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Review

Navigating the Landscape of Antibody Drug Conjugates: Current Trends and Future Research Prospects in Acute Myeloid Leukemia

Aayushi C. Shah[1], Sunayna G. Saluja[1], Jeel D. Trivedi[1], Shreeja H. Mehta[1], Rajanikant Patel[2], Mehul R. Chorawala[1,3,*], Kiran Dudhat[4], Bhupendra G. Prajapati[5,6,*]

[1]Department of Pharmacology and Pharmacy Practice, L. M. College of Pharmacy, 380009 Ahmedabad, Gujarat, India
[2]Department of Product Development, Granules Pharmaceuticals Inc., Chantilly, VA 20151, USA
[3]Centre for Research Impact & Outcome, Chitkara College of Pharmacy, Chitkara University, 140401 Rajpura, Punjab, India
[4]School of Pharmacy, RK University, 360020 Rajkot, Gujarat, India
[5]Department of Industrial Pharmacy, Faculty of Pharmacy, Silpakorn University, 73000 Nakhon Pathom, Thailand
[6]Now with Department of Pharmaceutics, Parul Institute of Pharmacy, Faculty of Pharmacy, Parul University, 391760 Vadodara, Gujarat, India
*Correspondence: mchorawalaresearch@gmail.com; mehul.chorawala@lmcp.ac.in (Mehul R. Chorawala); bhupen27@gmail.co; bhupendra.prajapati40731@paruluniversity.ac.in (Bhupendra G. Prajapati)

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Abstract

Acute myeloid leukemia (AML) is a hematologic malignancy with a poor prognosis and high relapse rates, especially in high-risk patients and older adults. Conventional treatment modalities confer limited benefit, specifically in relapsed and refractory cases. Antibody drug conjugates (ADCs) are a rapidly advancing treatment option that provides a novel approach to treating AML. The design and mechanistic aspects of ADCs have also been discussed. ADCs combine cytotoxic chemotherapeutic drugs with the specificity of monoclonal antibodies. This review primarily focuses on the current role of ADCs in the treatment of AML, including approved agents such as gemtuzumab ozogamicin, as well as others. Moreover, challenges associated with the use of ADCs have been explored, including resistance mechanisms, drug stability, immunogenicity, and cost. This review also highlights and summarizes various ongoing and completed clinical trials, which may provide insight into this treatment approach. Future advancements in AML treatment, including the use of nanoparticles or nanostructures, have also been discussed. In conclusion, this comprehensive review sheds light on the current and prospective future directions of ADCs in the treatment of AML, highlighting their potential to significantly alter the therapeutic landscape for this cancer.

Keywords: antibody drug conjugates; acute myeloid leukemia; targeted therapy; combination therapies; cancer; clinical trial

1. Introduction

Leukemia can be identified as a diverse group of hematologic malignancies that evolve from abnormal growth of white blood cells (WBCs) [1]. Simply, leukemia occurs due to disturbances in the normal hematopoiesis process, where there is dysregulated proliferation of pluripotent myeloid and lymphoid cell lineages [2]. Based on the swiftness of disease progression, leukemia can be distinguished as acute leukemia (AL) and chronic leukemia. Additionally, leukemia can be classified into myelocytic and lymphocytic subtypes based on the cell of origin. While the human body typically comprises 1–5% of immature or developing blast cells, a confirmatory diagnosis of AL can be obtained when the blood content of blast cells exceeds 20%, which further leads to the rapid onset of clinical leukemia manifestations. AL, based on the cell of origin, can be classified into two main types: acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL). Analyzing data from 2016, as cited by GLOBOCAN, which provides global cancer statistics and estimates, approximately 25,000 children are diagnosed with cancer annually in India [3]. Out of which, 9000 are diagnosed with leukemia; this equates to almost 90,000 children being affected by leukemia in the nation over a decade [4].

