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REVIEW

Biological therapy in elderly patients with acute myeloid leukemia

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ABSTRACT

Introduction: The introduction of target molecules and immunological therapies is changing the treatment landscape of acute myeloid leukemia (AML).

Areas covered: We recapitulate the biological therapies that can be employed in the treatment of elderly patients with AML. Alongside small molecules inhibitors that target specific gene mutations, antibodies, tumor microenvironment modulators, and cellular therapies are being developed for the cure of the disease. Here, we report the biological activities, the efficacy and toxicities of humanized antibodies and antibody-drug conjugates that targets surface antigens as CD33 (gemtuzumab ozogamicine) or CD123 (pivekimab sunirine). We further explore mechanisms and effectiveness of medications that modify the microenvironment, such as glasdegib, or that harness the immune system against leukemia, such as CD47 antibody magrolimab, PD1/PDL1 inhibitors pembrolizumab and nivolumab, TIM3 inhibitor sabatolimab, T-cell and NK-cell engagers. Cellular therapies are considered, even if a large trial is still pending for the feasibility of the approach. In this scenario, a brief overview of the mechanism of action of target agents is provided, particularly with respect to their biological mechanisms.

Expert opinion: Overall, this therapeutic armamentarium will constitute the basis for multimodal and personalized combinations that, in the idea of precision medicine, will enormously benefit elderly AML patients.

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Acute myeloid leukaemia - AML; elderly; target therapy; biological therapy; immunotherapy; natural killer (NK)

1. Introduction

In recent years, significant progress has been made in the treatment of Acute Myeloid Leukemia (AML). However, the median age at diagnosis is 68 years, and more than half of newly diagnosed patients are over 65 years[1], which results in a large proportion of patients that may be ineligible for conventional intensive treatment. Elderly patients present more frequently with unfavorable biological characteristics, such as adverse-risk molecular and cytogenetic features, resulting in highly refractory phenotypes to conventional chemotherapy [2,3]. Not least, the high burden of comorbidity present in some patients leads to unacceptable treatment-related mortality, limiting possible therapeutic choices [4].

The introduction of target molecules and immunological therapies is changing the treatment landscape of AML. The combination of bcl2-inhibitor venetoclax (VEN) and hypomethylating agents (HMAs) is now representing the standard of care for patients with newly diagnosed AML who are not eligible for intensive treatment [5,6]. Despite demonstrated high response rate and advantage of survival, relapse rates are still high, especially for patients harboring high-risk profiles such as complex karyotype and/or TP53 loss of function [7,8]. As already mentioned, those characteristics are not uncommon in older patients that still display a highly unfavorable prognosis even with this promising combination. Furthermore, tolerability and safety of the new molecules have yet to be defined, especially in patients that harbored other comorbidities and with polypharmacy in place, opening an only partially explored scenario in the clinical management of elderly patients.

In this review, we have summarized the current biological therapies available in the setting of elderly patients with AML, their clinical implications, possible future strategies, and a promising combination with the actual therapeutic backbone, as summarized in Figure 1.

2. Monoclonal antibodies

2.1. CD33

Gemtuzumab ozogamicin (GO) is the first-in-class anti-CD33 immuno-conjugate approved in AML: it is composed of a humanized immunoglobulin conjugated to the calicheamicin with anti-mitotic activity[9]. The first studies conducted on this molecule in combination with standard chemotherapy used dosages higher than 3 mg/m2 and a single-dose schedule, failing to demonstrate an efficacy and survival advantage in the face of an increase in toxicity [10,11].

Subsequent randomized trials demonstrated a survival advantage in low- and intermediate-risk patients with acceptable safety profiles and also in elderly patients [12,13]. In

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Article highlights

- Antibody-drug conjugates Gemtuzumab-ozogomicine and Pivekimab sunirine represent, to date, the most promising strategies to target CD33 and CD123 positive AML, respectively.
- A high number of T- and non-T-cell engagers have been developed, some promising results are appearing especially in terms of target and exposure optimizations.
- Non-canonical immune checkpoints are being explored in AML; the scientific community acquired promising early clinical results, especially addressing the macrophage-inactivator checkpoint SIRPa-CD47 and the terminal T-cell exhaustion molecule TIM3.
- Cellular therapies still represent the most fascinating way to acquire long-term control and eradicate AML, offering drug-dependent and -independent immune memory; in the field, waiting for the optimal design of chimeric-antigen receptors, non-edited allogeneic NK cells are being employed with preliminary effectiveness.
- Multimodal therapies that rely on biological activities and immunity may provide strong and less-toxic options to achieve long-term remission.

This box summarizes key points contained in the article.

particular, the open-label, phase 3 ALFA-0701 trial, conducted by the French group, evaluated the efficacy of addition to standard chemotherapy of low fractionated-dose GO in older adults with de-novo AML (dn-AML). The median age was 62 (range 58–66 years), in the experimental arm GO was given on days 1,4, and 7 during the '3 + 7' induction course at a 3 mg/m^2 dosage. Two-year OS and EFS results were significantly better in the GO arm vs control (53% vs 42%, p = 0.037 and 41% vs 17%, p = 0.0003 respectively). Persistent thrombocytopenia and delayed platelet recovery were higher in the GO arm nevertheless rate of treatment-related death and early mortality was similar in the two groups [12]. Similarly, the NCRI AML16 randomized trial demonstrated non-increased toxicity and premature mortality in older patients (median age 67, range 51–84) randomized to receive standard chemotherapy (daunorubicin and cytarabine or daunorubicin and clofarabine) with or without GO. In all analyzed groups, 3-years cumulative incidence of relapse was lower in the GO arm with associated better survival [13]. Interestingly in both the reported experience the advantage was highlighted in all age groups despite a lower rate of favorable cytogenetic profiles. Finally, a meta-analysis on more than 3300 patients confirmed the survival advantage of GO addition in patients with favorable (like core binding factor AML), and intermediate cytogenetics leading to re-approval of GO by the FDA in 2017 and by the EMA in 2018 for adult patients with dn-AML expressing CD33 [14].

To reduce GO's toxicity and enhance effectiveness, also in older patients, a combination with HMAs has been proposed. Azacytidine (AZA) is known to induce maturation of AML blasts and increased surface expression of CD33 leading to a greater GO uptake [15]. AZA also increases the expression of protein kinase Syk and SHP1 phosphatase; these molecules are critical for GO-mediated cytotoxic activity and their down-regulation represents a resistance mechanism [15,16].

A phase II study was conducted to evaluate the safety and effectiveness of AZA (75 mg/m^2 for 7 days) and GO (3 mg/m^2 on day 8) in 99 newly diagnosed AML patients with ages

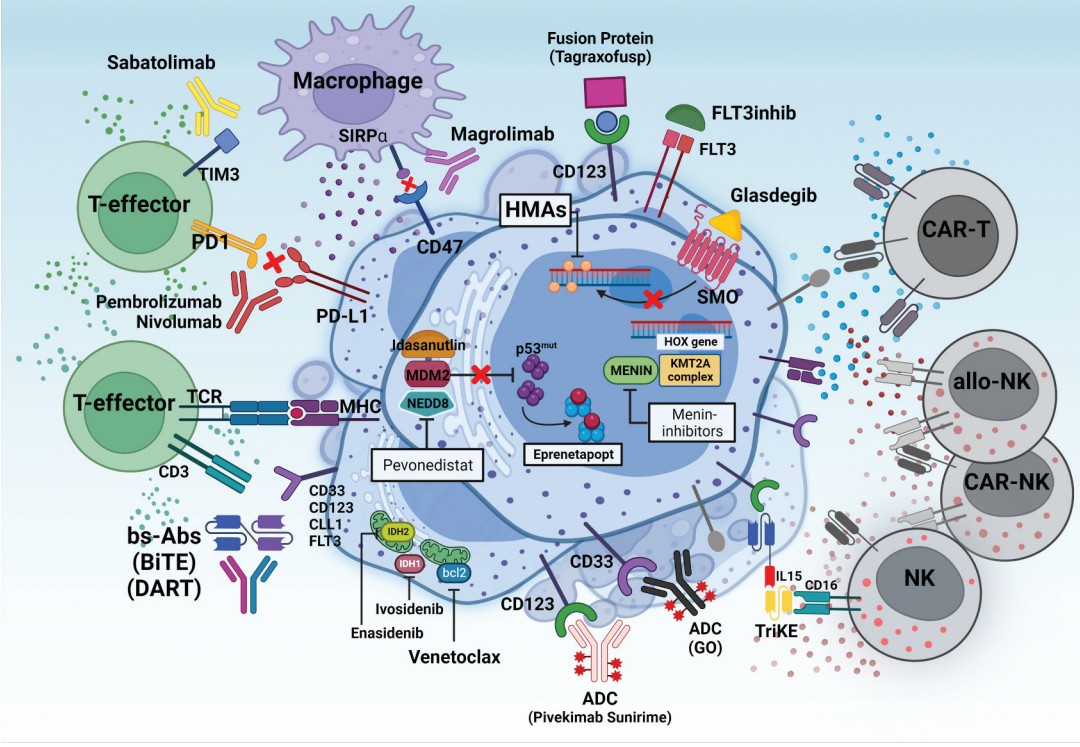

Figure 1. All the main therapeutic strategies presented in the text are summarized in this image. Adoptive cell therapy such as allogeneic NK cells, CAR-T and CAR-NK are represented in gray as they are still in the earliest stages of clinical experimentation.

