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Recent Developments in Immunotherapy for Acute Lymphoblastic Leukemia

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Abstract

Despite high rates of initial response to frontline therapy, the prognosis of adult patients with acute lymphoblastic leukemia (ALL) is still unsatisfactory, as many of them fail to reach a stable complete molecular response and they ultimately relapse. Intensification of chemotherapy regimens has determined a survival improvement especially in younger patients, but this strategy is less effective in case of unfavourable, high-risk cytogenetics and it is not feasible in unfit patients.

The number of target therapies for ALL patients has rapidly increased in the recent years. In particular, the use of drugs targeting either CD19 (blinatumomab and Chimeric Antigen Receptor-T cells) or CD22 (inotuzumab ozogamicin) led to unexpected high rates of deep/complete molecular response also in patients with relapsed/refractory disease after several lines of treatment, including allogeneic hematopoietic stem cell transplantation (HSCT). Unfortunately, this huge degree of response has determined so far only a small improvement of survival due to the short duration of remission, in particular when early consolidation with HSCT could not be performed. Early employment of immunotherapy, either at diagnosis or at first remission, seems a promising strategy to be tested in future prospective trials.

Keywords: Leukemia; Immunotherapy; Lymphoblastic

Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous disease arising from the malignant transformation and uncontrolled clonal expansion of early lymphoid progenitor cells [1]. Depending on the cell of origin, ALL are classified in B-cell precursor (BCP) and T-lineage subtypes. While long-term survival in children exceeds 80%, prognosis of adult patients still remains less satisfactory, with 40-50% overall survival (OS) and disease-free survival (DFS) rates also in recent years [2,3]. Actually, the majority of patients achieve complete response (CR) after frontline treatment; however, many of them either fail to reach a complete molecular response or relapse, often with a chemoresistant disease, thus showing typically poor OS [4,5]. The incorporation of intensive, pediatric-inspired chemotherapy regimens in the treatment of adult ALL has led to survival improvements as compared to historical controls [6,7]. However, it is very unlikely that the sole intensification of chemotherapeutic regimens can continue to improve prognosis substantially, particularly in elderly patients where a successful treatment is hampered by the poor tolerance of intensive regimens and the difficulty to perform allogeneic hematopoietic stem cell transplantation (HSCT) [8].

In the last decades, cytogenetic and molecular studies unraveled partially the biological complexity of ALL, indicating the leukemogenic role of recurrent abnormalities and identifying in some cases novel prognostic markers and molecular targets [9]. Unfortunately, excluding the substantial therapeutic improvements of tyrosine kinase inhibitors in Philadelphia chromosome (Ph)-positive ALL [10,11], target drugs are still unavailable for most of the newly defined molecular ALL subtypes. In this setting, immunotherapy represents a promising treatment strategy, because surface target molecules are expressed in the vast majority of ALL patients and novel immunological agents display a distinct, generally mild, toxicity profile that does not overlap chemotherapy.

Here we will review the role of immunotherapy in ALL, with a particular focus on the efficacy data in the higher risk subsets, i.e. elderly or relapsed/refractory patients.

The Role of Immunological Control in ALL

The role of the immune system in the control of tumorigenesis was preliminarily shown in 1891 by William Cooley, who developed the first successful immunotherapy against cancer

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Table I: B-cell ALL cell surface targets and available monoclonal antibodies.

Antigen	Function	Monoclonal antibody	Type of antibody
CD19	Type I transmembrane protein of the Ig superfamily, involved in B-cell differentiation	Blinatumomab Denintuzumab mafodotin Combotox	BiTE Ab-drug conjugate Ab-toxin conjugate
CD20	Calcium channel, regulates cell cycle and differentiation and modulates levels of proapoptotic proteins	Rituximab Ofatumumab	Naked antibody Naked antibody
CD22	Syaloglycoprotein binding Ig-like family of adhesion molecules, regulates B-cell activation and interaction of B-cells with T-cells and antigen-presenting cells	Inotuzumab Epratuzumab Moxetumomab pasudotox Combotox	Ab-drug conjugate Naked antibody Ab-toxin conjugate Ab-toxin conjugate
CD52	Glycoprotein involved in the induction of $CD4^+$ T regulatory cells	Alemtuzumab	Naked antibody

by injecting streptococcal cultures to cure sarcoma patients. At present, it is known that the immune system plays an ambivalent role, both suppressing and promoting the growth of cancer cells, thus favouring their escape from the immune-mediated clearance through several mechanisms (e.g. loss of MHC I, reduced antigen expression, development of immunosuppressive microenvironment, etc.) [12]. However, the definite curative role of HSCT in a subset of refractory cancers, including ALL, witnesses the possibility to overcome the immunotolerance state induced by cancer cells. The concept of eliciting an anti-tumor, post-transplant immunological effect, i.e. graft-*vs*.-leukemia (GvL), has been shown first in '70s [13] and extensively documented in ALL patients [14,15], leading to the use of donor lymphocyte infusions (DLI) to treat leukemic relapse after HSCT [16]. Various mechanisms have been hypothesized to explain GvL, including the reversibility of T-cell exhaustion (i.e. the reactivation of quiescent T cells immunosuppressed by the chronic exposure to tumor antigens) and the interaction with tumor-specific antigens or aberrant proteins [17]. Although the GvL effect in ALL is less potent than in myeloid leukemias [18], there is clear evidence for a T cell-mediated GvL against minor histocompatibility antigens or self-antigens, such as the Wilms Tumor Antigen-1 (WT1) [19,20]. A preventing DLI strategy seems to be more effective than salvage therapies for overt relapse: 16 patients (8 ALL) who became molecularly positive after HSCT were treated with pre-emptive DLI and had 100% CR rate with 1-year OS of 93.8%, significantly higher than that obtained in a comparison cohort of 11 patients (3 ALL) treated with DLI in overt relapse (CR rate 63.6% and 1-year OS 27.3%) [21]. Many efforts are still addressed to separate GvL from Graft-versus-Host Disease (GvHD) effect, in order to maximize the antitumor alloreactivity while limiting acute and chronic toxicity.

