INO-VATE study, a cutoff value of 90% positivity on ALL cells was not a significant determinant of InO response (79.2% *versus* 82.4%) [17]. However, both the percentage of positivity and the intensity of expression for CD22 antigen appear to be heterogeneous, ranging from 1% to 99% as a percentage of positive blasts, and from dim to very strong expression, as determined by the antigen expression level in single blast cells [24,25]. The implication of these findings may be of clinical value and could guide MRD detection [26], since it is known that immune-targeted therapies such as InO induce a rapid receptor down-modulation, forcing the flow cytometry operator to use alternative strategies to detect MRD [27]. In this regard, the significance of MRD among R/R ALL exposed to monoclonal antibody therapies such InO as salvage treatments, has been recently questioned [28].

Mechanisms of cell resistance may play a role in B-ALL exposed to InO. The effective destruction of target cells by InO requires the completion of a number of steps, including the successful delivery of antibody–drug conjugate to the tumor microenvironment, binding of the CD22 molecule, receptor internalization, hydrolysis of the chemical linker, activation of calicheamicin by cytoplasmic thiols, and the action of calicheamicin on DNA before cellular efflux [29]. It is unknown which of these factors may explain differences in efficacy observed for InO-treated ALL. Although drug efflux has not been investigated in this setting, previous studies with gemtuzumab ozogamicin showed that increased expression of efflux pumps for calicheamicin-reduced gemtuzumab efficacy [30]. Furthermore, patients in first salvage had a higher rate of response than those in second salvage (87.7% *versus* 66.7%). On the contrary, age was not a determining factor in the response rate to InO, since patients below or above 55 years old had similarly high response rates to InO (80.3% *versus* 81.4%). Finally, disease extension may be considered a predicting factor, as patients with a high disease burden (50% bone marrow blasts) showed a high response rate to InO treatment compared to SC (86.7% *versus* 77.9%) [31].

3.2. Blinatumomab

A bi-specific T cell engager monoclonal antibody that links CD19-positive cells to CD3-positive T-lymphocytes, was shown to be one of the most interesting new drugs used for R/R ALL to date. The multicenter, single-arm phase-2 study conducted by *Topp et al.* among 189 adult patients with primary R/R Ph-negative ALL disclosed a surprisingly high CR rate of 43%. The majority of patients who achieved CR did so within the first cycle (78%), and 82% of these patients had an MRD response. Of those patients achieving a response, approximately 40% went on to receive an allogeneic HCT [32]. In a subsequent international phase-3 clinical trial conducted on 405 pretreated adults affected by R/R Ph-negative ALL, blinatumomab (BLI) demonstrated superior rates of OS (7.7 *versus* 4 months), EFS (31 *versus* 12% at 6 months), and CR (34 *versus* 16%) as compared to cytarabine-based chemotherapy (Table 2). Lower marrow blast counts were associated with increased CR (65% *versus* 34.4% for marrow blasts <50% or ≥50%, respectively), suggesting that the BLI effectiveness requires low-tumor burden [33]. The favourable results obtained with the incorporation of BLI in sequential combination with Hyper-cyclophosphamide-vincristine-doxorubicin-dexamethasone (CVAD) among newly diagnosed ALL adult patients could also serve as an effective association in the R/R ALL setting [34].

The most frequent AEs were nonspecific symptoms, like fever, headache, fatigue, and infections. Cytokine release syndrome (CRS) and neurotoxicity appear to be a BLI-specific reaction, commonly observed also with anti-CD19 CAR-T cells mediated by activated T cells and pro-inflammatory interleukins [35]. The clinical hallmark of CRS is represented by high-grade fever into a systemic inflammatory response, leading, in the most severe situations, to capillary

leak syndrome associated with hypoxia and hypotension, requiring intensive care support. High leukemia burden seems to correlate with higher risk of developing severe CRS [36]. Corticosteroids are the mainstay treatment of grade 3/4 CRS, while anti-interleukin-6 may be effective, as already observed with CAR-T-associated CRS [37]. Neurologic toxicity is dose-limiting, may appear as an acute or subacute process and may be life-threatening [38]. Its underlying mechanism is still unknown, although recent evidence indicates a putative role played by both T and B-lymphocytes, and macrophages, within a disrupted blood–brain barrier. Endothelial activation and high levels of cytokines in the cerebrospinal fluid may also be implicated in its pathophysiology. Fascinating new suggestions propose an unexpected role of interleukin-1 in anti-CD19 immunotherapy-associated neurologic toxicity [39]. BLI-associated neurotoxicity includes a wide spectrum of clinical pictures, ranging from dizziness, paraesthesia, tremor, to more serious acute delirium, encephalopathy, convulsions, dysarthria, and ataxia. Treatment, whenever indicated, is based on corticosteroids.