ADC: Antibody drug conjugate; BCL-2, B-cell leukemia/lymphoma-2; BiTE: Bispecific T-cell engager; DART: Dual-Affinity Re-Targeting; FLT3, FMS-like tyrosine kinase 3; GO, gemtuzumab ozogamicin; HMAs: hypomethylating agents; IDH, isocitrate dehydrogenase; MDM2: murine double minute 2; MHC-1, major histocompatibility complex; PD1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TCR: T-cell receptor; TriKE: Trispecifica Killer Engagers. Created by G Ciotti with BioRender.com.

above 60 years old [17]. The population was stratified as 'Good-risk patients,' defined as those aged 60 to 69 years or performance status of 0–1, and 'Poor-risk patients,' defined as at least 70 years old and performance status of 2 or 3. The study failed to demonstrate a significant difference between the two groups in terms of CR/CR1 rate (44% in good-risk vs 35% in poor-risk, p = .38), RFS (8 months in good-risk vs 10 months in poor-risk, p = .78) and OS (11 months in both groups). Early mortality was also found not to be significantly different. The most frequent non-hematological AEs were febrile neutropenia and infections, no veno-occlusive disease (VOD) were reported. Despite response rate results comparable with historical data on conventional regimens [18], the advantage of this combination in terms of safety is unclear, especially in the good-risk group [17]. Since the treatment regimen includes only four maintenance cycles of azacitidine, prolongation of AZA therapy may be a safe strategy to enhance efficacy and improve survival.

Recently, a phase I/II study on the r/r-AML adult patient was published. The established maximum tolerated dose (MTD) defined in phase I part was azacitidine75 mg/m^2 daily for 6 days, followed by GO 6 mg/m^2 on days 7 and 21. The treatment regimen consisted of only 1 cycle of GO and AZA and the response rate was modest and comparable to those seen in GO used as a single agent with a higher dosage [19]. Conversely, no dose-limiting toxicity was seen in phase I, and no serious AE was specifically attributed to GO administration [20].

A retrospective study evaluated the safety and efficacy of GO+AZA combination in the r/r-AML patients not candidates for intensive treatment [21]. The overall response rate was 64.7% and in univariate analysis was not influenced by age (p > 0.05) or prognostic risk group (p > 0.05). To note, 10/17 patients achieve to perform allo-HSCT and no major AEs or VOD cases were reported [21].

Remaining in the setting of the elderly r/r-AML patients, coadministration of GO, AZA, and histone deacetylases (HDAC) inhibitors vorinostat showed an ORR of 42% at the MTD (vorinostat 400 mg/day from Days 1–9, AZA 75 mg/m^2 from Days 1–7, and GO 3 mg/m^2 on Days 4 and 8) with a manageable safety profile [22].

The discrepancy between reported experiences may be explained by extremely variable dose and administration schedules of GO in association with other compounds. The best regimen and combination with the best safety/efficacy ratio are yet to be defined.

Recently, the novel CD33 antibody-drug conjugate *vadastuximab talirine* (SGN-CD33A) showed as single agent ORR of 54%, to note almost 50% of patients with underlying myelodysplasia managed to achieve a response [23]. Following this promising result, combination with HMAs were explored as front-line therapy for elderly patient (median age 75 years) with good effectiveness in terms of response: 73% of CR/Cri rate and 43% of responding patients were MRD-negative by flow-cytometry. Remarkably, most of the population had intermediate or high-risk features. Most common grade 3 or above AEs, excluding hematological, were lung infections and febrile neutropenia [24]. Despite promising results, the randomized phase III trial comparing vadastuximab + decitabine/azacitidine with placebo + decitabine/azacitidine (CASCADE study NCT02785900) was terminated prematurely due to safety issues; specifically, a higher rate of deaths, including fatal infection, in the experimental arm [25].

Actinium-lintuzumab (*225Ac-lintuzumab*) is a humanized anti-CD33 radio-conjugated antibody, with proven single-agent activity in AMLr/r at clinical stage[26]. Inducing dramatic DNA double-strand breaks, 225Ac-lintuzumab seems to restore the expression of antiapoptotic proteins such as MCL-1, leading to re-sensitization of AML-cells to venetoclax [27]. Resistance is an emerging problem in the treatment of AML, especially in the elderly setting where biological characteristics confer primary and precocious resistance to current therapy. Overexpression of antiapoptotic BCL-2 family effectors, such as MCL-1, has been proposed as a primary and secondary mechanism of resistance to venetoclax [28]. Down-regulation of these molecules could represent a strategy to prolong the response to current therapies and re-sensitize cancer cells. Preliminary results of the combination of 225Ac-lintuzumab and VEN in three patients were recently presented at ASH. One patient was 75 years old and no dose-limiting toxicities (DLT) or non-hematologic adverse events (AEs) greater than Grade 2 were noticed [29]. The real safety and efficacy of this compound and its associations are yet to be established, phase I/II trial (NCT03867682) is still recruiting.

2.2. CD123

The alfa-subunit of the interleukin-3 (IL-3) receptor-chain α (IL3RA or CD123) is strongly expressed in myeloid blast and CD34+/CD38- leukemia stem cells [30–32]. Recent evidence showed as CD123 is highly expressed on AML-NPM1 mutated cells and particularly NPM1/FLT3 double-mutated AML [30]. Given its expression both at the level of the stem compartment and in cells with granulocytic and monocytic differentiation, IL3RA is becoming a promising target in myeloid malignancies. However, CD123 expression is not limited to the hematopoietic system, rising concern about the possible toxic off-target effects, especially in elderly and multi-treated settings [33].

Tagraxofusp (SL-401) is a recombinant fusion protein composed of IL3 fused with a truncated diphtheria toxin with promising activities on CD123 positive hematological malignancies [34–36]. In December 2018, the Food and Drugs Administration (FDA) approved tagraxofusp for patients affected by blastic plasmacytoid dendritic cell neoplasm (BPDCN) of all ages following the outstanding results of a non-randomized, open-label, NCT02113982 multicenter trial [37]. In a population of 47 patients, the median age was 70 years and almost all patients were treated with a dosage of 12 μg/kg. Complete response and complete clinical response reached 72% in previously untreated patients with a non-negligible number of patients bridged to transplant. To note, almost 80% of patients experienced AEs of grade 3 or higher. Serious concerns were hepatic toxicity, thrombocytopenia, and vascular leak syndrome, the latter occurred in 18% of patients, all in the first cycle [37]. Since tagraxofusp showed preclinical efficacy in AML samples, clinical studies were conducted also in this setting [32]. A phase I trial on 45 r/r AML

patients showed a maximum tolerated dose (MTD) of 12,5 µg/kg and major AEs were reversible transaminitis, fever, and vascular leak syndrome with associated hypoalbuminemia, liquid retains, and hypotension. Almost 80% of the population was > 60 years and clinical efficacy was low [36]. In this study the few responses seemed to be related to a lower burden of disease at the start of treatment; since CD123 seems to be expressed on LSCs, the use of tagraxofusp in remission may be a valid strategy to prolong response in patients where intensive consolidation strategies are not an option. The NCT02270463 phase I/II trial was conducted in AML patients in CR1 or CR2 who were not candidates for allo-HSCT or at high-risk of relapse. Safety was acceptable with no DLT and MTD at the highest tested dose of 12 µg/kg, the actual effectiveness is unknown as the data are not yet published [38].

Loss or reduction of diphthamide synthesis pathway expression due to downregulation of DPH1 and subsequent insensitivity to diphtheria toxin is been demonstrated as a mechanism of resistance to tagraxofusp that is partially mediated by altered DNA-methylation [39]. Combination with AZA may overcome this resistance pathway and enhance SL-401 effectiveness. Combination with azacytidine alone or with AZA + VEN is currently under investigation (NCT03113643) and the trial is in recruiting phase.