The therapeutic care plan for patients with AL is determined based on risk factors, as well as the genotypic and phenotypic traits of the affected individual [5]. Traditionally, the therapeutic regimen for AL encompasses the primary phase of induction therapy, which is subsequently followed by intense post-remission therapy, which is otherwise called consolidation therapy [5,6]. Intricated chemotherapeutic regimens for AL are determined considering patient-specific aspects, such as age, previous records of myelodysplasia or chemotherapy, along with the performance status score [6]. Conventionally, the “7+3” regimen is prescribed for induction therapy, which involves 7 days of continuous cytarabine infusion followed by 3 days of anthracycline administration. The primary goal of induction therapy is to achieve a complete remission (CR). Regrettably, measurable residual disease remains present in CR. Hence, an affirmative response to induction therapy suggests progression to consolidation therapy

to abolish any residual disease and accomplish long-term remission. Conceivable choices for consolidation therapy constitute chemotherapy and allogenic hematopoietic stem cell transplantation (Allo-HSCT) [7]. Affected individuals who refrain from receiving post-remission therapy have preeminent chances of recurrence within 6–9 months [6]. Nonetheless, the outcome of these conventional chemotherapies is displeasing due to unswerving DNA injury, inducing the death of malignant as well as normal cells [8,9]. The potency of these drugs is limited due to their action on unintended targets and a narrow therapeutic window, as well as the multidrug-resistant nature of tumors [9]. Despite the administration of these intensified chemotherapeutic agents, achieving long-term remission is challenging, and only a few patients may be perceived to have long-lasting survival [8,10].

In the medical context, visualizing the current scenario, several therapeutic antibodies are being used to manage specific types of solid tumors and liquid tumors, providing effective care. Alternatively, advancements in cancer knowledge have facilitated the entire process of bringing new drugs to market, paving the way toward a more effective cure, despite their potential off-target toxicities [9]. The competence of established antineoplastic agents somehow serves to imprecise chemotherapy that is quite lethal and inappropriately targeted therapy, leading to deficient cytostatic outcomes. This indicates a high level of significance and the need to implement a theory that aims to develop a molecule possessing the combined properties of chemotherapy and targeted therapy [11]. Considering the same aspect, the concept of ADCs has been established and approved by the United States Food and Drug Administration (FDA) since 2017, to achieve a better net result and mitigate certain other detrimental effects related to tumors [10]. The idea of ADCs was initially proposed by Paul Ehrlich, a German scientist and physician, in 1913. ADCs can recognize their targets while sparing damage to normal body cells and are thus referred to as “magic bullets” [12]. The cardinal components involved in framing an ADC molecule include a monoclonal antibody (mAb), a small drug particle named as payload, and a linker that attaches the drug to the antibody. These antibodies then get connected to the foreign particles (antigens) that are excessively expressed on the surface of cancerous cells [13]. The aggregation of all the aforementioned three components accounts for the formation of a thoroughly effective ADC molecule that provides direct drug delivery to tumor cells and exhibits extraordinary selectivity and efficacy against its targets [14]. The fragment antigen-binding (Fab) region of the antibody is responsible for recognizing the antigen and enabling the ADC to bind to it. The payload is a small fragment that ideally serves as the root of killing abnormal cells, as it can permeate through cell membranes and reach the DNA. The payloads are attached to the mAbs via linkers that can be either cleavable or non-cleavable. The hydrophobic nature of the linker is necessary for the tissue distribution of the ADCs. The fragment crystallizable (Fc) region binds to specific receptors and stimulates the operations of immune effectors such as antibody-dependent cytotoxicity [15].