The ability of BLI to induce undetectable MRD in the phase II (BLAST) study of 116 B-ALL adults in hematologic remission with MRD-positivity led to FDA and EMA-approval. An impressive 78% of the evaluable patients achieved MRD negativity after one 28-days cycle, which was associated with longer OS and EFS than in nonresponders. Any grade CRS and grade 3/4 neurotoxicity occurred in 3% and 13% of patients, respectively. Although 67% of the patients received an allogeneic HCT, the study was not powered to test the role of transplant in this setting [40]. The subsequent long-term follow-up analysis showed a net survival benefit for those patients achieving MRD-negative CR after cycle 1 and for those who received an allogeneic HCT without MRD at the time of transplantation [41].

Recent data on long-term follow-up of 35 R/R ALL treated in a phase II trial with BLI showed that, of the 19 patients who achieved a response (84% with MRD-negativity), 16 did relapse at a median of 3 months, 25% of whom with CD-19 negative relapses. Seventeen patients received an allogeneic HCT. The median OS was 10.6 months. Long-term survivors were observed only among those who underwent an allogeneic HCT either as a consolidation approach after BLI or as part of a salvage treatment after other anti-CD19 therapies, including CAR-T infusions [42].

There are various mechanisms of resistance to BLI in ALL. Although CD19 modifications, including epitope-loss or alternative splicing, or even complete antigen loss variants, have been observed [43], CD19-negative relapses after BLI exposure are not so frequent, as CD19 down-regulation by ALL blasts is quite uncommon [44]. Lineage-switch to acute myeloid leukemia, although infrequent, has also been reported [45]. Upregulation of programmed death-ligand 1 (PD-L1) offers ALL cells another viable immunological escape from BLI [46]. Simultaneous PD-L1-blockade may foster BLI activity, offering a novel potential therapeutic platform. A recent report from a phase I dose-escalation trial confirmed the safety and efficacy of BLI with nivolumab, with a reported MRD-negative CR rate of 80% among eight treated patients [47]. Dose escalation will also include anti-CTLA4 ipilimumab, in order to augment BLI cytotoxicity (*NCT02879695*), as observed in preclinical models [48]. The ongoing *NCT03160079* clinical trial is also testing the efficacy of a BLI and pembrolizumab combination among R/R ALL patients. Irrespective of the exact mechanism of resistance, ALL patients failing BLI have a poor outcome, with a median OS of 5.2 months estimated [49].

3.3. CAR-T Cell Therapy

CD19-directedCAR-T have generated unforeseen clinical responses among notoriously hard to treat R/R ALL patient population. However, the majority of the remissions achieved to date are not durable, and do need consolidation with an allogeneic HCT. The most frequently observed anti-CD19 CAR-T-associated toxicities are, as already mentioned for BLI, CRS and neurotoxicity, either of which can be fatal. We have summarized below some of the most influential clinical experiences with CAR-T in R/R ALL patients.

ELIANA was the first global CAR-T cell therapy registration trial ever undertaken. It led to FDA-approval, in August 2017, of 4-1BB/CD3ζ Tisagenlecleucel for pediatric and young adults below 25 years of age with R/R ALL [50]. It enrolled a total of 92 patients (median age 11, range: 3–23) with a median of three prior lines of therapy and 61% of post-allogeneic HCT relapses. At six months EFS and OS were 67% and 78%, respectively (Table 4). In the update analysis of 75 treated patients (53 of them evaluable for response), with a median follow-up of 13.1 months, the ORR was 81%, with 60% of CRs. CTL019 was detected in the blood up to 20 months after the infusion. The 12-month EFS and OS were 50% and 76%, respectively. A total of 46% of patients developed CRS grade 3 or 4, and 47% were admitted to the intensive care unit (ICU) for aggressive management. Neurologic events were observed in 40% of patients within eight weeks from infusion with 13% grade 3 but no grade 4 reported. Only eight patients went on to receive allogeneic HCT, and all of them were alive at last follow-up [51]. Results from the long-term follow-up of the Memorial Sloan Kettering Cancer Centre home-manufactured CAR-T cell phase-I trial among 53 R/R B-ALL heavily pretreated adult patients were recently published [52]. At a median follow-up of 29 (range, 1–65) months, median EFS and OS were 6.1 and 12.9 months, respectively, with a CR rate of 83%. Patients in hematologic CR prior to CAR-T infusion had enhanced remission duration and survival, with median EFS and OS of 10.6 and 20.1 months, respectively. Sixteen of the thirty-two patients who had a MRD-negative CR suffered disease relapse, including 25% of CD19-negative relapses. A total of 17 patients

