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Novel monoclonal antibody-based therapies for acute myeloid leukemia

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ABSTRACT

There has been long-standing interest in using monoclonal antibodies to improve outcomes of people with acute myeloid leukemia (AML). While several candidate therapeutics have failed at various stages of clinical testing, improved survival of some patients receiving the CD33 antibody-drug conjugate gemtuzumab ozogamicin has provided first evidence that monoclonal antibodies have a role in the armamentarium against AML. Over the last several years, work to improve the success of monoclonal antibody-based therapies in AML has focused on the identification and exploration of new antigen targets as much as on the development of novel treatment formats such as use of unconjugated engineered monoclonal antibodies and conjugated antibodies, delivering highly potent small molecule drugs or radionuclides to AML cells. Here, we will provide a brief overview of current efforts with such investigational monoclonal antibody-based therapeutics.

1. Introduction

In 2019, an estimated 21,450 people will develop acute myeloid leukemia (AML) in the U.S. alone [1]. Until recently, treatment options were relatively limited and decision-making followed an algorithm that has been invariant for several decades [2,3]. Despite gradual improvements in outcome over the years – primarily due to better supportive care measures that have enabled safer administration of intensive multiagent chemotherapies and allogeneic hematopoietic cell transplantation (HCT) – curative-intent treatment has often remained unsuccessful, with only a minority of patients surviving long-term. Monoclonal antibody-based therapies have long been pursued as one means to improve these outcomes [4]. While numerous candidate drugs have failed at various stages of clinical testing, better survival in some patients treated with gemtuzumab ozogamicin (GO), a humanized IgG_4 CD33 antibody conjugated to a toxic calicheamicin moiety, has provided first evidence that monoclonal antibodies have a role in the armamentarium against AML [5]. Over the last several years, work to improve the success of antibody-based therapies in AML has focused on the identification and exploration of new antigen targets as much as on the development of novel formats of unconjugated or conjugated monoclonal antibodies that have high anti-leukemia efficacy and potency. Here, we will provide a brief overview of current efforts with such investigational therapeutics.

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2. CD25-TARGETING monoclonal antibodies

CD25, the alpha chain of the interleukin 2 receptor, is normally expressed on activated T-lymphocytes and is involved in their proliferation and differentiation [6]. This antigen is expressed on AML blasts in around 20% of patients and is thought to be displayed on leukemic stem cells (LSCs) [7]. Contributing to its appeal as a therapeutic target is the observation CD25 expression on AML cells is an independent risk factor for poor outcome [8,9]. A first attempt to direct AML therapy to CD25 clinically was made with ADCT-301 (camidanlumab tesirine), an antibody-drug conjugate composed of a human CD25-targeting antibody conjugated to a cytotoxic pyrrolobenzodiazepine (PBD) dimer after the drug showed potent anti-tumor activity in mouse xenograft models of CD25-expressing hematologic malignancies [10,11]. A phase 1 study in relapsed/refractory $CD25^+$ AML patients (NCT02588092) is completed. Preliminary results of 29 patients (median age: 67 years) show tolerance of this antibody-drug conjugate but suggest insufficient single-agent activity, with none of the treated patients achieving a response [12].