The FDA has approved numerous ADCs for clinical use in oncology, including brentuximab vedotin (BV), inotuzumab ozogamicin (InO), belantamab mafodotin, fam-trastuzumab deruxtecan, ado-trastuzumab emtansine, gemtuzumab ozogamicin (GO), enfortumab vedotin, polatuzumab vedotin, sacituzumab govitecan, and loncastuximab tesirine. Amongst these ten ADCs, no more than six have been employed to treat hematological malignancies: GO, BV, InO, polatuzumab vedotin, belantamab mafodotin, and loncastuximab tesirine. Meanwhile, GO, InO, and moxetumomab pasudotox are currently used clinically to treat leukemia [10,16]. The first-generation ADCs encompassed murine antibodies and chemotherapeutic drugs linked by a non-cleavable linker. GO, with the commercial name Mylotarg®, is a first-generation ADC that involves a humanized antibody and a calicheamicin payload, targeting cluster of differentiation 33 (CD33) on leukemic cells [17]. GO was the first ADC to be approved by the FDA in 2000, before being withdrawn from the market owing to fatal toxicities and lack of beneficial evidence in 2011. Nonetheless, GO was reapproved in 2017 after assessing the associated efficacy of the drug at lower doses with fewer toxicities [18]. However, several disadvantages, including unstable linkers and poor chemical properties, led to the development of second-generation ADCs. InO, BV, and ado-trastuzumab emtansine are examples of second-generation ADCs that address the drawbacks of first-generation ADCs. Moreover, the shortcomings, such as rapid clearance and toxicity, associated with second-generation ADCs led to the development of third-generation ADCs, which resulted in conjugates with enhanced pharmacokinetics [17]. In 2017, the US FDA approved InO, a targeted therapy directed against the CD22 antigen on leukemic cells, for the treatment of relapsed or refractory B-cell acute lymphoblastic leukemia (B-ALL) [16]. Notably, B-ALL is a hematologic malignancy characterized by the accumulation of immature B cell precursors resulting from genetic mutations that impair normal B cell development and promote unchecked proliferation. InO has been associated with improved overall survival (OS) and increased remission rates [19]. Moxetumomab pasudotox targets CD22 and has been approved by the FDA for refractory or relapsed hairy cell leukemia since 2018. However, it was announced in 2022 that Moxetumomab pasudotox would be withdrawn from the market in July 2023, simply due to insufficient use and the availability of other treatment options [20]. Several ADCs are under investigation in clinical trials, paving the way for new advances and novel treatments in the management of leukemias. In this review, we provide an overview of the basic framework of ADCs, along with an in-depth understanding of their mechanism of action. Fur-

thermore, we focus on the clinical use of ADCs in AML and ALL, with substantiation derived from clinical trials, as well as the challenges encountered when implementing ADCs in therapeutic regimens.

2. ADC Design: Structure, Anatomy, and Function

The intricate design of ADCs represents a revolutionary leap in targeted cancer therapy. ADCs, hailed for their precision and efficacy, derive their power from a meticulously crafted structure comprising monoclonal antibodies, payloads, and linkers. This section highlights the structural intricacies of ADCs, scrutinizing their anatomy and functional mechanisms. From the careful selection of antibodies to the strategic choice of payloads and the crucial role of linkers, each component plays a pivotal role in framing the therapeutic potential of ADCs. Meanwhile, understanding the structural nuances is essential for unlocking the therapeutic potential of these innovative biotherapeutics. The individual components of ADCs have been diagrammatically represented in Fig. 1. This diagram provides a comprehensive overview of the structural anatomy of an ADC, highlighting the key components essential for its targeted therapeutic action in cancer treatment.