Table 4 | Chimeric antigen receptor T cells (CAR-T) trials.

Drug	Study	No. of Patients	ORR (%)	Median OS (Months)
Anti-CD19 CAR T cell	Maude *et al.* [51]	40	81	Not reached
19–28z - Anti-CD19 CAR T cell	Park *et al.* [52]	75 (53 evaluable)	83	12.9
Anti-CD19 CAR T cell	Turtle *et al.* [53]	29	93	Not available
41BB - Anti-CD22 CAR T cell	Fry *et al.* [59]	21	57	Not available

did receive an allogeneic HCT as a consolidation treatment after CAR-T cell-therapy. Among patients who achieved MRD-negative CR after CAR-T cell infusion, the authors did not observe a survival advantage of those who received a subsequent allogeneic HCT (n = 16) as compared to those who were not transplanted (n = 16). Severe CRS occurred in fourteen patients with one death reported. Patients without morphologic remission before treatment or with extramedullary disease had a greater incidence of CRS, neurologic toxicity, and shorter long-term survival. In contrast with other studies, there was no correlation between the persistence of *in vivo* CAR-T cells and survival rates, while higher ratios of peak CAR-T cell concentrations to tumor burden at baseline were significantly correlated to survival. Turtle *et al.* reported on the Fred Hutchinson Cancer Research Centre's experience with their phase I/II trial of anti-CD19 CAR-T cell construct with a 4-1BB costimulatory domain and a fixed CD4/CD8 cell ratio in adults with R/R ALL [53]. The enrolled patients had previously received a median of three lines of intensive therapy regimens, and 37% had relapsed after a prior allogeneic HCT. The authors reported MRD-negative CR in 27 of 29 patients treated with CAR-T cells. Severe neurotoxicity occurred in 50% and CRS in 83% of the patients (28% of them requiring ICU care). Interestingly, the addition of fludarabine in the preparative regimen prolonged CAR-T cell persistence and EFS rates. Their recently published long-term (median 30.9 months) follow-up analysis, not surprisingly, showed that patients who achieved MRD-negative CR before CAR-T infusion showed superior EFS (median 7.6 *versus* 0.8 months) and OS (median 20 *versus* 5 months) rates as compared to those with MRD-positive status. High levels of platelets and low lactate dehydrogenase serum concentration before cell infusion, and the incorporation of fludarabine in the lymphodepletive conditioning regimen, predicted lower survival rates among MRD-negative CR patients. Of note, a total of 22 of the 45 patients who reached MRD-negative CR suffered disease relapse, at a median of 3.5 months after CAR-T infusion. CD19-associated relapses were associated with CAR-T cell disappearance, while CD19-negative relapses were noted with ongoing CAR-T cell activity in the patient's blood. Of the 45 patients who achieved a CR after cell therapy, eighteen received an allogeneic HCT and displayed longer, as EFS compared to those who did not undergo a transplant (hazard ratio 0.31 95% CI 0.13–0.79) [54]. As already discussed, antigen loss is a cause of resistance to CD19-targeted immunotherapy. Acquired mutations and/or alternative splicing are possible mechanisms of immunological-escape from antiCD19 CAR-T [55]. CD19 isoforms could preexist at diagnosis and could, under therapy-induced selective pressure, evolve as a dominant clone [56]. Immunologic escape through the acquisition of a myeloid phenotype has been described for anti-CD19 CAR-T therapy [57]. Of relevant interest, preclinical models suggest that a state of T cell exhaustion may influence the response to CAR-T therapy. A complex network of cytokine milieu, produced by ALL blasts, promotes persistent up-regulation of inhibitory receptors, including PD1, Tim3, and LAG3 on T cell surface. Such T cell dysfunctional state does persist following the introduction of CAR-T constructs, impairing their activity and functionality [58]. CD22 is usually retained by ALL leukemic blasts, even after CD19 loss, representing a potential therapeutic target for cell-based therapies. Encouraging results came from a phase I trial, conducted with a novel lentiviral vector-transduced anti-CD22 CAR-T, among 21 R/R ALL patients, including 17 who relapsed after a previous exposure to anti-CD19 CAR-T cell. CR was observed in 57% of the patients with a reported median response duration of 6 months. Reduced and variable CD22 antigen site density on B-ALL cells represented the putative primary resistance factor in almost all relapsed patients [59]. In order to minimize ALL blast cells' immunologic escape after traditional mono-directed CAR-T cell therapy, the scientific community is evaluating the feasibility and efficacy of bi-target antigen CAR-T cell formulations. Investigators from Stanford University presented their data from a Phase I trial with a bi-specific CD19/22 CAR-T construct. A total of seven adult patients affected by B-cell malignancies (two ALL) were treated, and a subsequent expansion study with estimated 60 patients will follow [60]. The same Institution performed another phase I escalation study among pediatric patients with R/R ALL with promising results [61]. *Gardner et al.* reported on Seattle's Children experience with a dual transduced CD19/22 CAR-T cell product among seven pediatric patients with R/R ALL. Five of the seven subjects achieved CR, four of which were MRD-negative. The separate dual lentiviral transduction process generated a mixture of three different *in vivo* CAR-T populations (anti-CD19; anti-CD22; anti-CD19 + CD22). Surprisingly, the authors observed a selective *in vivo* expansion of the CD19 CAR T cell population over the remaining counterparts [62]. Investigators from China developed a bi-specific product from autologous sequentially-transduced CD19 and CD22 CAR-T cell constructs. Data from the phase I trial conducted among 19 R/R ALL adult patients showed a 95% ORR at day 30, with 94.4% MRD negativity. Of the fourteen patients who received a subsequent allogeneic HCT after a median of 61 days, none relapsed, while 75% of non-transplanted patients suffered from both CD19- and CD22-positive disease relapse [63].