Monoclonal Antibodies in ALL

The use of monoclonal antibodies in ALL has rapidly grown in recent years [22]. Lymphoblasts express several antigens in a quite specific manner and/or at higher density on their cell surface as compared to normal lymphocytes, thus allowing a selective membrane targeting and cell killing (Table 1). There are four categories of monoclonal antibodies that are currently available for ALL: a) naked antibodies killing the target cells through antibody-dependent cell-mediated cytotoxicity (ADCC) (e.g. rituximab, ofatumumab, alemtuzumab, epratuzumab); b) antibody-drug conjugates and c) antibody-immunotoxin conjugates, which exert their cytotoxic effect by vehiculating against the target cells either chemotherapeutic agents (e.g. inotuzumab ozogamicin and denintuzumab mafodotin) or natural toxins (such as *Pseudomonas aeruginosa* or diphtheria toxin) (e.g. moxetumomab pasudotox), respectively; and d) bi-specific T-cell engager (BiTE) antibodies tethering autologous resting T lymphocytes to cancer cells, which in turn trigger T cell-mediated oncolytic functions (e.g. blinatumomab) [23]. Chimeric antigen receptor (CAR) T cells are often joined to anti-CD19 antibodies in many reviews, but they represent a substantially different treatment strategy: although CD19 is the only available target of CAR T-cells so far, this technology might be applied to many other antigens: therefore, they will be reviewed in a separate chapter.

Anti-CD19 monoclonal antibodies

Nearly all cases of BCP ALL display a bright expression of CD19 [24], making this marker an ideal candidate for target therapy. However, the cellular internalization of CD19 when bound by specific antibodies and the poor results described in the first studies with naked anti-CD19 compounds have hampered the development of therapeutic naked anti-CD19 antibodies [25]. New hints came from the development of anti-CD19 antibodies conjugates with cytotoxic drugs (denintuzumab) or toxins (Combotox, SAR3419), and above all from the novel BiTE technology (blinatumomab).

Denintuzumab mafodotin (SGN-CD19A): Denintuzumab mafodotin, formerly indicated as SGN-CD19A, is a humanized anti-CD19 monoclonal antibody conjugated to the powerful monomethyl auristatin F (MMAF), a microtubule-disrupting agent, which is released directly into the target cells upon CD19 internalization. A phase 1 study has recently closed the enrollment of adult and pediatric patients with R/R BCP ALL, Burkitt leukemia/lymphoma, and B-lineage lymphoblastic lymphoma (n=91). A preliminary analysis on 49 patients (40 with BCP ALL) showed a 30% response rate, with 6 out of 8 CR/CRp patients achieving a complete MRD response. No responses were observed in pediatric patients or in patients with Burkitt leukemia/lymphoma. AEs associated with denintuzumab treatment included severe kerathopaty in 4 adult patients, partially reduced by prophylactic steroid eye drops [26].

Combotox and coltuximab ravtansine: Combotox is a mixture of antibodies targeting both the CD19 and CD22 antigens, conjugated with a deglycosylated ricin A chain. A phase 1 study demonstrated transient responses in adults [27] and this agent is currently evaluated in combination with chemotherapy for the treatment of patients with R/R BCP ALL. Coltuximab ravtansine (also known as SAR3419) is a humanized anti-CD19 antibody conjugated with maytansin, a potent microtubule inhibitor. In a phase 2 study in relapsed BCP ALL (n=36) SAR3419 was well tolerated, although associated with a small clinical response rate (about 25%, with a median duration of response of only 1.9 months); consequently, the study was prematurely discontinued and this agent was no longer developed for ALL therapy [28].

Blinatumomab: Blinatumomab is a first-in-class BiTE antibody that engages cytotoxic $CD3^+$ T-cells and drives them in contact with $CD19^+$ cells [29]. First description of blinatumomab activity in humans involved 38 patients with relapsed Non Hodgkin's B-cell lymphoma: tumor regression occurred in a dose-dependent manner, and blinatumomab doses as low as 15 $\mu g/m^2$/day led to depletion of

Table II: Blinatumomab clinical trials in BCP ALL.