2.1 Antibody Selection

The human body can fight against a substance that triggers an immunological response by forming a considerable number of antibodies [21]. Typically, when a normal immune response is triggered, polyclonal antibodies are released that are derived from different B cells. Each of these antibodies has a respective and distinct target binding site, with diverse epitopes or similar epitopes that exhibit distinct affinities. However, it is equally feasible to frame an antibody from a single B lymphocyte. Henceforth, lots of mAbs have been developed as newer drugs since 1985 [22]. Various kinds of mAbs are used in the treatment of malignancies, such as naked mAbs, bispecific mAbs, and conjugated mAbs that include radiolabeled antibodies and ADCs [21]. Therapies, including mAbs, showcase major advantages, as these treatments provide a precise treatment option that targets only specifically marked proteins and cells. In comparison to chemotherapy or radiation therapy (RT), mAb-related treatments have a lower risk of adverse effects. In terms of therapeutic impact, mAb-related treatments are quite fast-acting and also have the potential for long-lasting immunity [23]. Despite the numerous benefits of mAbs, they still carry a potential for several side effects, including chills, weakness, diarrhea, decreased blood pressure, headache, rashes, fever, nausea, vomiting, and others [24]. The mAbs have an affinity to bind with a particular portion of the molecule or to a specific segment of the foreign component, which is identified by the immunoglobulin (Ig) [23]. Five IgGs are most preferably used as mAbs in ADCs: IgG1, which is used primarily because it has high effectiveness in activating the immune response. IgG2 and IgG4 are less effective than IgG1 but are still utilized in ADCs. IgG3 is also highly efficacious in ADCs; however, a shortcoming hinders its application, as the associated elimination half-life is relatively short [9]. As a consequence of this exceptional characteristic of mAbs, there has been significant advancement in the field of cancer, particularly in leukemias. One such drug approved by the FDA as an ADC for human use was GO, which received approval in 2000 for the treatment of AML in patients with the CD33-positive antigen [25]. GO is an ADC that consists of a humanized recombinant mAbs, specifically IgG4 kappa, due to its high affinity toward cancerous cells that display the CD33 antigen on their surface [26]. The IgG4 antibody comprises four peptide chains: two heavy chains of 50 kD and two light chains of 25 kD, linked by four disulfide bridges [23]. The IgG4 antibody leads to antibody-dependent cell-mediated phagocytosis; however, it is less efficacious and has an incompetent targeting action owing to its association with Fab arm interchange [27].

2.2 Payload Selection

The payload of ADCs, referred to as the warhead, contains the drug that employs its cytotoxic effect on tumor cells [28]. Cytotoxicity refers to the cell-killing impact exerted by a chemotherapeutic drug, resulting in apoptosis and the eradication of cancer cells. These cytotoxic drugs are highly toxic and can, therefore, be incorporated into ADCs for targeted delivery. The cytotoxic drugs should fulfill several conditions before being employed as a payload, including potency, systemic stability, specific target site, hydrophilicity, and small molecular weight [9,28]. The cytotoxic drugs must also comprise a functional group that would act as the binding site for the antibody [29]. Moreover, the binding site of the payload to the antibody should be such that the drug maintains its potency after conjugation with the antibody. Additionally, the internalization of ADCs to their binding site should not be altered by the chemistry of the payload drug [30]. From the plethora of drugs available, only a few can fulfil the technical requirements to be used as a payload. DNA-damaging agents and microtubule inhibitors are the most commonly used payloads [31]. DNA-damaging payloads, which are not cell cycle specific, damage the DNA through cross-linking the DNA strands, breakage of double-stranded DNA, or by DNA alkylation and consist of calicheamicin, pyrrolobenzodiazepine (PBD) dimers, and duocarmycin [10]. Maytansine and auristatins are microtubule inhibitors that disrupt tubulin formation and are found to be cell-cycle specific. The leading benefits of DNA-targeting warheads compared to microtubule-targeting warheads are believed to be higher potency and activity throughout all phases of the cell cycle, resulting in potent activity against both dividing and non-dividing cells [28]. GO comprises a cytotoxic payload that targets DNA and is a calicheamicin disulfide derivative