3. Targeting the CD27/CD70 axis with monoclonal antibodies

CD27 is a tumor necrosis factor receptor that regulates cellular activity in the hematopoietic system. It is expressed on early thymocytes as well as naive $CD4^+$ and $CD8^+$ T-cells and is upregulated upon T-cell activation [13]. Expression of its ligand, CD70, is highly restricted, with only transient display on the surface of T-, B- and dendritic cells upon activation [14]. CD27 and CD70 are both expressed on AML blasts in the majority of patients: in a small series, CD27 has been found on 36 of 42 (86%) blood and 24 of 25 (96%) of bone marrow samples, whereas CD70 was expressed on 22 of 23 (96%) blood and 20 of 20 (100%) bone marrow samples. Consistent with significant expression of CD27 and CD70 in AML, soluble CD27, an *in vivo* marker for the extent of CD70/CD27 interactions, is markedly increased in sera of patients with newly-diagnosed AML and has been identified as independent prognostic marker for poor survival [15]. Some studies have also identified CD27 expression on LSCs. In these cells, binding of CD70 to CD27 increases expression of Wnt target genes, proliferation and cellular differentiation. Conversely, similar to what has been observed in murine models of chronic myeloid leukemia (CML) [16], blocking of the CD27/CD70 interaction with a monoclonal CD70 antibody delayed disease progression, reduced the number of LSCs and prolonged survival in murine AML xenografts [15]. *In vitro*, such a blocking antibody led to asymmetrical cell divisions of LSCs and promotion of cellular differentiation, whereas it did not affect normal hematopoietic stem cells (HSCs) of healthy donors. As an additional difference between normal and malignant hematopoietic cells, *in vitro* studies indicated decitabine upregulates CD70 expression on $CD34^+$ progenitor cells of AML patients, but not cells from healthy controls. Combining decitabine with CD70 blockade reduced colony-forming and re-plating capacity *in vitro* compared to single agent treatment and eradicated human $CD34^+$ AML stem and progenitor cells *in vivo* in murine xenograft models [17]. These preclinical data provided the rationale for pursuing blockage of the CD27/CD70 axis by use of CD70 monoclonal antibodies as treatment for AML. As a first translation, a phase 1/2 study was developed evaluating the safety and feasibility of standard-dose azacitidine (75 mg/kg subcutaneously for 7 days every 28 days) with ARGX-110 (cusatuzumab), a defucosylated IgG_1 monoclonal antibody targeting CD70 that was originally generated in llamas and then germlined to 95% human identity [18], in newly diagnosed elderly AML patients (NCT03030612). In this trial, ARGX-110 was administered at 1–20 mg/kg once every 2 weeks. Preliminary results on 12 patients suggest significant anti-leukemia activity of this treatment strategy: 11 of the 12 patients (92%) achieved either a complete remission (CR), CR with incomplete hematologic recovery (CRi), partial remission (PR), or morphologic leukemia-free status (MLFS), with CR/CRi observed in 9 of 11 evaluable patients (82%). Remission could be deep, as indicated by 5 or 11 (45%) of patients achieving MRD negativity by flow cytometry. The combination of ARGX-110 with azacitidine was overall well tolerated and no dose-limiting toxicities (DLTs) were observed. Consistent with what would be expected from azacitidine, hematological toxicities were the most frequently reported adverse events [19].

4. CD33-TARGETING monoclonal antibodies

Most efforts to date with antibody-based therapeutics in AML have focused on the myeloid differentiation antigen CD33, which is displayed on at least a subset of the leukemic blasts in almost all cases and possibly LSCs in some [20,21]. From the beginning, explored therapeutics not only included unconjugated antibodies but also antibodies conjugated with small molecule drugs, plant toxins, or radionuclides. That is because CD33 is internalized when bound by an antibody, permitting intracellular delivery of toxins [20,21]. Explored in patients since the late 1980s, however, success with CD33-targeted therapeutics has been limited, with several agents showing unsatisfactory clinical activity [22–25]. However, positive results from several randomized trials with GO for patients with newly-diagnosed AML, particularly when added to conventional induction chemotherapy, validate CD33 as the first and so far, only immunotherapeutic target for this disease [5]. Still, many patients with $CD33^+$ AML do not benefit from GO, prompting ongoing interest in developing improved, more effective CD33-directed immunotherapies. Besides bispecific antibodies and chimeric antigen receptor (CAR)-modified T-cells, these include a number of monoclonal antibody-based drugs.

4.1. Unconjugated CD33 antibodies

Unmodified monoclonal CD33 antibodies lack sufficient clinical anti-leukemia activity, as indicated by the experience with the humanized IgG_1 antibody lintuzumab (HuM195, SGN-33), which is no longer pursued as therapeutic after randomized trials showed no survival improvement when added to mitoxantrone, etoposide, and cytarabine (MEC) in patients with relapsed/refractory AML (NCT00006045) or to low-dose cytarabine in older adults with untreated AML (NCT0052833) [22,23]. Whether unconjugated CD33

antibodies with augmented immune effector cell functions, e.g. via Fc-engineering to enhance interactions with the low-affinity activating Fc receptor CD16 (FcγRIIIa) on natural killer (NK) cells, will fare any better is currently studied with BI 836858 (mAb 33.1) [26]. BI 836858 is a fully human IgG_1 CD33 antibody with 2 amino acid substitutions in the Fc CH2 domain that exerts potent single-agent NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) against $CD33^+$ blasts from patients with AML [27]. Anti-tumor properties of BI 836858 can be enhanced *in vitro* with azanucleosides (e.g. decitabine) providing some rationale for pursuing this combination therapy in the clinic. A single-agent phase 1 trial (NCT01690624) in adults with either $CD33^+$ AML and failure of at least one prior treatment for AML or achievement of CR, but high risk of relapse, has been completed, but results are not yet reported. Ongoing are investigations in combination with azanucleosides, either azacitidine (NCT03013998) or decitabine (NCT02632721), or the immunocytokine F16-IL2 (teleukin), a noncovalent homodimeric recombinant fusion protein consisting of a human antibody fragment against the extra-domain A1 domain of tenascin-C (a glycoprotein of the extracellular matrix, F16) fused to human IL-2, that may have immunostimulatory and antineoplastic activity in AML [26] (NCT03207191).