Study [ref]	Phase	Setting	Dose	Patients (M/F)	Age, median (range)	CR/CRh rate	MRD-negative rate	Median OS
MT103-202 [31,32]	2	adult MRD+	15 μg/m^2/day	21 (12/9)	47 (20-77)	already in CR	80%	n.r. (RFS 65% at 33 months)
MT103-203 [33,34]	2	adult MRD+	15 μg/m^2/day	116 (68/48)	45 (18-76)	already in CR	78%	36.5 months (40.4 in MRD neg *vs.* 12 in not responders)
MT103-205 [39]	1/2	pediatric R/R	5 → 15 μg/m^2/day	39 (24/15)	9 (2-16)	31%	42%*	4.3 months
MT103-206 [35]	2	adult R/R	5 → 15 μg/m^2/day	36 (22/14)	32 (18-77)	69%	88%*	9.8 months
MT103-211 [36]	2	adult R/R	9 → 28 μg/day	189 (119/70)	39 (18-79)	43%	82%*	6.1 months
Tower [37]	3	adult R/R Ph-	9 → 28 μg/day	405 (271 in the blina arm)	37 (n.r.)	46%	n.r.	7.8 months (vs. 4.0 months for SOC chemo)
Alcantara [36]	2	adult R/R Ph+	9 → 28 μg/day	45 (24/21)	55 (23-78)	36%	88%*	7.1 months
E1910 (NCT02003222)	3	newly diagnosed Ph-	9 → 28 μg/day	recruiting	---	---	---	---

CR/CRh: Complete Remission/Complete Remission with Incomplete Hematological Recovery; MRD: Minimal Residual Disease; RFS: Relapse-Free Survival; R/R: Relapsed/Refractory; SOC chemo: Standard of Care Chemotherapy; n.r.: Not Reported; *: MRD-negative state among CR/CRh responders

tumor cells in the majority of bone marrow infiltrates, while higher doses were required to obtain measurable effect on lymph nodes [30]. This observation paved the way for the further development of blinatumomab-based strategies in the treatment of BCP ALL. Table 2 summarizes the available evidence of the clinical activity of blinatumomab in different settings.

Blinatumomab in MRD-positive BCP ALL: In a phase 2 pilot study on 21 patients with B-ALL in complete hematologic remission (CHR) but MRD persistance or MRD relapse (>10^{-4}), blinatumomab was administered at a fixed dose of 15 μg/m^2/day 4 weeks on and 2 weeks off, until a maximum of 4 cycles. Sixteen of 20 evaluable patients (80%) obtained a complete MRD response after 1 cycle of blinatumomab, without differences between patients with MRD persistance or relapse [31]. After blinatumomab treatment, 9 patients underwent allogeneic HSCT and 11 did not: relapse-free survival (RFS) at a median observation of 33 months was 65% in transplanted patients and 60% in non-transplanted patients, respectively. Of note, two late relapses (19 and 31 months after treatment, respectively) were observed in transplanted patients, while no further relapses were documented after more than 7 months from blinatumomab treatment in patients not undergoing HSCT [32].

A confirmatory multicenter phase 2 study (BLAST study) was carried out to evaluate the rate of complete MRD response after one cycle of blinatumomab. Inclusion and exclusion criteria were similar, but patients were required to have a higher MRD load (≥10^{-3}). The primary endpoint of the study was achieved by 88 out of 113 evaluable patients (78%) and 2 additional patients obtained complete MRD response after more than 1 cycle of blinatumomab. MRD response was achieved by all subgroups, irrespectively of gender, age, number of prior relapses and MRD level at study entry [33]. However, patients treated in first CR had longer RFS than patients treated in later remission (median 24.6 vs. 11 months, respectively). After blinatumomab treatment, 90 patients underwent HSCT and 26 did not: no differences were observed in OS and RFS between transplanted and non transplanted patients [34].

Blinatumomab in relapsed-refractory BCP ALL: A dose-finding phase 2 study was conducted by the GMALL group on 36 patients refractory to chemotherapy (n=3), or relapsed after induction and consolidation (n=18) or after HSCT (n=15). CR or CR without hematological recovery (CRh) were reached by 25 out of 36 patients (69%) and 22 of them also achieved MRD negativity, the majority after one cycle of treatment. A non statistically significant higher rate of CR/CRh was observed among patients in first salvage (100%) as compared to patients in second or greater salvage (60%) or relapsed after HSCT (53%). A dose-step strategy (5 μg/m^2/day for the first week of the first cycle, 15 μg/m^2/day thereafter) was found to be safer and was chosen for the subsequent blinatumomab studies [35].

These results were confirmed in a large international multicentric study, including 189 patients with R/R BCP ALL at very high risk of unfavourable outcome (primary refractory, relapsing after less than 12 months from first remission, in second or subsequent salvage, or relapsing less than 12 months after HSCT). Patients should have more than 10% bone marrow (BM) blasts at the time of blinatumomab start. The primary endpoint of the study was CR/CRh after 2 cycles of blinatumomab and was achieved by 81 patients (43%). Of note, the probability of response was higher in patients with <50% BM blasts than those with ≥50% BM blasts (73% vs. 29%, respectively), while no differences in response were observed according to gender, age groups, previous lines of salvage or HSCT. After CR/CRh, 32 patients underwent HSCT, with a transplantability rate of 40%. Median RFS for patients in CR/CRh was 5.9 months and median OS 6.1 months [36].

Following these results from phase 2 studies, blinatumomab reached approval by the U.S. Food and Drug Administation (expedite) and by the EMA agency (conditional) for the treatment of Ph-negative BCP R/R ALL. Results of a randomized phase 3 study comparing blinatumomab to standard of care (SOC) chemotherapy were recently reported. A total of 405 patients were randomized in a 2:1 ratio to blinatumomab (n=271) or SOC (n=134). Median OS was significantly superior in patients randomized to blinatumomab than to SOC (7.8 vs. 4.0 months, respectively) and the study was prematurely stopped because of efficacy. Improvement in OS was consistent between subgroups based on age, prior salvage therapy and prior allogeneic HSCT [37].