Despite preclinical and initial promising results, several studies failed to demonstrate a clinical advantage of other unconjugated CD123-directed compounds [40,41]. Phase 3 trial on decitabine plus talacotuzumab versus decitabine alone in patients ineligible for intensive chemotherapy (NCT02472145) showed limited efficacy at the expense of increased toxicity [42].

Pivekimab Sunirine (IMGN632) is a novel CD123-targeting antibody-drug conjugated (ADC) with a highly DNA alkylating payload that has shown potent preclinical activity in AML models, including those with known multidrug resistance and poor prognostic features [43]. In phase I/II clinical trial IMGN632 was tested as a single agent in 74 patients, 67 with r/r AML and 7 with r/r BPDCN, in two dose schedules: one infusion on day 1 or fractionated dosing on days 1, 4, and 8. The median age of all population was 69 years, to note 70% of evaluable AML patients showed high-risk assets and 27% were primary refractory. Despite extremely unfavorable features, ORR was 20% in the AML population (32% in non-secondary AML) with a recommended dose of 0.045 mg/kg given on day 1 every 21 days. Of the seven BPDCN patients, 43% obtained an objective response (CR, CRi, PR), and two other patients reached stable disease. Adverse events were mainly febrile neutropenia and diarrhea with a 30-day mortality of 8%; two episodes of VOD occurred at dose levels ≥0.18 mg/kg and no sign of transaminitis or mayor cytopenia occurred with lower doses [44]. The safety profile, therefore, appears to be acceptable in pre-treated and elderly patients. Combination with AZA and/or venetoclax is currently under evaluation in the phase I/II trial (NCT04086264). Preliminary results on 35 elderly r/r AML patients showed a manageable toxicity profile: rates of febrile neutropenia and infections were consistent with those found in r/r AML patients treated with standard AZA + VEN regimen [45]. The rate of composite CR (cCR) seems promising in the higher intensity dose cohort (cCR of 40%) and in VEN naïve patients (cCR 60%); interestingly, patients with FLT3 mutations showed a CCR of 70%. New triplets including IMGN362 appear to be promising in AML and may represent a strategy also in older patients. Its role in MRD-eradication is also under evaluation in clinical trial (NCT04401748) [45,46].

3. Effector cell engagers

Rapid and specific activation of immune effector cells can lead to more effective and lasting cytotoxic responses. For that purpose, bispecific antibodies (bsAbs) were developed to simultaneously bind an effector cell (T-cell, NK-cell, and macrophage) to a specific target expressed on the cancer cell surface. Unlike the adoptive cell strategy, bsAbs doesn't require in vivo expansion or ex vivo manipulation; moreover, a direct and effective link between the effector and the target cell can bypass immune escape mechanisms put in place by tumor cells [47]. Following the outstanding example of blinatumomab for B-cell malignancies, different types of constructs and technology have been investigated in AML with different targets.

The bispecific T-cell engager (BiTE®) *AMG 330* was developed to redirect effective T cells against CD33-expressing AML blast showing robust preclinical activity[48,49]. In the phase 1 dose-escalation trial (NCT02520427) on 55 r/rAML patients (median age 58 years, range 18–80), AMG 330 showed quite modest results in terms of efficacy [50]. Updated results were recently published by Ravandi et al, on 42 evaluable patients, eight were considered responders. Preliminary data showed a correlation between higher exposure to AMG 333 and lower tumor burden at treatment start [51]. Cytokine release syndrome was found in almost 70% of patients and was generally reversible and dose-dependent. The safety profile was generally favorable. Recent evidence has demonstrated that effector T cell engagers induce a proinflammatory condition with possible upregulation of immune checkpoints on target and effector cells. Cytokines-inducted overexpression of immune regulators such as PD-1/PD-L1 can represent a mechanism of resistance to the action of bsAbs and at the same time a rationale for combination strategy with immune checkpoint molecules [52]. A phase I clinical trial (KEYNOTE-613, NCT04478695) is evaluating AMG 330 and pembrolizumab association in r/rAML patients, results are awaited but study was ended by decision of the sponsor. Most of these molecules need to be infused continuously requiring long hospitalizations, several efforts are being made to simplify the methods of administration. *AMG 673 (Emerfetamab)* is an interesting new anti-CD33xCD3 BiTe with a weekly infusion schedule, due to its increased half-life. In the phase 1 study (NCT03224819), the rate of AEs grade ≥ 3 was 50%, the most common represented by transaminitis (17%), CRS (17%), and hematological toxicity. Overall, toxicity was acceptable, and treatment was well tolerated, but conclusive data on efficacy are still missing [53]. Similarly, *AMV564* is a novel anti-CD33xCD3 Tandem Diabody with a longer half-life and possible subcutaneous administration, under investigation in both AML as a single agent and solid tumor in association with pembrolizumab [54,55].

In addition to CD33, other antigens have been considered as bsAbs targets. *Flotetuzumab* (MGD006) is a dual-affinity retargeting (DART) antibody that binds to CD3 T-lymphocytes and CD123 target antigen (CD123 x CD3) promoting an immunological synapse and redirecting polyclonal lymphocytes as effectors against the tumor. Preclinical studies have shown significant antitumor activity against CD123+ AML blasts [56,57]. Recently, the results of a phase 1/2 study (NCT02152956) were published in which flotetuzumab was used in 88 patients (median age 64 years, range 29 to 84) with r/r AML: 42 patients had entered a dose-finding phase 1 and a total of 46 patients received the recommended phase 2 dose (RP2D) of 500 ng/kg/day as a continuous 28 days infusion [58]. Almost the entire population treated at the RP2D experienced CRS and infusion-related reactions (IRR), generally grade 1–2. In most cases, these complications were effectively contained with adequate steroid therapy, timely use of tocilizumab, and/or interruptions of the infusion [59]. No association was seen between the grade of IRR/CRS and effectiveness or disease burden [59,60]. In terms of efficacy, ORR was 13.6%; a higher ORR of 24% (CR 18%) was observed in patients who received the RP2D of 500 ng/kg/day or higher. Interestingly, in primary refractory and early relapsed (within six months) patients, treatment with RP2D dose was associated with a CR/CRi rate of 30%. Among patients who achieved a response, the median OS was 10.2 months (range 1.9–27) with a 6- and 12-month probability of survival of 75% and 50%, respectively. The response rate among patients with intermediate-adverse risk (according to ELN 2017) was 28.6% (8 of 28 patients), while it was 40% among sAML (4 of 10 patients). Fewer previous lines of therapy were associated with a greater likelihood of response to flotetuzumab [58]. The greater efficacy of flotetuzumab in primary refractory/early relapsed patients has been attributed to the prevalent immune-enriched and IFN-γ-dominant tumor microenvironment highlighted in this setting. These patients also show an exhausted phenotype signature and increased PD-L1 expression suggesting that association with immune checkpoint inhibitors (ICIs) may represent a future strategy to enhance response to flotetuzumab immunotherapy [61]. Altogether, these results suggest that flotetuzumab may be further explored in primary refractory, early relapsed, and TP53 mutated patients [62,63], even if effectiveness is limited and the compound is poorly clinically manageable. NCT02152956 study has completed recruitment, and final results on safety and efficacy are pending; however, the programmed expanded access program was withdrawn prematurely.

Vibecotamab (XmAb14045) is a new potent bsAb directed on CD123xCD3 with intermitted dosage schedule. In a phase I study on 104 patients, no MTD has been identified; 66% of patients experienced CRS symptoms, mostly grade 1–2, infusion-related reactions were mild and generally reversible and no particular hematological toxicity was seen. Similarly to previous reported data, the response seems to be correlated to lower disease burden and higher dose level with no responders patient at dosage < 0.75 μg/kg [64]. Overall, considering the usually long times infusion schedule and the need for hospitalization due to the high rate of IRR/CRS, some concerns remain about the use of bsAbs in unfit and elderly subjects.

Is indeed true that immunotherapy in AML still suffers from the lack of an optimal target. A novel promising molecular target is the C-type lectin domain family 12 member A (CLEC12A), also known as CLL-1 or CD371 [65]. CLL-1 is widely expressed AML and can be detected in more than 90% of blast and also leukemic stem cells (LSCs). Interestingly, CLEC12A antigen is not expressed in other hematopoietic lineage or non-malignant hematopoietic stem cells making it an attractive target for immunotherapy strategies. Several clinical agents against CLEC12A were developed and preclinical data suggest that they might express higher specify toward disease and consequent better safety profile [66,67]. A phase 1 trial on elderly AML patients, is exploring *Tepoditamab* (MCLA-117), a CLEC12AxCD3 BiTE, which is still ongoing; preliminary results showed acceptable toxicity with most events being reversible [68]. Optimization of the schedule may be important to avoid T cell exhaustion.