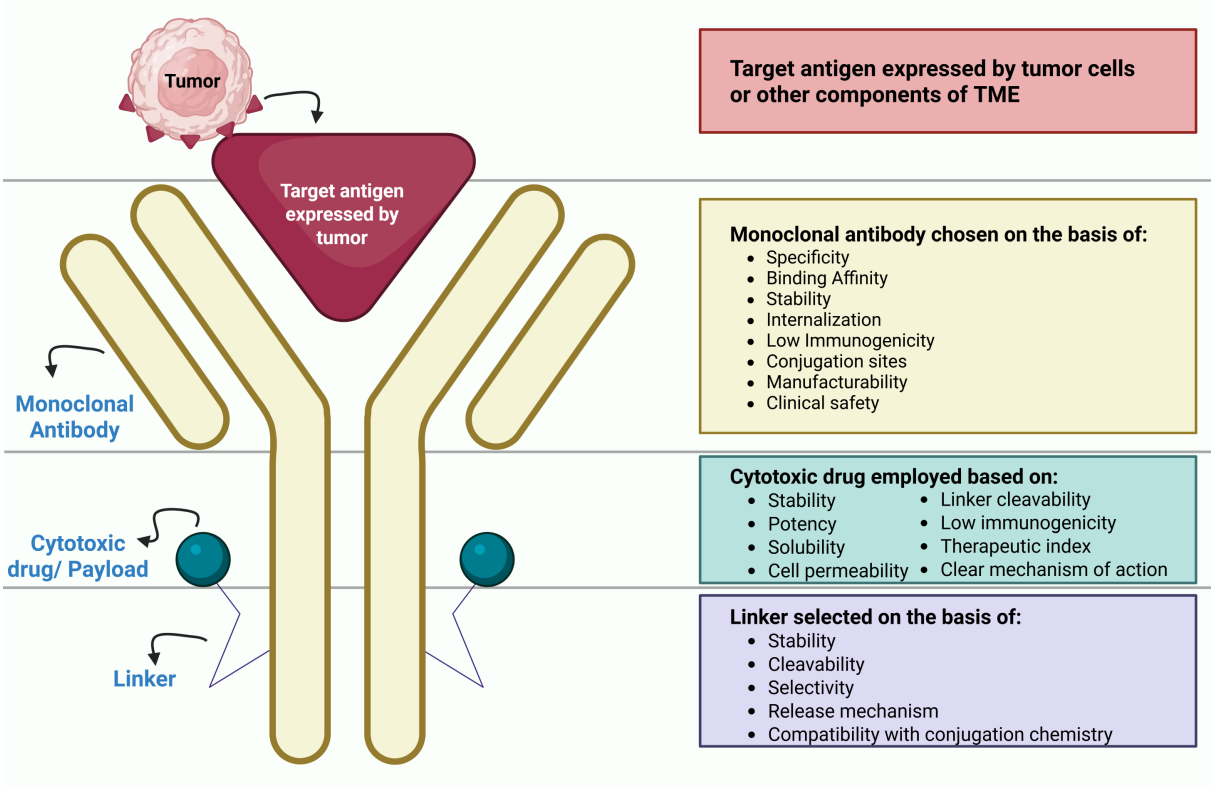

Fig. 1. Diagrammatic representation of antibody–drug conjugate components. The diagram illustrates the fundamental components of an antibody–drug conjugate (ADC), which include a monoclonal antibody (mAb), a cytotoxic drug payload, and a cleavable or non-cleavable linker. The mAb serves as the targeting moiety, recognizing and binding to specific antigens on the surface of tumor cells. This selective binding minimizes off-target effects and enhances the specificity of drug delivery. The cytotoxic drug payload, often referred to as the "warhead", exerts its cell-killing effect upon internalization by tumor cells. Various cytotoxic drugs, including DNA-damaging agents and microtubule inhibitors, can be utilized as payloads depending on their potency, stability, and target specificity. The linker acts as the bridge between the mAb and the cytotoxic drug payload. Cleavable linkers contain chemical triggers that facilitate the release of the payload within the TME. In contrast, non-cleavable linkers rely on lysosomal enzymatic degradation for the release of the payload.

of N-acetyl gamma-calicheamicin-dimethyl hydrazide [8]. Calicheamicin causes the cleavage of DNA double strands, intercalation or cross-linking of DNA strands, and transcription impairment, ultimately leading to cell apoptosis by binding to the minor groove of DNA [9,30]. The various analogs of calicheamicin include iodinated analogs, such as α2I, α3I, β1I, and δ1I, as well as brominated analogs, including β1Br and γ1Br. The disulfide derivative of natural calicheamicin endows a functional group for its conjugation with the antibody. The calicheamicin disulfide derivative of (N-acetyl gamma-calicheamicin-dimethyl hydrazide) was preferred for GO owing to its greater stability [28].