4.2. CD33 antibody-drug conjugates

The experience with GO (for review, see Ref. [5]) validates the use of antibody-drug conjugates targeting CD33 in AML. However, not all attempts with CD33-directed antibody-drug conjugates have been successful. For example, a humanized CD33 antibody conjugated to a thiol-containing maytansinoid derivative, AVE9633 (huMy9-6-DM4), showed only minimal activity in adults with relapsed/refractory AML in phase 1 studies and its clinical development has been terminated [24]. Also currently no longer pursued as therapeutic, is the newer-generation antibody-drug conjugate SGN-CD33A (vadastuximab talirine). Compared to GO, SGN-CD33A not only integrated advancements in conjugation and linker technology but, with use of highly active, synthetic DNA cross-linking PBD dimer, also potency of the cytotoxic payload and maintained anti-leukemia efficacy in the presence of drug transporters in preclinical AML models [28].

SGN-CD33A has been tested alone and with other anti-leukemia agents in several clinical trials. In a phase 1 trial of 131 adults aged 26–89 years (median: 73 years), a dose of 40 μg/kg was chosen as the recommended monotherapy dose for phase 2 testing after consideration of anti-leukemia efficacy and toxicity [29]. At this dose, a CR or CRi was obtained in 11% and 17% of patients, respectively, for a combined CR/CRi rate of 28% among the 18 treated patients with relapsed/refractory AML. A total of 27 patients with previously untreated AML received SGN-CD33A at 40 μg/kg within this phase 1 trial. Six and 9 patients obtained a CR (22%) or CRi (33%), for a CR/CRi rate of 56% (15/27), with bone marrow blast clearance achieved in 19/27 (70%) patients [29]. Moreover, a cohort of 53 patients was given SGN-CD33A (10 μg/kg every 4 weeks on the last day of azanucleoside administration) in combination with azacitidine or decitabine [30]. As with single agent treatment, 30- and 60-day mortality was low (2% and 8%, respectively). Among all treated patients, 43% and 26% achieved a CR or CRi for a combined CR/CRi rate of 70%, with high CR/CRi rates observed in several high-risk patient subgroups including adverse-risk cytogenetics (80%), *TP53*-mutated AML (6/7, 86%), secondary AML (75%), and age ≥75 years (62%); median overall and relapse-free survival was estimated at 11.3 and 7.7 months [30]. These results provided the basis for a randomized phase 3 study testing the addition of SGN-CD33A to azacitidine or decitabine in patients with intermediate- or adverse-risk AML eligible for treatment with an azanucleoside (CASCADE trial). So far only preliminary data were reported on 67 patients, age 18–65 years, who received SGN-CD33A together with 7 + 3 [31]. Across different dosing schedules, a CR/CRi rate of 76% was observed, with 44 of 56 evaluable patients with bone marrow blast clearance (an entity that includes patients who achieved a CR, CRi or MLFS) testing negative for measurable residual disease (MRD) by centralized multiparameter flow cytometric assessment [31].

With SGN-CD33A monotherapy, non-hematologic adverse events included fatigue, nausea, and diarrhea. In the phase 1 trial, no cases of sinusoidal obstruction syndrome (SOS or veno-occlusive disease [VOD]) – a classic and feared complication observed with GO therapy [5] – were observed in patients who did not undergo allogeneic HCT, but one patient who subsequently underwent haploidentical HCT developed fatal SOS/VOD [29]. Concerns over liver toxicity led the U.S. Food & Drug Administration (FDA) to place a hold or partial hold on several of the trials with SGN-CD33A to further evaluate the potential risks of the drug when administered before or after allogeneic HCT [5]. These holds arose from the identification of 6 patients experiencing hepatotoxicity, including several cases of SOS/VOD, with 4 resulting in death. Ultimately, patient enrollment and treatment in all ongoing trials of SGN-CD33A was suspended and the CASCADE trial permanently discontinued after interim analyses indicated a higher rate of deaths, including fatal infections, in the SGN-CD33A-containing arm of the CASCADE trial [26].

One CD33 antibody-drug conjugate still in clinical testing is IMGN779. IMGN779 consists of a humanized CD33 antibody, a cleavable disulfide linker and a DNA cross-linking indolino-benzodiazepine dimer containing a mono-imine moiety [32]. Preliminary results have been reported on the first 50 patients aged 27–84 years (median: 68 years) with relapsed/refractory $CD33^+$ AML who received IMGN779 either biweekly (0.02–1.5 mg/kg on days 1 and 15 of a 28-day cycle) or weekly (0.39–0.7 mg/kg on days 1, 8, 15, and 22 of a 28-day cycle) as part of the first-in-human trial (NCT02674763) [33]. No dose-limiting toxicities (DLTs) have been observed on either schedule. The most frequent grade ≥3 adverse events included febrile neutropenia, bacteremia, pneumonia and anemia. Early data indicate IMGN779 has some, albeit perhaps modest, clinical anti-AML activity as evidenced by decreases in blast numbers in the first week of therapy among 19 of 24 patients with measurable circulating blasts (79%). In the subset of 27 patients treated at 0.39 mg/kg or above across both dosing schedules, 11 (41%) had a > 30% reduction in bone marrow blasts [33].