An attractive immunotherapy approach is the Tri-Specific Killer Engager (TriKE) platform. Natural killer-based therapy seems to have a better safety profile than T-cell engager, TriKE agents are new, trispecific nanobody-constructs, designed to bridge natural killer cells to cancer cells. These molecules are composed of three functional domains: a humanized camelid-derived nanobody able to bind CD16 on NK cells, a human IL-15 molecule that acts as costimulatory enhancing effectiveness, expansion, and survival of NK cells, and a single-chain variable fragment directed against a specific target [69]. Several targets are being tested in the preclinical stage: GTB-3650 (CD16xIL-15xCD33) is currently in phase I/II clinical trial for patients with high-risk myelodysplastic syndromes and r/rAML (NCT03214666). Preliminary results were recently presented at ASH-2020 and no infusion-related reactions or DLT have been observed [70]. CLEC12A-directed TriKe are also under develop to target AML blast and LSCs. Results on in-vivo models are promising [71].

The presence of a co-stimulator (IL-15) within the construct allows to avoid cytokines systemic administration, displaying a limited risk of CRS. Several studies are still needed to define the effective clinical role of these compounds and the best target; however, first data on their safety profile make them an attractive therapeutic strategy even in elderly patients.

Monoclonal antibodies, ADCs and cell engagers with clinical activity in elderly AML are summarized in Table 1.

4. Immune checkpoint inhibitors

Immune checkpoint inhibitors (ICIs), mostly targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death receptor1 (PD-1), have recently gained attention for their outstanding results in solid tumor treatment. However, their effectiveness on hematological malignancies is still unsatisfactory except for Hodgkin Lymphoma, Primary Mediastinal B-cell Lymphoma, and other few exceptions. As already mentioned, several evidence demonstrates a T-cell exhaustion phenotype in AML patients and upregulation of PD-1 and other immune regulators, especially at the time of progression and after multiple lines of treatment. Moreover, overexpression of B7 family ligands (such as PD-L1 and PD-L2) on AML blast cells might be responsible for HMAs resistance [72,73].

Table 1. Monoclonal-antibodies with clinical activity in elderly AML. Toxicity was reported in order of frequency.

Target/Type	Study	Combination	Pop.	Efficacy/Outcome	Toxicity	Ref.
ADC anti-CD33						
Gemtuzumab-ozogamicin[1]	Phase 3	+ '3 + 7'	ND N = 139	No difference in response rate GO arm vs control: • 2 yrs EFS: 41% vs 17% • 2 yrs OS: 53% vs 42%	Persistent thrombocytopenia Prolonged myelosuppression Higher grade ≥3 AEs	[12]
	Phase 3	+ sCHT	ND N = 559	No difference in response rate GO arm vs control: • 3 yrs RFS: 21% vs 16% • 3 yrs OS: 25% v 20%	No additional toxicity Low rate of VOD (mostly arising during sepsis)	[13]
	Phase 2	+ AZA	ND N = 99	OS 11 mo, CR 35%-41%	Febrile neutropenia Consistent with AZA alone	[17]
	Phase 1/2	+ AZA	R/R N = 50	No advantage vs GO single-agent	Consistent with AZA alone No VOD events	[19,20]
	Phase 1/2	+ AZA + VORINOSTAT	R/R N = 52	ORR of 42% at the MTD	Febrile neutropenia Infections/Sepsis	[22]
Vadastuximab talirine	Phase 3	+ HMAs	ND N = 117	Terminated by Sponsor due safety issue	Higher rate of deaths, including fatal infection	[25]
Radioconjugate anti-CD33						
Lintuzumab-Ac225	Phase 1	+ sCHT	R/R N = 15	cCR 67%	Febrile neutropenia Infection Maculopapular rash	[26]
	Phase 1/2	+ VEN	R/R	Still recruiting (data on the first 3 enrolled patients)	No DLT No non-hematologic AEs ≥2	[29]
BiTE – CD33xCD3						
AMG-330	Phase 1	Alone	R/R N = 55	ORR 19%	CRS (reversible and dose-dependent)	[50,51]
Emerfetamab	Phase 1	Alone	R/R N = 30	Missing	Hematological toxicity Transaminitis CRS	[53]
Fusion protein anti-CD123						
Tagrazofusp[2]	Phase 1/2	Alone	R/R N = 45	Low efficacy Better results on low-burden leukemia	CLS Fever Transaminitis Hypoalbuminemia (all AEs mostly reversible)	[36]
ADC anti-CD123						

(*Continued*)

Table 1. (Continued).

Target/Type	Study	Combination	Pop.	Efficacy/Outcome	Toxicity	Ref.
Pivekimab sunirine	Phase 1/2	Alone	R/R N = 67	ORR 20%	Febrile neutropenia Diarrhea	[44]
	Phase 1/2	+ AZA + VEN	R/R N= 35	All: cCR of 40%; VEN naïve patients: cCR 60%; FLT3mut: cCR of 70%	Consistent with AZA+VEN	[45]
DART – CD123xCD3						
Flotetuzumab	Phase 1/2	Alone	R/R N = 88	ORR: 13.6%; CR/CRi 30% in refractory/early relapsed In responders: OS of 10.2 mo	CRS IRR (grade 1–2)	[58,59]
Vibecotamab	Phase 1	Alone	R/R N = 104	ORR 14%	CRS No myelosuppression	[64]
BiTE – CLEC12AxCD3						
Tepoditamab	Phase 1	Alone	R/R N = 50	Missing	No DLT Fever CRS Transaminitis (all AEs mostly reversible)	[68]

[1]Approved by FDA and EMA in combination with daunorubicin and cytarabine and as a monotherapy for adult patients with ND, de-novo, CD33-positive AML.
[2]Approved by FDA (for adults and pediatrics) and EMA (only adults) as monotherapy for the first-line treatment of blastic plasmacytoid dendritic cell neoplasm (BPDCN).
ADC: antibody-drug conjugate; AEs: adverse events; AZA: azacitidine; BiTE: Bispecific T-Cell Engager; CLS: capillary leak syndrome; CRS: cytokines release syndrome; CR/cCR: complete response/composite complete response; DART: dual-affinity re-targeting antibody; DLT: dose-limiting toxicities; EMA: European Medicines Agency; FDA: Food and Drugs Administraton; FLT3: FMS-like tyrosine kinase 3; FLT3i: FLT3 – inhibitor; HMAs: Hypomethylating agents; IRR: infusion-related reaction; MTD: maximum tolerated dose; N: number of patients; ND: newly diagnose; OS: overall survival; ORR: overall response rate; R/R: relapsed/refractory; sCHT: standard chemotherapy; VEN: venetoclax; VOD: veno-occlusive disease.

The innate immune system also plays a critical role in AML immune-escaping. Leukemia cells overexpose of CD47 on their surface leading to inhibition of macrophage-mediated phagocytosis through CD47-Signal Regulatory Protein alpha (SIRPα) interaction [74,75]. Given the rationale, clinical investigation on ICIs in AML, especially in combination therapy, is still worth the effort and several trials are ongoing.

4.1. PD-L1 inhibitors

In elderly (over 65 years) AML patients, the addition of *durvalumab* (anti-PD-L1) to AZA did not show any improvement in effectiveness over AZA single-agent in the first-line setting [76]. Similarly, *avelumab* (anti-PD-L1) and HMA combination failed to demonstrate a clinical advantage in the r/rAML population, despite a good safety profile [77]. Both studies highlighted a PD-L2 overexpression on AML cells at baseline and during treatment. Moreover, the hypomethylation promoted by combination therapy, leads to upregulation of PD-L1 on differentiated cells, such as monocytes, suggesting that the AML microenvironment might act as a suppressor of the immune response versus leukemia. Altogether, the latter results might depict novel AML cells immune escape mechanisms. Despite acceptable toxicities, PD-L1 inhibition doesn't seem to be the better strategy in AML, given the lack of immunological changes; PD-1/PD-L1 inhibition combination treatment has been proposed and evaluation studies are underway.