2.3 Linker Selection

The core structure of the ADC consists of an antibody and a payload connected by a linker through covalent conjugation [32,33]. The properties of a linker indirectly influence the therapeutic effectiveness and pharmacokinetic parameters of ADCs since the aggregation of the payload and its release in tumor cells are reliant on the stability of the linker [34,35]. Ideal features that linkers have exhibited are as follows: (A) Exhibit stability in bloodstream circulation and (B) deliver target tissue-specific liberation of payload after formation of the antigen–antibody complex to minimize off-target toxicities [34]. Considering the presence or absence of chemical triggers, linkers have been broadly distinguished into cleavable linkers and non-cleavable linkers. Cleavable linkers are a type of linker that contains chemical triggers in their configuration, which, upon cleavage, release the chemotherapeutic payload in the tumor microenvironment (TME) [36]. Cleavable linkers are further subdivided into enzymatically cleavable linkers and chemically cleavable linkers [32]. For instance, in GO, the chemotherapeutic drug and mAb are connected through a cleavable linker, namely 4-(4-acetylphenoxy) butanoic acid (AcBut linker), via an amide bond with the antibody and a hydrazone bond with the payload drug [37]. Alternatively, there is an absence of chemical triggers and stable bonds in non-cleavable linkers, where the cytotoxic payload is liberated through lysosomal enzymatic degradation of the antibody, resulting in the concomitant removal of the linker [23]. Non-cleavable linkers are further subdivided into thioether

or maleimidocaproyl (MC) [38]. Numerous limitations of linkers have been noted, which include: (a) the unspecific delivery of chemotherapeutic payloads that result in toxicities in normal cells, along with the restricted therapeutic index of ADCs [39]. GO, developed by Pfizer, was initially withdrawn in 2010 due to toxicities caused by an unstable N-acyl hydrazone linker, which led to the premature release of calicheamicin in plasma. However, GO was later redesigned and reapproved by the FDA in 2017 under the name Mylotarg [40]. (b) The effectiveness of ADCs is minimized due to the occurrence of the retro-Michael elimination reaction, which results from the attachment of maleimide. (c) The confined linker–payload attachment is inadequate for the accelerated expansion of chemotherapeutic payloads. To overcome these limitations, significant progress has been made in the design and optimization of ADC linkers. These advancements include: (i) improving the specificity of chemical triggers currently in use and developing novel chemical triggers for highly selective linker activation; (ii) enhancing linker–antibody conjugation methods to produce more stable and consistent ADC constructs; (iii) expanding the diversity of linker–payload conjugation strategies to enable broader payload incorporation; (iv) optimizing linkers to improve the pharmacokinetic properties of ADCs, thereby enhancing their therapeutic performance [33].

3. Mechanism of Action of ADCs

The major advantages of ADCs as a treatment for leukemia are their specificity in targeting the destruction of the cancerous cells and their property to exhibit minimal toxicity toward non-cancerous cells [41]. Various antigens that are highly expressed on cancerous cells can be utilized as targets by ADCs [18]. Meanwhile, an appropriate mAb must be selected based on its ability to rapidly uptake into cellular targets [42]. The payload present in ADCs operates on any microtubules or DNA molecules, leading to apoptosis. It has been found that the payload tends to function as a tubulin inhibitor on microtubules, similar to the effects of maytansines and auristatins, or to cause damage to DNA molecules, leading to apoptosis, as observed with PBD and calicheamicin [11]. Linkers in ADCs function based on their type to release the payload in targeted cancerous cells. Cleavable linkers demonstrate their effect by releasing a chemotherapeutic payload in response to biochemical indicators, such as low pH and enzymes. In contrast, non-cleavable linkers release the payload upon lysis of the attached antibody [36,43].

The framework of ADCs exceptionally highlights the role of these conjugates in specifically binding to the target site of the antigen, where they deliver a considerable quantity of drug directly onto the surface of the cancerous cells. Consequently, an antigen–ADC complex forms once the ADC binds to the oncogenic antigen [42]. This complex, then, with the support of receptor-mediated endocytosis (which can be clathrin- or caveolae-mediated endocytosis) or pinocytosis, penetrates the tumor cell and merges with the lysosomes. Before the complex joins with the lysosome, internalization proceeds to the formation of an early endosome, which later develops into a late endosome [14]. The fusion of the antigen–ADC complex with lysosomes further advances the processing and trafficking of ADCs within cells. Moreover, the fusion leads to a drop in the pH level, causing disunion of the ADC structure and delivery of the payload [44]. This results in the release of the drug from the ADC composition into the cytoplasm of the tumor cell. The drug then binds to targets such as topoisomerases, DNA, and tubulins, thereby halting the cell cycle and leading to cell death [45]. If the metabolites of the drug penetrate the cell membrane, they spread to adjacent cells and progress toward the death of non-participating cells. This mechanism addresses the ordinary functioning of the ADCs; nevertheless, the mAb in each ADC has a distinct mechanism for binding to a specific antigen. Additionally, for the sake of clarity, a comprehensive theory of how GO works has been presented previously [42].