Blinatumomab has been also tested in Ph-positive ALL patients relapsed after, or refractory to, at least one second generation (2G) TKI, or intolerant to 2G TKI and intolerant or refractory to imatinib. The primary endpoint of CR/CRh after 2 cycles of blinatumomab was met by 16 patients (36%), with similar response rates among patients with or without the T315I mutation, and in patients previously treated

with ponatinib. Similarly to Ph-negative R/R high risk patients, median RFS for patients in this cohort was 6.7 months and median OS was 7.1 months [38].

Blinatumomab in untreated BCP ALL: The ECOG group designed a randomized phase 3 study to compare blinatumomab to standard consolidation/maintenance chemotherapy after induction/intensification chemotherapy in newly diagnosed Ph-negative ALL patients aged 30-70: the study is ongoing (NCT02003222). Another trial about frontline treatment of elderly patients with blinatumomab in combination with low-dose chemotherapy or dasatinib in Ph-negative or Ph-positive patients is ongoing (NCT02143414). Similarly the GIMEMA group has planned a trial with dasatinib and blinatumomab in newly diagnosed adult Ph-positive ALL (D-ALBA, NCT02744768).

Blinatumomab in special populations (pediatric and older patients): In the pediatric setting, a phase 1/2 study was conducted by the BFM and Children's Oncology Group involving children with primary refractory disease, in second or greater BM relapse, or in BM relapse after allogeneic HSCT. Also in children, a dose-step approach was found to be safer to prevent cytokine release syndrome (CRS). CR rate was 31%, and 42% of responding patients reached also complete MRD response. Half of the responder patients underwent allogeneic HSCT, thus suggesting that blinatumomab can provide a bridge to transplant also in children who are resistant to salvage chemotherapy [39].

Recently, a pooled analysis of the phase 2 studies of blinatumomab in R/R patients according to patient age at screening was reported: of 36 adults older than 65 years (13.8% of all treated patients), 56% achieved CR/CRh compared to 46% of younger adults, and the rates of complete MRD response were comparable (60% and 70%, respectively). Despite a significantly inferior use of allogeneic HSCT in older patients, survival curves for RFS and OS overlapped for both age groups. A higher incidence of severe neurologic events was reported in older adults [40]. Similar rates of response between patients younger or older than 65 years were also reported in the confirmatory multicenter trial on MRD-positive patients [33], and in the R/R Ph-positive trial [38].

Blinatumomab safety: Common toxicities after blinatumomab administration included: pyrexia (81-88%), headache (38-47%), tremor (29-36%), chills (25%), fatigue (24-50%), nausea (22%) and vomiting (22%), severe lymphopenia (19%), and decrease of immunoglobulin serum levels. Fatal cases of infections occurred during or after treatment with blinatumomab, mainly in R/R non-responder patients [36]. Neurotoxicity represents the most significant treatment-emergent adverse event (AE) associated to blinatumomab treatment. Patients with history of either CNS relevant pathologies, autoimmune disease or CNS leukemic involvement were excluded from blinatumomab clinical trials. Neurologic events (including tremor, dizziness, confusion, ataxia, aphasia, and seizures) tipically appeared during the first days of treatment and in the majority of cases were low-grade, reversible and did not preclude the possibility of favourable responses [36]. However, CNS AEs were the main cause of treatment interruption in 31% and 15-17% of patients in MRD and R/R clinical trials, respectively. Stepwise dosing during the first course and a mandatory pre-phase with high-dose dexamethasone in cases of high tumor burden led to the reduction of both frequency and severity of neurotoxicity. Anticonvulsivant prophylaxis was reported to be effective in preventing further events in patients who interrupted blinatumomab for seizure, and in many cases these patients were able to resume blinatumomab treatment [35].

Anti-CD20 monoclonal antibodies

CD20 antigen expression on the surface of BCP ALL blasts is quite heterogeneous, ranging from 0% of pro-B to 30-40% of pre-B ALL cases and 100% of mature B-ALL [24]. Notably, its expression increases after corticosteroid treatment and in patients with persistent MRD after induction [41]. Two naked antibodies, rituximab and ofatumumab, have been used in ALL, while there are only preclinical data about obinutuzumab, another second-generation anti-CD20 antibody [42].