4.2. PD-1 and CTLA-4 inhibitors

Nivolumab (NIVO) and AZA combination was evaluated in a phase 2 trial on 70 r/rAML patients; the median age was 70 years (range 22–90) and the median number of prior lines was two. ORR was higher in HMAs naïve patients (52% vs 33%) and survival advantages over historical control were modest: OS of 6.3 months vs 4.6 months ($p = 0.013$) [78]. Immune-related AEs, grade ≥ 2, were reported in 23% of patients, and slightly more than 50% were pneumonitis. Almost 90% of episodes responded to steroids and were reversible. The majority of events occurred within the first 8 weeks of treatment and the rate of NIVO discontinuation was 13%. Subsequent analysis of non-responder patients demonstrated a CTLA-4 upregulation in the T-cell compartment and lower CD4+ and CD8 + T effective cells, setting the stage for a double inhibition (CTLA-4 and PD-1) strategy [78]. *Ipilimumab* (anti-CTLA-4) showed only modest activity as a single agent in r/rAML, with more competitive results in the post-transplant setting [79]. This can be explained by increased exposure of inhibitory checkpoint receptors on the donor-derived T-cells in patients who relapsed after allo-HSCT [80]. Ipilimumab in combination with NIVO and AZA was tested in a second cohort of the previously reported study (NCT02397720). Thirty-one r/rAML patients were treated with this triple combination therapy and the results compare favorably to AZA plus NIVO. On 24 evaluable patients, CR/Cri rate was 36%, with a median OS of 10,5 months and 1-year survival of 45%. Immune-related AEs greater than grade 2 were higher in this cohort over AZA + NIVO (25% vs 11%) but no death related to immune toxicity was reported [81]. NIVO was also investigated in the front-line setting in phase 2, a single-arm study where NIVO was combined with standard idarubicin plus cytarabine regimen. The enrolled population (N = 44) was younger than 60 years or deemed fit for intensive treatment (median age 42, range 22–66), and 50% were considered high risk according to ELN 2017. Composite CR rate was 78% with a high rate of MRD negative status by flow cytometry. Median OS was 15,5 months and the treatment was overall well tolerated with the most common adverse event being febrile neutropenia. Interestingly, the survival of a patient who underwent allo-HSCT (N = 43%) did not differ from that one observed in patients who continued treatment [82]. Considering the encouraging results, a phase 2 study explored NIVO as maintenance therapy for high-risk AML; however, the study failed to demonstrate an advantage in MRD eradication and in the prolongation of remission [83].

Pembrolizumab (PEM) plus HMAs combination was also explored. PEM plus AZA was evaluated in a phase 2 trial in r/rAML (cohort 1) and ≥65 years ndAML (cohort 2) patients. While in cohort 1 the efficacy was poor (CR/CRi rate: 14% and median OS: 10,8 months), response in ndAML was promising with a CR/CRi rate of 47% with a median OS of 13,1 months (not reached in CR/CRi patients). Remarkably PEM and AZA combination was safe and tolerate in both cohorts [84]. Similar results regarding safety and effectiveness were seen in PEM plus 10 days of decitabine evaluation trial in r/rAML [85]. In another study on 37 r/rAML patients, high-dose cytarabine was followed by PEM administration as salvage therapy. To note, most of the population was either refractory or early-relapsed, and more than half showed high-risk biological features. Composite CR rate in all populations was 38%, with higher response in first-salvage (CR/CRi rate: 46%) and younger (< 60 years, CR/CR1 rate: 45%) patients [86]. Nine of the responding patients were bridged to allo-HSCT. Prior exposure to PEM doesn't seem to correlate to a higher risk of GVHD or other post-transplant complications [87]. Two randomized phase-2 studies are ongoing to assess the effectiveness of PEM in eradicating MRD and ameliorating survival in ndAML patients, in combination with intensive chemotherapy (BLAST MRD AML-1, NCT04214249), and with AZA plus VEN (BLAST MRD AML-2, NCT04284787). The results are pending [88,89].

4.3. CD47/SIRPα blocking agents

Magrolimab (5F9) a first-in-class monoclonal humanized antibody directed against human CD47. Blocking CD47-SIRPα interaction, magrolimab restores macrophage-mediated phagocytosis on leukemia cells, inhibiting the 'don't eat me' signal on macrophages [90]. Proven his single-agent activity in preclinical models and clinical trials (CAMELLIA trial), magrolimab was investigated together with AZA in a phase 1b trial on previously untreated AML unfit patients and intermediate/high-risk MDS (NCT03248479). On preliminary results, treatment was well tolerated (median age: 72 years) and possible greater efficacy in TP53-mutated patients was observed with 75% of the AML harboring the mutation achieving a composite CR [91,92]. A further cohort was then designed to investigate this association in front-line

treatment of TP53 mutated AML. Magrolimab plus AZA was administrated in 72 AML patients. Major AEs were febrile neutropenia (45.8%), peripheral edema (30.6%), and hematological toxicity. Anemia was reposted in almost 30% of the treated population with 60-day mortalities of 18%. In evaluable patients, ORR was 48,6% (CR/CRi 41,6%) with a median time to response of 2 months. The Median OS of 10.8 months compared favorably with historical data [93]. Mature erythrocytes express CD47 and phagocytic mediators on their surface as inhibitory mechanisms against red cells phagocytosis by splenic macrophages. These pro-phagocytic signals are particularly expressed on older erythrocytes but not on reticulocytes. Several concerns have arisen about the risk of hemolytic anemia during magrolimab treatment. The use of a 'priming-dose' strategy with progressive exposure and dose escalation may induce compensatory reticulocytosis and reduce on-target anemia [94,95]. A phase 3, randomized trial with magrolimab plus AZA in untreated TP53-mutated AML patients is ongoing. Magrolimab was also investigated with AZA + VEN in both r/r and elderly ndAML patients. In this phase 1b/2 study, 38 patients were enrolled; the median age was 70 years and 13/38 patients were r/r to prior VEN exposure. No DLT was reported and in RP2D magrolimab was administrated with a priming-dose schedule (first cycle: 1 mg/kg on days 1 and 4, 15 mg/kg on day 8, and 30 mg/kg on day 11; subsequent doses: 30 mg/kg). Nearly half of the patients harbored TP53 mutations or other high-risk features. In ndAML patients (N = 17) ORR was 100% with a CR/CRi rate of 94%. As expected, response rate in r/rAML population was lower; nevertheless, r/rAML VEN-naïve patients (N = 8) still display competitive response with a CR/CRi of 63% vs 27% of VEN-failure patients. In patients who relapsed or were refractory after VEN-based therapy, survival is still dismal with a median OS of 3.1 months. Regarding toxicity, the most frequent grade ≥3 non-hematologic AEs were febrile neutropenia (32%) and pneumonia. The anemia was more pronounced during the first treatment administration but it was overall well tolerated and manageable [96]. Two major randomized phase-3 trials are in the process of recruiting with as primary outcome CR rate and survival: magrolimab plus AZA vs VEN plus AZA or 3 + 7 according to physician's choice, in TP53-mutant, untreated AML patients (ENHANCE-2, NCT04778397) and VEN + AZA plus magrolimab versus placebo in previously untreated AML patients who are ineligible for intensive chemotherapy (ENHANCE-3, NCT05079230) [97]. Results will help clarify the role of magrolimab in high-risk, TP53 mutated patients.

Trying to improve tolerance and reduce CD47-inhibition on normal cells, *Evorpacept* (ALX148) was generated to enhance antitumor activity without increasing toxicity. Evorpacept is a CD47-directed fusion protein composed of a SIRPα domain for high-affinity CD47-blocking and an inactive immunoglobulin Fc domain. His particular structure prevents the engagement of the Fc-gamma receptors, sparing normal cells from phagocytosis [98]. A similar phase 1b/2 study (ASPEN-5, NCT04755244) is evaluating the safety and efficacy of evorpacept plus AZA + VEN in AML patients.

4.4. T-cell immunoglobulin domain and mucin domain-3 (TIM-3) inhibition

TIM-3 is expressed on IFNγ-producing immune cells (like T cells, T regulatory cells, and antigen-presenting cells) and acts as a co-inhibitory receptor, suppressing immune cell response [99]. This immune checkpoint is proven to be expressed on blasts and LSCs surfaces but not on normal hematopoiesis. Autocrine production of Galectin-9 by leukemia cells mediated TIM-3 dependent LSC self-renewal, conversely, galectin-9/TIM-3 interaction on T effective cells leads to cell death, dampening T-cell mediate immune response versus leukemia [100]. TIM-3 inhibition is therefore a possible new therapeutic strategy in AML.