GO targets CD33, which is not highly expressed on mature cells of myeloid or lymphoid origin, nor on normal hematopoietic cells; however, CD33 is expressed on most blast cells in leukemia [9,46]. The humanized mAb in GO identifies and interacts with the CD33 antigen expressed on the surface of tumor cells, resulting in the binding of GO to CD33 and the formation of the GO–CD33 complex [46]. This complex is internalized into the cytoplasm from where the complex travels into the lysosome. The acidic environment of the lysosome leads to rapid hydrolysis of the complex, initiating the release of the payload, *i.e.*, calicheamicin [47]. The DNA-targeting calicheamicin involves two areas that differ structurally, one of which has a sugar residue that both delivers the drug to the target site and binds to the double-stranded DNA [28]. This DNA-targeting drug is converted into reactive species that bind to the minor groove of DNA, inducing irreparable double-strand breaks by abstracting two hydrogen atoms from the DNA structure [48,49]. This interaction of radicals with minor grooves results in DNA strand breaks, which, if not repaired, can lead to apoptosis. The phosphorylation of Bcl-2-associated protein x (BAX) and Bcl-2 homologous antagonist/killer (BAK) proteins triggers cell death through the activation of caspase 3 and caspase 9 in the intrinsic apoptotic pathway [47]. Furthermore, DNA double-strand breaks inhibit DNA synthesis, thereby directing the cell toward death [48]. Fig. 2 elucidates the intricate process of targeted cytotoxicity mediated by ADCs in targeted cancer therapy, using GO as an example to explain the process.

4. Clinical Utilization of ADCs in Acute Leukemias

The clinical application of ADCs emerged as a groundbreaking paradigm in the clinical landscape of ALs,