Rituximab: Rituximab is a type I chimeric monoclonal antibody inducing cell lysis through a complement-mediated mechanism as well as ADCC. Rituximab has no role as single agent in ALL, but some studies have explored its activity in combination to chemotherapy. The addition of 8 doses of rituximab (375 mg/m^2) to the first 4 cycles of HyperCVAD chemotherapy (fractionated cyclophophamide, vincristine, doxorubicine and dexamethasone, alternating with high-dose cytarabine and methotrexate) improved the prognosis of CD20-positive BCP ALL as compared to historical controls, but this benefit was observed only in younger patients: 3-year rates of CR duration in patients aged <60 years were 70% vs. 38% for rituximab *vs*. no rituximab patients, respectively, with a strong benefit in survival (3-years OS 75% vs. 47%, respectively), while no improvement in the outcome of older patients was found, mainly due to the increased fatal infection rate in CR during consolidation [43]. In a GMALL study, patients with CD20-positive newly diagnosed BCP ALL (age 15-55 years) were treated with (n=181) or without (n=82) the addition of rituximab to induction and consolidation therapy: although no differences were seen in the CR rate, there was a significantly higher rate of MRD complete response in patients treated with rituximab (90% vs. 9%), leading to improved 5-year OS (75% vs. 54%, respectively) [44]. The final results of a phase 3 randomized study of the French GRAALL group were reported at the American Society of Hematology (ASH) Meeting 2015: 209 patients with CD20-positive BCP ALL at diagnosis (median age: 40 years) were randomly assigned to receive a pediatric-like intensive induction/consolidation chemotherapy with or without rituximab (16 to 18 doses). CR rate and MRD response after induction and first consolidation blocks were similar in the two study arms. At a median follow-up of 30 months, relapse incidence was lower in patients treated with rituximab (18% vs. 30%), translating in a significantly higher 2-year EFS (65% vs. 52%, respectively). However, 2-year OS was not different in the two groups and was superior in the rituximab arm only when censoring for HSCT in first remission [45]. Of note, rituximab is also commonly used in addition to chemotherapy for mature B-ALL (Burkitt's leukemia). Even though in many reports the improvement of rituximab addition over rituximab-free chemotherapy regimens has been poorly significant [46-48] or not detected at all [49], a recent randomized phase 3 trial showed that the addition of rituximab to short intensive chemotherapy improved 3-year EFS in adults (n=260) with Burkitt's leukemia or lymphoma (75% in the rituximab vs. 62% in the no rituximab group) [50].

Ofatumumab: Ofatumumab is a second-generation anti-CD20 monoclonal antibody that binds to a different epitope on the target antigens as compared to rituximab, thus increasing efficacy of complement activation and ADCC. In a phase 2 study, ofatumumab has been tested as frontline therapy of 41 patients with CD20-positive

Table III: Inotuzumab ozogamicin clinical trials in BCP ALL.

Study [ref]	Phase	Setting	Dose	Patients	Age, median (range)	CR/CRh rate	MRD-negative rate*	Median OS
Kantarjian et al. [55]	2	Children and adult R/R	1.8 mg/m^2 every 3-4 weeks	49	36 (6-80)	57%	68%	5.1 months
Kantarjian et al. [56]	2	Children and adult R/R	0.8 mg/m^2 on day 1; 0.5 mg/m^2 on days 8 and 15 (courses repeated every 3-4 weeks)	41	39.5(4-84)	59%	71%	7.3 months
Advani et al. [58]	2	Adult R/R in second or later salvage	0.8 mg/m^2 on day 1; 0.5 mg/m^2 on days 8 and 15 (1.8 mg/m^2/cycle every 4 weeks)	35	34 (20-79)	66%	78%	7.4 months
INO-VATE [59]	3	Adult R/R (Ph- or Ph+)	0.8 mg/m^2 on day 1; 0.5 mg/m^2 on days 8 and 15 (courses repeated every 3-4 weeks)	109	47 (18-78)	81% (vs 29% of SOC)	78% (vs 28% of SOC)	7.7 months (vs 6.7 months of SOC)
Jabbour et al. [60]	2	Newly diagnosed	1.8 or 1.3 mg/m^2 on day 3 of mini-hyper-CVD	34	69 (60-79)	97%	100%	2 years OS: 70%

CR/CRh: Complete Remission/Complete Remission with Incomplete Hematological Recovery; MRD: Minimal Residual Disease; R/R: Relapsed/Refractory; SOC: Standard of Care Chemotherapy; *: MRD-negative state among CR/CRh responders

BCP ALL in association with HyperCVAD chemotherapy. Eight doses of ofatumumab were administered during the first 4 cycles. All but one patient achieved CR (97%) and 38 out of 40 responders (95%) had a complete MRD response. With a median follow-up of 15 months, the 2-year PFS and OS rates were 68% and 87%, respectively [51].

Anti-CD22 monoclonal antibodies

CD22 is expressed on the majority of B-cell malignancies, including more than 90% of cases of BCP ALL [52], while it is not expressed by stem cells and plasma cells. CD22 is an attractive target for antibody therapy, because it is not shed in the extracellular compartment and is internalized with high efficiency [53].

Inotuzumab ozogamicin: Inotuzumab ozogamicin (InO) is an anti-CD22 directed antibody conjugated with calicheamicin, a natural anti-tubulin cytotoxic drug, previously used also in the anti-CD33 antibody-drug conjugate gemtuzumab ozogamicin. When internalized upon CD22 binding, InO is rapidly degraded in the lysosomes; calicheamicin is then released into the target cell and moves to the nucleus, where it causes breaks in double-stranded DNA and cell apoptosis [54].

Table III summarizes the available evidence about the clinical activity of InO in different settings.

Inotuzumab in relapsed-refractory BCP ALL: A single-institution phase 2 trial at the MD Anderson Cancer Center involved 90 patients (children were included) in first or later salvage therapy. In a first cohort of 49 patients InO was given every 21-28 days as single dose of 1.8 mg/m^2 in adults and 1.3 mg/m^2 in pediatric patients, respectively. The overall response rate (including patients with marrow CR without hematologic recovery) was 57%, without significant differences among patients in first, second or further salvage therapy (69%, 46%, and 67%, respectively). The majority of responders (19/28, 68%) obtained the MRD-negative status. Median survival was 5.1 months in the whole cohort and 7.9 months in the responding patients; of note, survival was similar among patients who underwent SCT or those who did not [55]. In a second cohort of 41 patients, InO was administered on a weekly schedule (0.8 mg/m^2 on day 1, 0.5 mg/m^2 on day 8 and 15 of a 21- or 28-day cycle), which resulted in similar response rate and improved safety profile. The median duration of response was 7 months, and 37% of patients treated in first salvage line were alive at 1 year [56]. Patients with high peripheral blast count, low platelet count or poor karyotype [complex, t(9;22), t(4;11), and abn(17)] had a lower chance of CR and shorter OS [57].