Sabatolimab (MBG453) is a novel monoclonal antibody directed at TIM-3. In a phase 1b study sabatolimab in combination with HMAs showed an ORR of 40% (CR/CRi 30%) in 48 unfit ndAML with a median duration of response (DOR) of 12.6 months. The combination was safe, and AEs consistent with those observed in HMA alone. Interestingly, patients with high-risk biological features achieved an ORR of 53,8% [101]. The STIMULUS trial program was designed to evaluate sabatolimab in different settings (AML, high-risk MDS, and chronic myelomonocytic leukemia), in different combinations [102]. Preliminary results of the STIMULUS-AML1 phase 2 trial (NCT04150029) were recently presented at the EHA meeting. In this clinical trial, sabatolimab was investigated with AZA + VEN, data on the first 18 patients showed a safety profile comparable to AZA plus VEN therapy with hematological toxicity and febrile neutropenia as the most frequent events. Sabatolimab was tested in 2 dose levels (400 mg and 800 mg) and the expansion cohort at 800 mg dose is ongoing. Regarding efficacy, 13/18 patients (including both cohorts) were reported to reach the CR status. Still, the data are too premature for an appropriate evaluation in terms of efficacy [103].

5. Adoptive cell therapies

5.1. Natural killers (NK) cells

Natural Killer (NK) cells are leucocytes involved in both innate immune systems and acquired immunity. Their meaningful cytotoxic activity is proven to be effective both against cells infected with microorganisms (especially viruses) and dysfunctional cells origin of cancerous processes. The uniqueness of NK cells lies in their marked ability to recognize cellular stress and the high speed of reaction to cellular dysfunction [104]. Their activities are finely regulated by an extensive repertoire of multiple inhibitory and activating receptors exposed on the cell surface. Killer immunoglobulin-like receptors (KIRs) can bind MHC class I and are the best known and the most extensively characterized in their inhibitory and activating capacities; these receptors exhibit a high level of polymorphism which results in an extreme variability of expression of the KIR gene family between different individuals [105]. The allo-reactions mediated by the KIR-KIR ligand (KIR-L) mismatch between donor and recipient enhance graft versus leukemia (GVL) in haploidentical

transplantation and are proven to significantly impact the clinical outcome of AML patients [106–108]. Alloreactive donors' NK cells can also reduce graft-versus-host disease (GvHD) through the elimination of the recipient's antigen-presenting cells and T cells. Several studies show how a more rapid NK cell recovery after transplantation and NK-alloreactive donor grafts can prevent early relapse and prolong survival [109,110]. Given their critical role in the immune system, in recent years several NK cell-based strategies are under development in AML; in this review, we're going to focus on applications in non-transplantation settings.

One of the first experiences of adoptive NK cell transfer in non-transplanted AML patients was conducted by Miller et al. on 19 r/r AML patients: all populations were considered unfit for allo-HSCT and underwent intensive immune suppression regimen with high-dose cyclophosphamide (CTX) and fludarabine (FLU) prior infusion, lower intensity regimens showed no expansion of donor cells. Haploidentical NK cells were CD3 depleted and stimulated with IL-2 administration both in vivo and ex vivo. All procedure tourn out safe and effective in terms of NK expansion with the remarkable induction of CR in 5/19 patients enrolled [111]. In a pediatric experience, ten patients in first CR were exposed to low dose CTX and standard FLU followed by KIR-KIR-L mismatched and CD3-depleted NK cells, and IL-2 in vivo administration. Engraftment was transient (median 10 days) but in vivo expansion was seen in all patients. Toxicity was more than acceptable with no GVHD reported and all patients maintained the CR status. However, the population was all MRD-negative at the time of infusion and only one patient showed prolonged allogeneic NK persistence [112]. Curti et al investigate the safety and feasibility of haploidentical NK-cell transfer in a cohort of high-risk, and non-transplant eligible AML patients [113]. All patients with a suitable donor received high-dose CTX and FLU as immunosuppressive therapy followed by subcutaneous IL-2 administration for 2 weeks (6 doses in total) after NK infusion; no GVHD prophylaxis was provided. This study suggested a better efficacy of this strategy as maintenance therapy in CR patients or as a pre-emptive approach in early molecular relapses leading to a subsequent validation study [113,114]. The same Italian group explored haploidentical mismatched NK-cell in 17 elderly (median age 64 years) AML patients, unfit for allo-HSCT in the first morphological CR [115]. After a median FU of 55.5 months, 50% were disease-free; patients with positive MRD reached negativity and 2/3 of relapsed patients showed a prolonged CR phase before relapsing. Interestingly, a higher functional dose of infused alloreactive NK-cells (>2 × 105/kg) seems to improve OS and DFS and is associated with a lower number of Tregs [114,115]. No GVHD was observed and hematological toxicity did not differ from that seen with conventional chemotherapy regimens as well as infectious events related to aplasia [114]. These data show good applicability of NK-derived immunotherapy in elderly subjects, however, the need for immune suppressive therapy and the related AEs can still limit its feasibility for chemotherapy-fit patients.

Interestingly, NK cell is proven to exhibit adaptive and memory-like ability [116,117]. Memory-like NK (ML-NK) cells can be obtained following exposure to different antigens or stimulation of synergistic cytokine combinations such as IL-12, IL-15, and IL-18 [117,118]. Ex vivo exposure of alloreactive NK cells to this cytokine mix would lead to the acquisition of the memory-like phenotype with consequent prolonged and increased cytotoxicity against leukemia (CIML-NK: cytokine-induced memory-like NK). This approach may overcome the need for in vivo stimulation and lead to better leukemia control. Further studies are needed to explore ML-NK in non-transplant settings and elderly patients.

Taken together, data on adoptive NK-cell therapies seem to highlight an extremely low risk of GVHD, rare cytokine-related complications (such as CRS), and hematological toxicity consistent with the standard regimen [119]. Unsolved problems are the right expansion and activation technique (in vivo or ex vivo) for these cells such as administration schedule.

Several chimeric antigen receptors (CAR)-engineered NK are currently being tested in multiple clinical trials on hematological malignancy against different epitopes [120,121]. CIML-NK can be also used in CAR technology to enhance its efficacy [122]. Most of these studies are still in the preclinical phase [123] but preliminary data results on CD33-CAR-NK-92 cells showed a safe profile after salvage chemotherapy in relapsed AML patients; since irradiation of CAR NK-92 cells before infusion was necessary due to cell line origin, data on efficacy cannot be extrapolated [121]. CAR NK may represent an advantage over CAR T cell therapy due to their safer profile with low risk of CRS; they can be obtained from allogeneic donors without increased incidence of GVHD. Furthermore, the possibility of an 'off-the-shelf' NK cell-based may overcome current adoptive and CAR-T cell therapy issues, even in elderly patients.

5.2. CAR-T: find the right target

Currently, most of the approved CAR-T cells are directed against the CD19 antigen and are used in non-Hodgkin's lymphomas and acute lymphoblastic leukemia with outstanding results. Several efforts have been made to exploit this platform also in the context of other hematological diseases, including AML. More than 60 studies are ongoing and recruiting to investigate the efficacy and feasibility of CAR-T therapy, directed against different cellular antigens [124]. Finding the best molecular target is extremely challenging due to the frequent expression of these antigens also on non-tumor cells and the antigen-loss mechanism during the course of the treatments. Most of the CAR-T products that have reached phase 1/2 clinical trial are directed against CD33, CD123, CLL1, FLT3, CD70, TIM3, NKG2D, CD44v6 or have 'bispecific' activity such as novel CLL1-CD33 CAR-T cells. In a recent experience, a CLL1-CD33 compound CAR-T was able to induce MRD negativity in 7/9 r/r patients of which 6 were successfully bridged to allo-HSCT. Nevertheless, all patients who experienced grade 4 myelosuppression, CRS and neurotoxicity were frequent but always reversible [125]. Bispecific CAR-T may reduce the risk of prolonged pancytopenia and on-target/off-tumor effects on other normal tissues. Several new technologies are under evaluation to reduce CAT-T toxicity such as switching-off construct. Despite the impressive number of ongoing studies,

efficacy data are still scarce to hypothesize a rapid introduction of CAR-T into clinical practice.

6. Small molecule inhibitors: beyond VEN plus HMAs combination

Given the extremely heterogeneous biological landscape of AML of the elderly, biological therapies, as a solo actor, seem to be insufficient in the eradication of such an intrinsically resistant disease. Several small target inhibitors have been developed and showed solid clinical results, and will potentially be included in combination strategies, concurrently or sequentially with biological therapies. In this section, we will briefly focus on compounds that have shown the most interesting rationale.