4.3. CD33 immunotoxins

Conjugates between CD33 antibodies and toxic bacterial or plant proteins have not been widely explored. One agent, lintuzumab

carrying recombinant gelonin (HuM195/rGel), was tested in a small number of patients, but then discontinued after it did not produce any CR or PR among 22 evaluable patients with advanced myeloid malignancies [25]. There are currently no CD33-directed immunotoxins in clinical testing.

4.4. CD33 radioimmunoconjugates

Unsurprisingly given that AML cells are highly sensitive to radiation [34–36], there has been long-standing interest in radionuclides as payloads for CD33 antibodies. Early studies with antibodies labeled with the β-emitting radionuclide iodine-131 (^{131}I) demonstrated anti-leukemia effects (and profound cytopenias at higher drug doses) in many patients with relapsed/refractory AML when given as single agent. In most of the studies, such antibodies have been used as part of preparative regimens before allogeneic HCT. In this setting, remissions were achieved without engraftment complications in the majority of patients and with little toxicity related to the radioisotope except perhaps some increase in liver toxicity [37–41]. More recent efforts have focused on labeling CD33 antibodies with α-emitters such as bismuth-213 (^{213}Bi) or actinium-225 (^{225}Ac). α-emitters are characterized by a high decay energy (5–8 MeV) deposited over short distances (55–70 μm) for potent, precise and efficient cell kill of target cells and minimized toxicity to normal, non-targeted surrounding cells compared to β-emitters that have lower decay energies (0.66–2.3 MeV) delivered over longer path lengths (0.3–2.3 mm) [42,43]. For several α-emitters, studies have documented as few as 10 hits/cell or less kill hematopoietic neoplasms [44–47] allowing use even for lower-density targets including CD33.

A first α-emitting CD33 antibody tested in patients was ^{213}Bi-labeled lintuzumab, which showed moderate activity in relapsed/refractory AML when given as single agent or, used sequentially after cytarabine, in patients with newly-diagnosed or relapsed/refractory AML [48–50]. Because ^{213}Bi requires high activities to achieve clinical effects and an onsite generator for drug preparation (half-life of ^{213}Bi: 46 min), actinium-225 (^{225}Ac) was then explored (half-life of ^{225}Ac: 10 days). ^{225}Ac is 1000–10,000 times more potent than ^{213}Bi when bound to antibodies [50]. A phase 1 trial of an ^{225}Ac-labeled lintuzumab showed elimination of peripheral blood blasts in 10/16 adults with relapsed/refractory AML, with 3 patients treated with doses $\geq$1 μCi/kg achieving marrow blasts of $\leq$5% [50,51]. A subsequent 1/2 study used ^{225}Ac-lintuzumab with low-dose cytarabine in older adults with previously untreated AML and reported a response rate of 69% among 13 patients who received a 2.0 μCi/kg/dose. However, consistent with "on-target, off-leukemia cell" toxicity, many patients suffered from prolonged severe thrombocytopenia and neutropenia. This led to deaths from infections in some, necessitating reduction to 1.5 μCi/kg/dose for further evaluation [52,53]. At this dose, objective responses were much less common (4/18 treated patients) and the study was closed early [53].

5. CD38-TARGETING monoclonal antibodies

CD38 is a transmembrane glycoprotein and multifunctional ecto-enzyme involved in the regulation of cell migration and catabolism of NAD + and NADP [54,55]. CD38 is broadly expressed on lymphoid and myeloid cells, erythrocytes, NK-cells and platelets, with the highest expression found on plasma cells. It is also displayed on non-hematopoietic cells, including epithelial, Purkinje, pancreatic β-, retinal and sarcolemma of smooth muscle cells and osteoclasts [55]. High and homogenous expression of CD38 on malignant plasma cells led to the development of CD38-targeted immunotherapy in multiple myeloma. Several monoclonal CD38 antibodies (e.g. daratumumab, isatuximab, and MOR202) have shown efficacy in patients with relapsed or refractory multiple myeloma and their use is now progressing towards frontline therapy [56], with daratumumab being approved by the FDA for the treatment of multiple myeloma since 2015. Extensive use of daratumumab in multiple myeloma has shown this antibody has an acceptable toxicity profile, with mild pancytopenia and well-manageable infusion-related respiratory problems being the most common.