Genome-editing technologies such as transcription activator-like effector nuclease (TALEN) and CRISPR-Caspase 9 represent ideal platforms to generate third-party, off-the-shelf CAR-T cells [64]. The international, multi-centre phase I clinical trials of the off-the-shelf CAR-T product UCART19 has recently started recruitment for adult (from 16 to < 70 years) R/R ALL (*NCT02746952*, CALM study).

4. ALLOGENEIC HCT

Allogeneic HCT is the standard frontline therapy for patients with Ph-negative ALL at high risk of relapse. Historical donor *versus* no-donor studies have clearly shown improved outcome following allogeneic HCT, as compared to conventional chemotherapy in this setting [65,66]. Such data, together with the evidence of a strong graft-*versus*-leukemia effect in ALL [67], prompted the use of allogeneic HCT in first CR in order to prevent leukemia relapse. Nevertheless, the role of allogeneic HCT in ALL has been recently redefined by major changes in clinical practice, including the introduction of pediatric-like regimens [68], the availability of novel salvage therapies, and the widespread use of MRD assessment. Unfortunately, allogeneic HCT still carries significant NRM risks, which should be counterweighted by the expected reduced risk of relapse, considering disease biology (cytogenetics, phenotype, elevated initial white blood cell count) and kinetics of response to induction chemotherapy. Recently, the Adult Acute Lymphoblastic Leukemia (EWALL) and the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation (EBMT) groups joined together with the aim of producing a position statement on transplantation in ALL [69]. There is broad