FMS-like tyrosine kinase 3 (FLT3) mutations are found overall in nearly 30% of all newly diagnosed AML (ndAML). Since mutations on FLT3 seem to be a later event in leukemogenesis, they are frequently preceded by alterations in genes involved in clonal hematopoiesis, and the incidence of FLT3-mutated AML increases with age [126]. First-generation FLT3 inhibitors, such as *sorafenib* and *midostaurin*, showed only limited activity as a single agent and might be burdened with increased off-target effects [127–130]. Second-generation inhibitors have shown much greater efficacy; major studies and results are reported in Table 2 [131–140]. Currently, several trials are investigating triplet therapies of FLT3 inhibitors with HMAs and VEN. Preliminary results on 25 patients, addiction of an FLT3 inhibitor to DEC + VEN achieved a CR rate of 92% and 62% in ndAML and r/rAML, respectively. Newly-diagnosed AML also reached 2-years OS of 80% [141]. Phase 1/2 trial (NCT04140487), preliminary results on gilteritinib plus AZA + VEN were recently presented at ASH. Among the 11 ndAML patients, the CR rate was 73%; in the r/r cohort (N = 15), the ORR rate was 67% with a median duration of response of 9 months. Of note, 33% of r/r patients had already been exposed to a previous FLT3 inhibitor [142]. Extremely promising results of triplet therapy have to be balanced with the higher rate of prolonged myelosuppression, febrile neutropenia, and infection events that often requires dose adjustment, drug interruption, and hospitalizations. The best combination and right administration schedule are yet to be found.

Isocitrate dehydrogenase (IDH) mutations can be detected in almost 20% of AML cases [143]. Given their role in leukemogenesis, several small inhibitors have reached clinical investigation. IDH2-inhibitor *enasidenib* [144] and IDH1-inhibitor *ivosidenib* showed the most solid data and are both currently investigated in association with VEN, with or without AZA [145]. Based on phase 3 AGILE trial the IVO plus AZA combination was recently approved by Food and Drug Administration (FDA) in elderly or unfit newly diagnosed $IDH1^{R132}$-mutated AML (ndAML) patients [146]. The preliminary results on IVO plus VEN showed a pretty impressive composite CR rate of 87% with 63% of patients achieving MRD negativity by multiparametric flow cytometry (MFC). Median OS was 42 months and 4/30 patients experienced differentiation syndrome; toxicity was overall expected and almost all grade ≥3 events were reversible [147]. Despite solid data, enasidenib and ivosidenib have not yet received approval from European Medicines Agency (EMA), limiting their use outside the clinical trial in Europe.

Glasdegib is a small, selective hedgehog pathway inhibitor that has recently been approved for older (> 75 years) or unfit for intensive treatment, AML patients in combination with low-dose cytarabine (LDAC) [148]. Despite survival benefit was modest (only 4 months longer than the comparator arm), other studies are undergoing to evaluate glasdegib in

Table 2. Immune check-point inhibitors with promising clinical activity in elderly AML. Toxicity was reported in order of frequency.

Drug name mAb anti-PD1	Study	Combination	Population	Efficacy/outcome	Toxicity	Ref.
Nivolumab	Phase 2	+ AZA	R/R N = 70	Global low survival advantage HMAs-naïve vs HMAs.exposed: • ORR: 52% vs 33%; • OS: of 6.3 mo vs 4.6 mo	Reversible immune-relate events (mostly pneumonitis)	[78]
	Phase 2	+ AZA + ipilimumab	R/R N = 31	CR/Cri: 36% OS: 10,5mo	Reversible immune-relate events (higher rate than NIVO+AZA)	[81]
Pembrolizumab	Phase 2	+ AZA	R/R & ND N = 37	Low efficacy in R/R ND: cCR:47%, OS 13 mo	Consistent with AZA alone	[84]
	Phase 1	+ DEC10d	R/R N = 6	ORR 66%	Hematological AEs Infections (pneumonia)	[85]
mAb anti-CD47						
Magrolibab	Phase 2	+ AZA	ND p53mut N = 72	ORR: 48,6% (CR/CRi 41,6%) OS of 10.8mo	Febrile neutropenia Peripheral edema Anemia (manageable with priming-dose schedule)	[93]
	Phase 1/2	+ AZA + VEN	R/R & ND N = 38	ND: ORR 100% (CR/CRi 94%); R/R after VEN-failure: cCR 27%; R/R VEN-naïve: cCR 63%	Febrile neutropenia Pneumonia Anemia (as above)	[96]
mAb anti-TIM3						
Sabatolimab	Phase 1	+ HMAs	ND N = 48	ORR: 40% (cCR 30%) High-risk: ORR 53,8% DOR: 12.6 mo	Consistent with HMAs alone	[101]
	Phase 2	+ AZA + VEN	ND N = 18	CR 72% (preliminary) Including high-risk MDS, and CMML	Febrile neutropenia Hematological toxicity	[103]

AEs: adverse events; AZA: azacitidine; CMML: chronic myelomonocytic leukemia; CR/cCR: complete response/composite complete response; DEC10d: decitabine for 10 days administration; DOR: duration of response; HMAs: Hypomethylating agents; mAb: monoclonal antibody; MDS: myelodysplastic syndrome; N = number of patients; ND: newly diagnose; ORR: overall response rate; OS: overall survival; R/R: relapsed/refractory; VEN: venetoclax;

Table 3. Small molecules with proven clinical activity in elderly AML patients and promising combination with standard treatments. Toxicity was reported in order of frequency/clinical relevance.

Setting	Combination	Study	Population	Efficacy/Outcome	Toxicity	Ref.
FLT3-mutated						
Gilteritinib	Alone[1]	Phase 3	R/R	CR 34%, OS 9.8 mo in GILTE arm	Lower than salvage CHT	[131,132]
(both ITD/TKD)	+ VEN	Phase 2	R/R	cCR 83.8% Also effective in pts already treated with FLT3i	Febrile neutropenia Myelosuppression	[133]
	+ AZA	Phase 3	ND	No advantage in CR and OS	Consistent with AZA alone	[134]
	+ AZA + VEN	Phase 1/2	R/R and ND	CR 73% in ND, ORR 67% in R/R	Febrile neutropenia Prolonged myelosuppression Infections	[142]
Quizartinib	Alone[2]	Phase 3	R/R	OS: 6.2 mo in the experimental arm Survival advantage only vs LDSC	Febrile neutropenia Sepsis QT-prolongation Nausea	[135]
(only ITD)	+ LDAC or AZA	Phase 1/2	R/R and ND	cCR 87% in ND, 64% in R/R Better survival in QUIZ/AZA arm	Febrile neutropenia Pneumonia Infections	[136]
	+ DEC + VEN	Phase 1/2	R/R	cCR 90%, OS at 6mo 86%	Febrile neutropenia	[137]
	+ '3 + 7'[3]	Phase 3	ND Up to 75 yrs	Median OS: 31.9 mo with QUIZ vs 15.1 mo with PBO*	Consistent with '3 + 7'+ PBO QT-prolongation Higher rate of G3 neutropenia	[138]
Crenolanib	+ '3 + 7'	Phase 2	ND	(34%) pts were >60 yrs	Febrile neutropenia	[139, 140]
(including D835)			Up to 75 yrs	CR 86% after 2 cycles, OS 20.2 mo	Diarrhea	
IDH-mutated						
Enasidenib	+ AZA	Phase 1/2	ND	ORR 74%	Consistent with AZA alone	[145]
(IDH2-inhibitor)				Modest OS advantage		
Ivosidenib	+ AZA[4]	Phase 3	ND	Experimental arm: OS 24 mo, CR 47%	Consistent with AZA alone	[146]
(IDH1-inhibitor)	+ VEN ± AZA	Phase 1/2	R/R and ND	cCR 87%, OS 42 mo	Febrile neutropenia Pneumonia	[147]
NPM1-mutated/MRD eradication						
Glasdegib	+ LDAC[1]	Phase 2	ND	Modest OS advantage vs LDAC alone: 59,8% at 1-years vs 38,2%	Consistent with LDAC alone	[148]
(hedgehog-inhibitor)						
Mutated-TP53						
Idasanutlin	+ VEN	Phase 1/2	R/R	Only modest efficacy cCR 26%, OS 5.1 mo	Diarrhea and vomiting Febrile neutropenia Infections (sepsi, pneumonia)	[150]
Pevonedistat	+ AZA	Phase 3	ND - low blast	No advantage in CR rate and OS vs AZA alone	Consistent with AZA alone No increased toxicities	[152]
(NEDD8-inhibitor)	+ AZA + VEN	Phase 1/2	ND ≥75 yrs 43%	cCR 64% OS: 8.9 mo in TP53mut vs 18 mo	Consistent with AZA+VEN Hypophosphatemia	[153]
Eprenetapopt	+ AZA	Phase 2	ND – Low blast	ORR 69% (including MDS patients)	Consistent with AZA aloneAtaxia (reversible)	[155]

(*Continued*)

Table 3. (Continued).