CD38 is heterogeneously expressed on AML blasts and a significant reduction in AML tumor burden could be demonstrated with daratumumab *in vitro* and in xenograft models *in vivo* [57,58]. Efficacy of daratumumab as single agent in relapsed/refractory AML and high-risk myelodysplastic syndrome (MDS) is currently explored in a phase 2 trial (NCT03067571). In parallel, several strategies with CD38 antibody-containing combination therapies are tested. *In vitro* studies have demonstrated all-trans retinoic acid (ATRA) upregulates CD38 not only on plasma cells but also AML cells and enhances the efficacy of daratumumab as well as CD38 CAR T-cell therapy [59–62]. Also upregulating CD38 expression, is the orally available, synthetic retinoid SY-1425 (tamibarotene), which enhances the anti-tumor efficacy of daratumumab *ex vivo* [63,64]. SY-1425 in combination with azacitidine or daratumumab is investigated in an ongoing phase 2 study in patients with relapsed/refractory AML or MDS (NCT02807558). While toxicities appear acceptable, preliminary efficacy results are rather disappointing, with all 4 evaluable patients in the daratumumab arm experiencing progressive disease [65].

6. CD44-TARGETING monoclonal antibodies

The CD44 receptor belongs to a transmembrane protein family with multiple functions in humans related to, among others, immunological processes, hematopoiesis and organogenesis [66]. CD44 is expressed on hematopoietic precursors, including long-term culture-initiating cells, colony forming unit-granulocyte macrophages and leukemic blasts [67]. CD44's main ligand is hyaluronic acid, an extracellular matrix glycosaminoglycan. For LSCs to facilitate their cellular adhesion and migration in the bone marrow microenvironment, interactions between CD44 and hyaluronic acid are essential [68]. Conversely, activating monoclonal CD44 antibodies can eradicate human LSCs in immunocompromised mice [69].

One CD44 antibody tested in the clinic is RG7356, a humanized IgG_1 monoclonal antibody that inhibits cell adhesion by blocking

the interaction between CD44 and hyaluronic acid [70]. With RG7356, disruption of the tumor microenvironment triggers the release of specific chemo-attractants that recruit and activate macrophages, leading to the phagocytosis of RG7356-opsonized tumor cells. RG7356 has been used in a phase 1 dose-escalation study in 44 patients primarily with relapsed/refractory AML. The antibody was given intravenously at doses $\leq$2400 mg every other week or $\leq$1200 mg weekly or twice weekly; dose escalation started at 300 mg. Although the majority of adverse events was mild or moderate, one DLT (grade 3 hemolysis) occurred. Infusion-related reactions occurred in 64% of patients mainly during the first treatment cycle, and 2 patients experienced grade 3 drug-related aseptic meningitis. Overall, responses were disappointing, with the majority of patients having progressive disease. Only 1 patient each obtained a CRi, PR, or hematologic improvement [70].

7. CD45-TARGETING monoclonal antibodies

CD45 is a tyrosine phosphatase with expression restricted to cells of the hematopoietic system that is a pivotal modulator of signal transduction processes in these cells [71]. CD45, which, unlike CD33, is not internalized after antibody engagement [72], is widely expressed on AML blasts and may play a role in AML development [73]. So far, therapeutic targeting of CD45 in AML has most commonly been pursued in the form of radiolabeled monoclonal antibodies. Similar to CD33-directed radioimmunotherapy, initial work focused on β-emitters as payloads (e.g. ^{131}I or ^{90}Y), with more recent efforts exploring the value of α-emitters, in particular astatine-211 (^{211}At), primarily as augmentation of HCT conditioning regimens. Several proof-of-principle studies have documented the feasibility of this strategy [74,75]. However, although introduced in the clinic over 20 years ago, studies were typically small, single-arm trials without appropriate control groups, leaving the question whether such radioimmunotherapies indeed improve outcomes in AML unanswered. This void may be filled with the first-ever conducted randomized phase 3 trial (SIERRA; NCT02665065), which currently explores the role of a ^{131}I-labeled CD45 antibody (Iomab-B, apamistamab) as part of conditioning prior to allogeneic HCT. In SIERRA, adults > 55 years of age with relapsed/refractory AML are randomized 1:1 to either the experimental arm with Iomab-B followed 12 days later by allogeneic HCT with fludarabine/2 Gy total body irradiation (TBI) as transplant conditioning or the standard-of-care control arm, in which conventional salvage chemotherapy as per physician choice is given and the option of crossing-over is offered if no response is obtained. An interim analysis performed on 38 evaluable patients showed most enrollees in the control arm (88%) failed standard salvage chemotherapy and 65% crossed over to receive Iomab-B. All patients receiving Iomab-B engrafted, with almost all patients reaching $\geq$95% donor chimerism within 100 days of HCT. Grade 3 adverse events occurring in > 10% of patients in both arms included febrile neutropenia, stomatitis, sepsis, hypotension, hyperbilirubinemia and fatigue, but no grade 3+ infusion-related adverse events due to Iomab-B were reported [76].