consensus that allogeneic HCT should be offered to R/R ALL patients, after they enter a second remission. Any available therapeutic options employed as salvage treatment should be attempted to bring patients into remission at the time of transplant, as the outcome of patients with active disease or even positive MRD at the time of transplant is poor [70–72]. The indications for allogeneic HCT in first CR are significantly more heterogeneous: currently, most authors recommend offering transplant to "younger adults" (defined as <50–65 years, the cut-off age limits being highly variable among experts) in first CR with MRD positivity after induction/early consolidation, or to patients with adverse disease characteristics, irrespectively of MRD status. Among these criteria, MRD is the most consistent, although with different cut-offs and evaluation time points. Overall, MRD positivity after early consolidation is considered a strong predictor of relapse and, therefore, such patients should be offered allogeneic HCT. Older adults have a significantly poorer outcome following allogeneic HCT with a reported long-term EFS of less than 35% [73]. In fact, there is no consensus about indications for transplant in this setting, and most authors consider transplant an "option" which should be carefully evaluated balancing comorbidity score, kind of donor, and disease characteristics. Matched sibling donor (MSD) is the preferred donor source for allogeneic transplant in ALL, but 10/10-matched unrelated donor (MUD) is considered an acceptable alternative [74]. If no MSD or 10/10 MUD are available, there is no agreement among experts on the preferred donor source. There is evidence that increase in HLA disparities leads to higher NRM [75], but no prospective study has ever compared different donor sources in ALL. In the last decade, T-replete haploidentical transplantation has been increasingly performed worldwide, following the development of the posttransplant cyclophosphamide platform, with promising results in different hematological malignancies, including ALL [76]. Nevertheless, recent evidence showed similar outcome for cord blood and matched/mismatched unrelated donors [77]. Therefore, the choice of the donor should be carefully weighted considering availability, timing, and costs. The use of autologous HCT in ALL has progressively decreased worldwide [78]; this strategy could represent an alternative approach for low risk, MRD-negative patients [79]. Currently, most transplants are performed using peripheral blood stem cells (PBSCs) worldwide, and ALL does not represent an exception. The inclusion of antithymocyte globulin in the preparatory regimen is associated with reduced incidence of chronic graft *versus* host disease without affecting EFS or OS, as evidenced by a recent EBMT study on patients with Ph-negative ALL undergoing transplant with PBSC from either MSD/MUD-HCT in first remission [80]. Historically, total body irradiation (TBI) has been the standard conditioning regimen for allogeneic HCT in ALL for many years [81]. TBI is usually combined with either cyclophosphamide or etoposide [82,83]. A great body of retrospective studies comparing TBI-*versus* chemotherapy-based protocols reported reduced relapse risk after TBI-including regimens. Nevertheless, it should be highlighted that TBI is associated with severe late effects, which include endocrine dysfunction, cardiovascular and pulmonary sequelae, and secondary tumors. Recent evidence from a retrospective analysis highlights the importance of delivering TBI during the conditioning regimen also in the R/R ALL cohort [84]. When a radiation-free regimen is selected, thiotepa represents an alkylator commonly included in the preparatory protocol, and recent retrospective data showed similar survival after thiotepa-based regimens as compared to TBI [85]. Nevertheless, there is no definitive evidence demonstrating the superiority of one approach over the other.

5. PRACTICAL MANAGEMENT CONSIDERATIONS

As already discussed above, the goal of reinduction therapy after relapse is to obtain a second CR to be consolidated as soon as possible by an allogeneic HCT. For patients suffering from disease relapse after two/three years from initial antileukemia treatments, traditional cytotoxic chemotherapy could represent a viable choice, after careful assessment of the patient's fitness and evaluation of toxic effects associated with previous regimens. In this scenario, the utilization of new drugs not previously used, such as CLO, may be potentially effective.

The vast majority of patients with early relapse need an urgent remission induction in order to be consolidated rapidly by allogeneic HCT. CAR-T cell therapy could be a feasible option for young patients aged ≤ 25 years among carefully selected institutions with skilled staff and proper resources. Recent insights are confirming that CAR-T responses may not be durable. The correct identification of patients at risk of disease relapse after CAR T cell will be crucial, in order to address them to consolidation strategies such as allogeneic HCT.

The choice between BLI and InO should be the result of a fine assessment between drug availability and associated costs, institution experience, expected patient tolerability estimated drug-induced toxicities, and disease biologic features (ALL blast antigenic expression profile).

6. CONCLUSIONS

After years of struggle, novel drugs are effectively changing the current scenario for R/R ALL patients, bringing the fraction of responding patients to unexpected high levels, enabling them to receive an allogeneic HCT as a consolidation treatment. More evidence is needed to better integrate allogeneic HCT with the novel immunotherapies currently available, in order to improve antileukemic control before and after transplantation.

CONFLICT OF INTEREST

There are no conflicts of interest to report.

AUTHORS' CONTRIBUTIONS

Enrico Maffini, Francesco Saraceni, and Francesco Lanza wrote, reviewed, and approved the manuscript.

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