The relevant activity of InO in R/R patients was confirmed by a multicenter phase 1/2 study in a particularly unfavourable population of patients in second or later salvage line: overall remission rate (CR/CRh) was 66% and median OS was 7.4 months [58].

Patient enrollment in a large randomized trial comparing InO with standard chemotherapy in patients with BCP ALL in first and second salvage line has been completed, and results of a preliminary analysis on the first 218 randomized patients have been recently published [59]. CR/CRi rate was significantly higher in patients treated with InO as compared to standard chemotherapy (80.7% vs. 29.4%; $p<0.0001$); similarly, the rates of MRD negativity were significantly different in the two arms (78.4% vs. 28.1%, respectively; $p<0.0001$). In subgroup analyses according to the patients' characteristics at baseline, the rate of response was better with InO as compared to standard chemotherapy for all the factors examined, except for the Ph-positive or t(4;11)-positive patients. Although the duration of remission (4.6 vs. 3.1 months) and PFS (5.0 vs. 1.8 months) were significantly longer for InO-treated than standard chemotherapy-treated patients, median OS was only slightly better for the InO group (7.7 months vs. 6.7 months, respectively; $p=0.04$) and the second primary objective of the trial (showing significantly longer OS in the InO arm) was not met. However, a significantly higher percentage of patients were able to proceed to SCT after treatment with InO as compared to patients receiving standard chemotherapy (41% vs. 11%) and further follow-up is required to determine the clinical benefit of InO treatment.

Inotuzumab in untreated BCP ALL: InO was combined with low-dose HyperCVD regimen (omission of anthracyclines, reduction of cyclophosphamide and steroids to 50% of the original dose, and reduction of methotrexate and cytarabine to 25% of the original dose) in untreated B-ALL elderly patients. InO was administered at single dose of 1.3 to 1.8 mg/m^2 with each of the first 4 courses, in addition to rituximab standard dose. In 34 newly diagnosed B-ALL patients (median age 69 years, range 60-79 years) the overall response rate was 97%, and all the responding patients achieved MRD negativity. The 2-year PFS and OS rates were 87% and 70%, respectively. These results were better than those achieved with full dose chemotherapy in a similar patient population (2 year OS 38%) and paved the way to a broader use of InO and "mild" chemotherapy in the frontline setting [60].

Inotuzumab in elderly patients: In the phase 3 INO-VATE trial comparing InO to standard chemotherapy as salvage treatment, 38% of patients were older than 55 years. Response rates in the InO arm were similar for older and younger patients (CR/CRi rates 81.4% and 80.3%, respectively), and also the rates of MRD negativity among

responders were not different (85.7% and 73.6%, respectively). Cytopenias were more common in older patients. Overall, the frequency of hepatic toxicities with InO was similar in all the age groups, but VOD was more common in older than in younger patients undergoing SCT following InO administration (33% vs. 17%, respectively) [61].

Inotuzumab safety: Transient myelosuppression, abnormalities in liver function tests, nausea, vomiting, abdominal pain, and fatigue are the most common toxicities reported during treatment with InO. Infusion-related reactions, including cytokine release syndrome, have been observed mainly during the first cycle. An increased risk of hepatic VOD was recognized as an emerging AEs potentially linked to InO, occurring in 8-23% of patients both during and after treatment with InO, also in non transplanted patients. Hepatic VOD has been also associated with the use of anti-CD33 calicheamicin-conjugate drug gemtuzumab ozogamicin, in CD33-positive acute myeloid leukemia patients [62]. In InO-treated patients, VOD was associated with more lines of therapy and more intensive myeloablative regimens [63]. Weekly infusions have been associated to a reduced risk of VOD as compared to single doses of higher concentrations [56]. VOD was observed in 11% of patients treated with frontline mini-Hyper-CVD and InO, mostly in non-transplanted patients in CR and ongoing consolidation chemotherapy [60].

Epratuzumab: Epratuzumab is a humanized monoclonal antibody targeting CD22 with limited activity as single agent. The combination of epratuzumab and reinduction chemotherapy in 114 pediatric patients with early bone marrow relapse did not improve the CR rate when compared to historical data obtained with the same chemotherapy regimen (65% vs. 68%, respectively); however, a trend towards improvement in MRD response with a more intensified schedule of epratuzumab administration (twice weekly) was noted [64]. A randomized phase 3 trial comparing standard chemotherapy with or without epratuzumab in children with relapsed B-ALL is ongoing.

In adults, epratuzumab was administered with a salvage chemotherapy consisting of clofarabine and cytarabine in 31 R/R patients. The overall response rate (CR/CRi) was 52%, significantly higher than 17% obtained with the same regimen in the historical cohort, but median OS was only 5 months [65]. To ameliorate these results, epratuzumab was either coniugated to a radionuclide (90Y-epratuzumab tetraxetan) or linked to a topoisomerase I inhibitor (epratuzumab-SN-38). In a phase 1 study, 17 patients were treated with 90Y-epratuzumab and 3 of them achieved CR [66]. Other clinical studies are ongoing with both agents.