8. CD47-TARGETING monoclonal antibodies

CD47 is ubiquitously expressed on normal and malignant tissues [77]. Through interaction with the myeloid inhibitory immunoreceptor signal-regulatory protein α (SIRPα) on phagocytic and dendritic cells, this transmembrane protein induces inhibitory signals that limit phagocytosis of CD47-expressing cells [78]. Via interaction with secreted thrombospondin-1, it also inhibits T-cell receptor signaling and antigen presentation by dendritic cells. With these properties, CD47 is a checkpoint regulating both innate and adaptive immunity, rending it appealing as target for immunotherapy. Many malignant cells overexpress CD47, with the degree of expression being independently correlated with poor clinical outcome in a variety of hematologic malignancies and solid tumors [79,80]. The notion that blockade of CD47 enhances phagocytosis and elimination of CD47-expressing cancer cells led to the development of the unconjugated humanized IgG_4 CD47 antibody Hu5F9-G4. In the preclinical setting, Hu5F9-G4 induces potent macrophage-mediated phagocytosis of primary human AML cells *in vitro* and eradicates human AML *in vivo* in patient-derived xenograft models and showed safety in non-human primates at clinically-relevant doses [81]. Encouraging clinical activity of Hu5F9-G4 was observed in a phase 1b study in patients with relapsed or refractory non-Hodgkin's lymphoma [82]. Hu5F9-G4 has been given to patients with hematologic malignancies including relapsed/refractory AML and high-risk MDS either as single agent (CAMELLIA trial, NCT02678338 [trial completed]; NCT03248479) or, in combination with azacitidine, to patients with previously untreated AML/MDS (NCT03248479). While evaluation of Hu5F9-G4 is ongoing, testing of CC-90002, another CD47 monoclonal antibody, was terminated early in relapsed/refractory AML and MDS patients because of insufficient activity (NCT02641002).

9. CD123-TARGETING monoclonal antibodies

The interleukin 3 (IL3) receptor α-chain (CD123) forms a heterodimer with the β-subunit of the IL-3 receptor upon ligand binding, inducing cellular differentiation and proliferation [83]. Within the hematopoietic system, CD123 is displayed on myeloid precursors, monocytes, basophils, plasmacytoid dendritic cells and a small subpopulation of B-cells. CD123 is also found on a broad variety of non-hematopoietic tissues, with high expression on multiple epithelial tissues of the respiratory and gastrointestinal tract [84]. Besides several other hematologic malignancies, CD123 is widely displayed on blast cells of patients with AML (50-nearly 100%) [85]. What makes CD123 particularly attractive, is that it is overexpressed on leukemic stem/progenitor cells in AML relative to normal hematopoietic stem/progenitor cells, which express little or no CD123 [85]. Contributing to its attractiveness as a target are studies reporting a correlation between higher $CD123^+$ leukemic stem/progenitor cell numbers and worse outcome with AML chemotherapy [86]. Because of this expression pattern, CD123 is an attractive pharmacologic target in AML for monoclonal antibodies but also bispecific antibodies, CAR T-cells and an immunotoxin using a recombinant IL-3 to deliver a truncated diphtheria toxin payload (SL-401; tagraxofusp) that was recently approved for the treatment of blastic plasmacytoid dendritic cell neoplasm

(BPDCN), a neoplasm characterized by high CD123 expression.

9.1. Unconjugated CD123 antibodies

Initial clinical attempts with unconjugated CD123 antibodies were made with CSL360, a recombinant, chimeric IgG_1 antibody. However, although this antibody demonstrated anti-leukemic activity *in vitro* [87] clinical efficacy was unsatisfactory, with only 2 out of 40 patients with advanced AML showing a response [88] and drug development discontinued. More recent attempts with unconjugated CD123 antibodies have focused on molecules with enhanced effector cell function. One example is CSL362 (JNJ-56022473, talacotuzumab), a fully humanized CD123 antibody with enhanced ADCC due to increased affinity for CD16. However, single-agent activity was also modest – for example, in the German SAMBA trial, only 1 patient each achieved a CRi or had hematologic improvement among 14 evaluable patients with AML or MDS who failed azanucleoside therapy [89]– and a phase 2/3 trial comparing decitabine to decitabine plus talacotuzumab (NCT02472145) in older adults with previously untreated AML was terminated early. Another example is KHK2823, a non-fucosylated fully human IgG_1 monoclonal CD123 antibody. Phase 1 trial testing of this antibody is planned (NCT02181699).