Setting	Combination	Study	Population	Efficacy/Outcome	Toxicity	Ref.
KMT2Ar & NPM1-mut						
Revumenib (Menin – inhibitors)	Alone	Phase 1/2	R/R	AML n = 56/68 pts cCR 38% with DOR 9.1 mo	QT-prolong (asymptomatic) Differentiation syndrome	[160]
Ziftomenib (Menin – inhibitors)	Alone	Phase 1/2	R/R	Dosage 600 mg (12pts) cCR 33%	Febrile neutropenia Cytopenias Differentiation syndrome	[61]
RARα- overexpressed						
Tamibarotene (RARα agonist)	+ AZA	Phase 2	ND	ORR 67%, CR/CRi 61% OS 8.4 mo (18 mo in CR/Cri pts) High rate of transfusion independence	Consistent with AZA alone Hypertriglyceridemia (G ≥ 3)	[163]

*Sub-analysis on patients > 60 years: not available.
[1]Approved by FDA and EMA.
[2]Only approved in Japan.
[3]Priority review by FDA.ss
[4]Approved by FDA, not by EMA.
AZA: azacitidine; CR/cCR: complete response/composite complete response; DEC: decitabine; DOR: duration of response; FDA: Food and Drugs Administraton; FLT3: FMS-like tyrosine kinase 3; FLT3i: FLT3 – inhibitor; EMA: European Medicines Agency HMAs: Hypomethylating agents; IDH: Isocitrate dehydrogenase; KMT2Ar: Lysine Methyltransferase 2A gene rearrangements; LDAC: low-dose cytarabine; LDSC: low-dose standard chemotherapy; mo: months; MRD: minimal residual disease; ND: newly diagnose; NPM1: nucleophosmin 1; OS: overall survival; ORR: overall response rate; PBO: placebo; R/R: relapsed/refractory; VEN: venetoclax.

combination with AZA and suggesting a possible role in MRD eradication strategy.

Overexpression of MDM2 ubiquitin-ligase is recognized as a mechanism of resistance of AML blasts to therapy and leads to inactivation of the p53 pathway by directing the p53 protein to proteasomal degradation. MDM2-inhibitors Idasanutlin failed to demonstrate effectiveness in combination with intermediate-dose of cytarabine in r/rAML patients [149] and association with VEN showed only modest improvement [150]. MDM2 also promotes conjugation of ubiquitin-like protein NEDD8 to p53 protein. MDM2-mediated NEDDylation inhibits the transcriptional activity of p53 with consequent downregulation of its grown-suppressing effects [151]. *Pevonedistat* is a first-in-class NEDD8-activating enzyme (NAE) inhibitor and it's being investigated in several clinical trials. Despite strong rationale, randomized phase 3 trial of pevonedistat plus AZA vs azacitidine alone failed to demonstrate advantage of the combination [152]. Triple therapy with VEN and AZA showed more promising activities in an elderly and unfavorable population, and randomized phase 3 trial results are expected [153].

Even in the era of VEN-based therapies, TP53-mutated AML still represents an unmet clinical need. *Eprenetapopt* (APR-246) is a novel, first-in-class, small molecule that selectively binds to cysteine residues on mutant p53, restoring a functional conformation and reactivating p53 pathways. It also induces oxidative stress promoting leukemia cell death [154]. Phase 2 study, in association with AZA, showed promising response rates in treatment-naïve TP53-mutated myelodysplastic syndrome (MDS, N = 74) and oligoblastic AML patients (N = 22). No higher toxicity was reported and the onset of neurological events, like ataxia, was always reversible [155]. The randomized phase 3 trial results are expected. Furthermore, emerging data about an interesting immunological activity warrants further investigation, especially on microenvironment-directed combinations [156].

Menin-inhibitors will also prominently enter the scene in the treatment of leukemias with aberrant overexpression of Homeobox (HOX) genes such as HOXA and/or HOXB. This specific expression signature can be found in Lysine Methyltransferase 2A (KMT2A)-rearranged and in NPM1 mutated leukemias [157,158]. Menin is an essential cofactor for the KMT2A complex in the binding HOX gene promoters, and this interaction seems to be more specific to leukemic cells than in normal hematopoiesis [159]. Several compounds are being investigated and *revumenib* and *ziftomenib* already display some impressive results as a single agent in heavily pretreated patients [160,161]. Upregulation of HOXA has been imputed as a mechanism of resistance to venetoclax and several authors are already suggesting that menin-inhibitors may be promising in patients resistant to current VEN-base therapy [162].

The most promising compounds, including the not mentioned *Tamibarotene*, and emerging data in association with the current standard of care are summarized in Table 3 [163].

7. Conclusion

Patients unsuitable for intensive treatment still represent the majority of the AML population and display high incidence of unfavorable cytogenetic and molecular features, concurrent comorbidities and poor tolerance to standard treatment also make this population extremely difficult to cure. Approved venetoclax-based therapies are still far from offering a true curative option in these patients and are burdened by high frequency of primary and secondary resistances. Biological therapies will offer new potential treatment to a poor prognosis population, definitively ameliorating the life expectation of people that to date have no active treatment.

8. Expert opinion

Venetoclax plus HMAs combination has dramatically changed the therapeutic landscape of ndAML patients who were ineligible for intensive chemotherapy. Despite CR rates close to 70% [7,164] the number of relapsing patients is still high and the prognosis for patients who relapse is extremely poor.

Novel therapies were proposed in order to offer significant treatment options sparing from toxicities, as we recapitulate in Table 1 and 2. Biological therapies are expected to enormously enlarge our armamentarium in the fight against AML. Particularly, the use of target agents, as small molecule inhibitors directed against recurrent mutations found in AML, the targeting of surface antigens with monoclonal antibodies, immune cell engagers and antibody-drug conjugates can offer a strong potential in selectively target AML cells. Immunological therapies are being investigated and can have an important role in allowing leukemia stem cell eradication and the achievement of long-term survival without leukemia recurrence, as it is for allogeneic transplant in fit population; even if underexplored, cell therapies may reveal the best activity in this sense. In future, the study of off-target beneficial activities, as the immunological effects of hypomethylating agents or of target drugs and the microenvironment modifier effect of check-point inhibitors, also with single cell and spatial technologies, will deliver fresh biological knowledge to orient triplet and quadruplet trials.

Biological therapies have significant strengths; first of all, their activity relies on a particular mechanism, thus allowing the delivery of a personalized treatment directed against a target. The therapeutic window of a biological therapy can be enormously enlarged selecting targets that are fundamental for blast cell proliferation while allow to spare healthy cells. The well-defined AML biology also allows to project treatments that harness the power of immune system, redirecting T-cell, NK and innate immune response. Finally, a toxicity profile that is not wide and as specific as for chemotherapy, allow multimodal combinations both with existing treatment schemes and between molecules with different mechanisms, also building synergistic and synthetically lethal combinations.

Due to these strengths, the population of elderly AML will potentially be the first in which we will achieve significant results with this kind of therapeutic interventions. Nowadays, we have in our and a large armamentarium. The differing biology, as well as the differing indications for treatment and the differing efficacy of treatment modalities, dictate patient-specific approaches. Furthermore, fitness evaluation, polypharmacotherapy and drug-drug interactions represents a significant problem in delivering a cancer treatment in the old population.

As the population included in clinical trials often represent only a moderate share of the patient population, there will be in the future an increasing need of registries and pragmatic studies that will be able to photograph the patient journey instead of a single treatment, also allowing to reflect in the optimal sequential combination of different treatment options and modalities. We also must enrich our knowledge on populations with significant frailty, comorbidities and polypharmacotherapy.

Most of all, it will be important to invest in large biological studies during the exploratory trials of novel agents, in order to have instruments to predict optimal delivery of treatment and avoid wasting of time, resources and, most important patients' life in large trials that try to win the largest battle instead of considering AML as it is, a conundrum of diseases with different biology.

It is true that some of these strategies could be weighed down by excessive costs, especially in the context of therapy with an indefinite duration. The cost-effectiveness of these compounds is largely unknown, and appropriate studies will be needed to evaluate how much these therapeutic strategies will be sustainable by the healthcare system. A possible strategy may be to include cost-effectiveness analysis in clinical trials. Recently, several studies showed that novel therapies are still cost-effective compared to standard approaches, especially in patients unable to perform intensive and fixed-duration strategies [165,166].

The field will be further expanded with novel multimodal therapies that combine hypomethylating agents, chemotherapy, small molecule inhibitors, antibodies, and immunologic interventions (including cell therapies and transplant). Thus, precision medicine is rising as a model that proposes the customization of healthcare tailored to a subgroup of patients, instead of a one-drug-fits-all model, taking into consideration fitness, accurate evaluation of AML biology, and optimization of the expected efficacy of treatments at the patient level. In the future, promising interventions are warranted to be translated in younger and fit population.

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