Moxetumomab pasudotox (Ha22): Moxetumomab pasudotox (MP) is a monoclonal anti-CD22 antibody fused to a 38-kDa truncated fragment of the *Pseudomonas aeruginosa* exotoxin A. A phase 1 study was conducted with a similar compound (known as BL22) in pediatric patients, demonstrating the decrease of leukemic blasts in 16 out of 23 B-ALL patients, but not CR [67]. MP was administered to 21 children with R/R B-ALL every other day for 6 doses every 3 weeks: of 17 evaluable patients, 24% achieved CR, 6% had partial response, and 47% had hematologic improvement for an overall activity rate of 70% [68]. In adults, MP was administered every other day for 6 doses (30 to 50 μg/kg) in 16 patients with R/R B-ALL, including patients previously treated with InO and allogeneic SCT. One patient with several prior treatments obtained CR, but no other responses according to standard criteria were observed. AEs included capillary leak syndrome, edema and elevation of liver enzymes [69].

Anti-CD52 monoclonal antibodies

CD52 is expressed in nearly all normal and malignant lymphoid cells, including both B- and T-lymphocytes, making it the less specific target for the immunological treatment of B-ALL.

Alemtuzumab: Alemtuzumab is a classic naked antibody targeting CD52 and inducing cell lysis through ADCC. In a pediatric study on R/R B-ALL (n=13), single agent alemtuzumab determined only one CR and the authors concluded that further studies with this compound were not justified [70]. The CALGB group tested the role of alemtuzumab in 24 newly diagnosed CD52-positive ALL patients as consolidation treatment after four post-remission chemotherapy courses. Alemtuzumab was administered 3 times a week up to a maximum dose of 30 mg. The median OS was 55 months, and 8 out of 11 patients with serial MRD measurements had at least 1-log reduction in MRD [71]. In a smaller cohort of 12 adult patients with R/R ALL alemtuzumab determined only transient responses [72]. Alemtuzumab was associated in all studies with significant toxicities, including severe invasive fungal and CMV infections.

Chimeric Antigen Receptor (CAR) T-Cells

The concept of CAR modified T-cells was introduced more than 20 years ago [73]. The aim of this approach was to induce autologous T cells to identify and destroy malignant cells through the interaction with tumor-specific antigens. Tipically, CAR molecules are composed by an extracellular antigen-binding domain linked to a cytosolic signalling domain, usually the ζ chain of the T-cell Receptor complex, through a transmembrane domain. CARs recognize the targeted surface antigen in a major histocompatibility complex (MHC)-independent manner, so their function is not influenced by the patient haplotype. First experiences with CARs reported poor efficacy and persistance of engineered T cells, with many cases of graft failure. This event has been attributed to the absence of intracellular co-stimulatory domains (CD28 or 4-1BB), which have been added starting from the second generation of CARs. Presently, "third generation" CARs incorporate two co-stimulatory domains (i.e. CD27, CD28, OX40/CD134 or 4-1BB/CD137) to favour engraftment and improve T-cell proliferation, functions and persistence in the host. [74].

Autologous T-cells are collected from the patient and CARs are inserted *in vitro* by a vector capable of being integrated inside the host cell genome [75]. Many ongoing clinical trials use retroviral or lentiviral vectors, with some technical differences: in particular, lentiviral vectors may transduce quiescent cells, while retrovirus need cells in mitosis. A non-viral system (so called "sleeping beauty") consists in transducing T cells with the gene of interest through the use of transposase and DNA plasmid [76]. However, despite inital concerns about the oncogenic potential of viral vectors, there is no evidence so far of clonal processes in patients treated with CARs, even if modified T-cells may persist in the host for many years after transfer [77]. Before reinfusion, autologous modified CAR-T cells have to be expanded *ex vivo* to reach at least a 1-3 x 10^6 cells/kg dose.

CD19 CAR-T cells in relapsed/refractory patients

CD19-targeting CAR T-cells were mostly studied and displayed significant activity in patients with refractory or relapsed B-cell clonal diseases. Table IV summarizes the evidence of efficacy and relevant CAR-T treatment-related emergent AEs.

Table IV: CAR T-cells clinical trials in BCP ALL.

Study [ref]	Type of CAR	Patients	Age, median (range)	CR/CRh rate	MRD-negative rate*	Median OS	Incidence of severe CRS	Neurotoxicity
Maude et al. [78]	UPenn 4-1BB	53	11(4-24)	94%	85%	78% at 1 year	27%	43%
Lee et al. [79]	NCI CD28	39	NR (4-25)	60% (low burden 81%; high burden 46%)	87%	9.7 months	28%	29%
Davila et al. [80]	MSKCC CD28	44	45 (22-74)	84%	83%	8.5 months	43%	25%
Turtle et al. [81]	Seattle 4-1BB	30	40 (20-73)	100%	93%	n.r.	83% (2 TRM)	50%

CR/CRh: Complete Remission/Complete Remission with Incomplete Hematological Recovery; MRD: Minimal Residual Disease; CRS: Cytokine Release Syndrome; *: MRD-negative state among CR/CRh responders