9.2. CD123 antibody-drug conjugates

Preclinically, antibody-drug conjugates targeting CD123, including SGN-CD123A and IMGN632, have shown high anti-AML activity [90,91]. SGN-CD123A is composed of a humanized CD123 antibody with an engineered cysteine residue in each heavy chain to enable site-specific conjugation. Via dipeptide linker, it carries a PBD payload. The drug has been tested in a phase 1 trial in adults with relapsed/refractory AML (NCT02848248) but this trial has been terminated early and study results have not yet been publicly reported. Still under clinical investigation is IMGN632, which contains a humanized CD123 antibody linked to an indolinobenzodiazepine pseudodimer (IGN), a DNA mono-alkylating payload. IMGN632 is currently tested in a phase 1 trial in patients with relapsed/refractory AML, BPDCN, acute lymphoblastic leukemia (ALL) and other CD123-expressing hematological malignancies (NCT03386513). Clinical activity of IMGN632 is indicated by interim analysis of 12 AML patients showing achievement of CR or CRi in 33% of patients during the initial stages of dose escalation [92].

10. FLT3- (CD135) TARGETING monoclonal antibodies

FMS-like tyrosine kinase 3 (FLT3, CD135) is one of the most commonly mutated genes and has therefore been a prime target for small molecule inhibitors. However, because FLT3 expression on normal hematopoietic cells is limited to immature cell subsets, but this antigen is displayed in 70–100% of AML cases [93], there is some interest targeting FLT3 with monoclonal antibodies. Currently under investigation is AGS62P1, a FLT3-targeting human IgG_1 antibody conjugated to a microtubule disrupting agent (AGL-0182-30) via an alkoxyamine linker [94,95]. After demonstration of potent preclinical activity [94], AGS62P1 has entered the clinic and is now tested in as monotherapy in relapsed/refractory AML (NCT02864290).

11. CD157-TARGETING monoclonal antibodies

CD157, also known as bone marrow stromal antigen 1 (BST-1), is a glycosyl-phospatidylinositol-anchored membrane protein expressed on multiple tissues, including dermal fibroblasts, peritoneal mesothelium and peripheral blood mononuclear cells [96]. During myeloid differentiation, CD157 expression arises from the stage of $CD34^{-/low}$ myeloblasts, with the highest expression on monocytes [97,98]. CD157 is almost universally expressed on blasts from AML patients (97% of 101 samples tested) although expression levels vary substantially between individual patients and are generally lower on immature cell fractions than leukemic bulk cells [99]. Mimicking physiologically high expression during monocytic cell differentiation, highest levels were found in patients with myelomonocytic and monocytic AMLs. One attempt targeting CD157 is made with MEN1112, an Fc-optimized CD157 antibody that showed high preclinical activity against AML cell lines and primary AML cells [99]. A phase 1 trial (ARMY) in patients with relapsed/refractory AML is ongoing (NCT02353143) with no results reported to date.

12. CXCR4- (CD184) TARGETING monoclonal antibodies

Binding of chemokine C-X-C motif receptor 4 (CXCR4) to its ligand, C-X-C motif ligand 12 (CXCL12) or SDF-1α, leads to signaling events that are crucial for survival, proliferation, engraftment and migration (homing and retention) of HSCs in the bone marrow [100]. While CXCR4 is displayed on many cell types, including $CD34^+$ hematopoietic progenitor cells, is CXCL12 more restrictedly expressed on bone marrow stromal cells [101,102]. Interruption of the CXCR4/CXCL12 signaling axis results in peripheral blood migration of hematopoietic progenitor cells from the bone marrow [103,104], an effect that is exploited in the clinic with small molecule inhibitors (e.g. AMD3100 [plerixafor] and BL-8040 [BTK-140]) for the purpose of hematopoietic stem/progenitor cell mobilization. Preclinical studies have also shown that blocking of CXCR4, via small molecule inhibitors or monoclonal antibodies, exerts antileukemic effects [105–108]. This observation led to the clinical testing of ulocuplumab (BMS- 936564), a fully human IgG_4 unconjugated antibody which inhibits the binding of CXCR4 to CXCL12. A phase 1 trial was conducted in 73 adults with relapsed/refractory AML who received ulocuplumab alone or in combination with mitoxantrone/etoposide/cytarabine (MEC). Ulocuplumab was given in doses up to 10 mg/kg without causing DLTs even with combination therapy. Forty-three patients received 10 mg/kg of

ulocuplumab in combination with MEC, with 51% achieving either CR or CRi. Of note, 4 patients entered CR/CRi after the pre-treatment phase with a single dose of ulocuplumab, indicating this antibody has some single-agent anti-leukemia activity. The only treatment-related adverse event with ulocuplumab monotherapy was mild and transient thrombocytopenia, whereas the safety profile of ulocuplumab when combined with MEC was similar to MEC alone [109]. Further testing of ulocuplumab in combination with low dose cytarabine in patients with newly diagnosed AML in a randomized phase 1/2 trial is planned (NCT02305563).

13. CLEC12A-TARGETING monoclonal antibodies

CLEC12A (also known as CLL-1 or CD371) is a type II transmembrane glycoprotein and member of the C-type lectin superfamily [110–112]. This superfamily has been implicated in a wide variety of cellular functions related to phagocytosis, cell adhesion, pathogen recognition, complement activation and cell adhesion [113,114], but the precise physiological functions of CLEC12A remain poorly understood. CLEC12A is primarily displayed on cells of the myeloid lineage during differentiation whereas normal hematopoietic stem and progenitor cells and most if not all non-hematopoietic cells do not express CLEC12A [84,115–118]. CLEC12A is widely displayed on leukemic blasts in AML, with expression ranging from 77.5 to 92% at diagnosis [84,115,119–121] and may also be found on LSCs, as indicated by studies showing CLEC12A expression on $CD34^+/CD38^-$ cells in 70–87% of cases of $CD34^+$ AML [122,123]. Of interest from a drug development perspective, antibody-induced crosslinking of CLEC12A results in internalization of the antigen [115,124].

The expression of CLEC12A on AML blasts and LSCs but not on normal stem/progenitor cells or any other tissues has raised interest in developing CLEC12A-directed therapies. Currently most advanced are efforts with bispecific antibodies (e.g. MCLA-117) and CAR T-cells, which have entered early-phase clinical testing [112]. In contrast, monoclonal CLEC12A antibodies have so far only been studied preclinically. For example, 2 unconjugated CLEC12A antibodies have been developed that mediate dose-dependent complement-dependent cytotoxicity (CDC) and ADCC against AML derived cell lines, freshly isolated AML blasts and lead to reduction of tumor size in xenograft models [119]. Moreover, a recently-developed CLEC12A antibody conjugated to a potent PBD dimer via a self-immolative disulfide linker effectively depleted $CLEC12A^+$ blasts from AML patients *in vitro* and tumor cells in AML xenograft models. Supporting use in the clinic, no target-independent toxicities were observed in cynomolgus monkeys at doses that depleted $CLEC12A^+$ myeloid cells [125].

14. Summary

Besides GO, the FDA has approved 7 additional drugs for the treatment of AML in the last 2 years. With these, the treatment options have substantially increased for people with newly-diagnosed and relapsed/refractory AML. Still, these drugs largely provide incremental benefits for subsets of patients and treatment outcomes remain unsatisfactory for many. Indeed, many affected individuals will die from consequences of leukemia or treatment-associated complications and only a minority will be long-term survivors. There is therefore ongoing need for effective, relatively well-tolerated new therapies. Success with monoclonal antibody-based therapies to improve treatment outcomes in AML has so far been limited but GO has provided first evidence that monoclonal antibodies should have a role in the clinical care of AML. As summarized in the previous sections, many parallel efforts are currently ongoing to identify new monoclonal antibodies with clinically-relevant anti-leukemia efficacy that could find a niche in the AML treatment armamentarium. Several recent failures of candidate therapeutics not only stress the fact that preclinical studies do not easily translate into clinical results, but also highlight that AML is, in general, a difficult disease to treat with antibodies. Nonetheless, some investigational agents shown efficacy in early clinical studies and more robust results from these drugs are eagerly awaited. As the experience with GO teaches, of great importance for the development of these (and other antibody) agents will be early identification and implementation of biomarkers, enabling the selection of patients most likely to benefit from such therapies as one strategy to minimize the risk of false-negative clinical testing and prevent that drugs are removed from consideration as therapeutics too early.

15. Research agenda/remaining questions

- Is there one ideal target antigen for the treatment of AML (if so, which one), or should efforts focus on approaches targeting more than one antigen simultaneously?
- Is targeting a combination of antigens the optimal treatment strategy to minimize not only the risk of development of treatment evasion/resistance but also the likelihood of on-target/off-leukemia toxicities?
- Is combination of treatment modalities (e.g. chemotherapy/targeted therapies) the most effective treatment regimen and if so, what combinations would be most potent?
- If combination of treatment modalities or compounds is the optimal strategy, what would be the optimal sequence for these therapies, regarding effectiveness and tolerability/toxicity?
- Which are useful biomarkers to identify patients that will benefit most from monoclonal antibody-based therapy?
- How can normal hematopoietic cells (and other normal target-positive cells) best be protected from the cytotoxic properties of potent AML-directed monoclonal antibodies?

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