In a phase 1 trial conducted at the University of Pennsylvania, 30 pediatric (n=25) and adult (n=5) patients with relapsed/refractory ALL were treated with lympho-depleting chemotherapy followed by infusion of autologous T cells engineered with a CD19-directed CAR (CTL019). Twenty-seven patients (90%) obtained CR 1 month after the infusion, with 6-month EFS and OS rates of 63% and 78%, respectively [78]. Another phase 1 dose-escalation trial was conducted at the National Cancer Institute (NCI) on 21 patients with R/R ALL or NHL. Age ranged from 1 to 30 years and all patients received fludarabine and cyclophosphamide before CD19-CAR T cell infusion. CR rate was 66% and MRD-negative complete response was observed in 60% of B-ALL patients (12 out of 20). OS was 51.6%, with a median follow up of 10 months [79]. Similar data were reported by Davila et al. [80] in 24 R/R B-ALL patients treated with salvage chemotherapy followed by infusion of 19-28z CAR T cells. CR was documented in 20 out of 22 evaluable patients (91%) and MRD-negative remission was observed in 80% of them. In a recent phase 1/2 trial, $CD8^+$ and $CD4^+$ autologous T cells were separately modified to express a CD19-directed CAR, and infused in a defined $CD4^+$:$CD8^+$ ratio. CR was achieved by all treated patients, while MRD complete response was observed in 27 out of 29 evaluable patients (93%). T-cell mediated anti-CAR immune response was identified in some patients as a mechanism of reduced CAR T-cell persistence, and increased relapse risk; addition of fludarabine to lympho-depleting regimen improved CAR T-cell persistence and DFS [81]. Emerging evidence from all these studies suggests that duration of response correlates with the proliferation degree of transferred CAR-T cells in the recipient and their persistence *in vivo*. Factors determining CAR-T cell expansion and persistence are not well defined, mainly because of differences in CAR-T cell manifacturing, doses administered to patients, and lympho-depleting chemotherapy. Relapses are most likely due to short persistence of engineered T-cells, but CD19-negative relapses have also been observed, as occurring in patients treated with blinatumomab. Regardless the significant percentage of relapse, approximately 50% of patients treated with CAR-T cells maintained CR and underwent allogeneic HSCT.

CD19 CAR-T cells safety

The most common and serious toxicity described after CAR-modified T-cell therapy is the cytokine release syndrome (CRS), which has been observed nearly in all patients a few hours or days after infusion and correlates with tumor load. CRS is an inflammatory process due to excessive T cell proliferation with consequent release of cytokines. Generally, this may cause a number of symptoms ranging from mild to severe, including fever, myalgia, hypotension, nausea and vomiting or even multi-organ failure syndrome. Life-threatening CRS have been observed in patients with high tumour load. In these cases the inhibitor of the IL-6 pathway tolicizumab has been successfully employed. Corticosteroids represent a second line-treatment, as they could prevent CAR T-cells function. Another treatment-related side effect is neurotoxicity, occurring in around 40% of patients: similarly to blinatumomab, neurotoxicity is often self-limited and can include confusion, aphasia, seizure or encephalopathy. Persistent B-cell aplasia is constant and due to the profound depletion of normal B lymphocytes, thus requiring long-term prophylactic administration of intravenous immunoglobulins. No GvHD has been described so far in patients treated with CAR-T cells because of relapse after prior allogeneic HSCT.

Conclusion and Future Perspectives

Novel immunotherapy represented a major improvement in the treatment of BCP ALL and determined previously unmet high rates of CR and MRD complete response also in heavily pretreated patients. Three agents are particularly promising: the anti-CD19/CD3 BiTE antibody blinatumomab, the anti-CD22 chalicheamicin-conjugated antibody inotuzumab ozogamicin, and anti-CD19 CAR-T cells. All these treatments were proved effective in R/R patients with particularly unfavourable characteristics (i.e. primary refractory, early relapse, and relapse after HSCT) and blinatumomab was effective also in determining complete MRD clearance in the majority of patients with high MRD burden. However, the median duration of response and OS obtained in these conditions is far from optimal. The mechanisms of resistance to these agents are not fully elucidated. Emergence of CD19-negative clones and extramedullary relapse in immunological *"sanctuaries"* were observed during the first studies with blinatumomab and CAR T-cells and postulated as the main causes of failure; nevertheless, longer follow-up and many more treated patients made clear that most of relapse cases are driven by $CD19^+$ cells and occur also in the bone marrow. Considering the rapidity of relapse also in patients with deep levels of molecular response, it was hypothesized that blinatumomab might neither affect the leukemic stem cell compartment nor prevent the emergence of genetically unstable subclones [82]. Also durability of response after CAR-T cells remains a major concern, and phase 2 studies should determine which manifacturing characteristics correlate with CAR-T cells persistence and EFS.

The main question to be addressed is the role of allogeneic HSCT in the immunotherapy era. To date, HSCT were proposed to all eligible patients, as immunotherapy was seen primarily as a bridge to transplant: however, no difference in OS were observed in R/R patients when censoring for HSCT both in blinatumomab [32] [35] and InO [56] trials, because the outcome of HSCT was influenced by high post-SCT mortality. One may speculate that anticipating immunotherapy during induction or early consolidation, i.e. before the occurrence of resistant subclone selection and in presence of relatively preserved immunocompetence, would lead to a better outcome, thus eventually representing a valid alternative to HSCT.

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