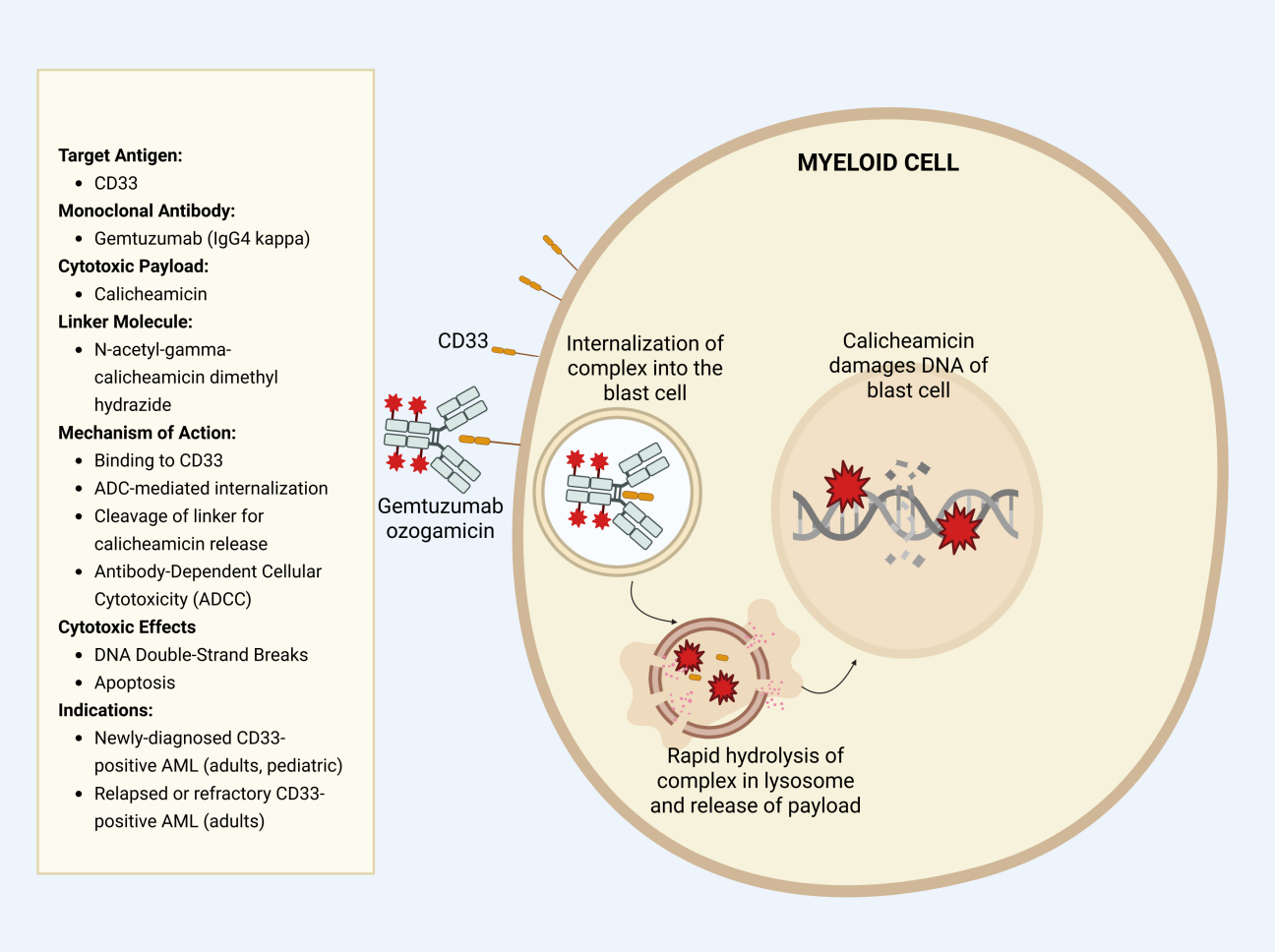

Fig. 2. Mechanism of action for GO, the first FDA-approved ADC. This diagram depicts the specific mechanism of action for GO, the pioneering ADC approved by the FDA for clinical use. GO targets the CD33 antigen, which is expressed on the surface of leukemia cells. Upon binding to CD33, GO is internalized and transported to the lysosome, where the cytotoxic payload, calicheamicin, is released. Calicheamicin induces DNA damage by causing DNA double-strand breaks, leading to cell death through apoptosis. This figure provides a detailed illustration of the unique mechanism through which GO selectively targets and eradicates leukemia cells.

ushering in a new era of targeted and precision medicine. This section provides a close examination of the practical application of ADCs, with a primary focus on their role in treating AML and ALL. Supported by evidence from clinical trials, specific ADCs, including GO and vadastuximab talirine, will be examined for their efficacy, safety profiles, and overall impact on patient outcomes. Through a clinical validation vision, this exploration sheds light on the transformative potential of ADCs in the treatment landscape for AL. The clinical progress and outcomes associated with various ADCs targeting AML in different therapeutic settings, ranging from newly diagnosed to relapsed/refractory cases, are outlined in Table 1, summarizing pivotal clinical trials discussed across subsections 4.1–4.9.

4.1 Gemtuzumab Ozogamicin

GO is a humanized IgG4 mAb that comprises N-acetyl-γ calicheamicin dimethyl hydrazide, a derivative of calicheamicin as warhead that is linked to the antibody via bifunctional AcBut linker [9]. Preclinical studies of GO, as summarized in Table 2 (Ref. [50,51]), have laid the foundation for these clinical investigations by demonstrating its target specificity, cytotoxic potency, and safety profile in relevant AML models. Moreover, preclinical studies are essential for characterizing the pharmacodynamics, pharmacokinetics, toxicity, and antitumor activity of ADCs before advancing to human trials. Importantly, one of the first ADCs to be approved, GO, which targets the CD33 antigen, was subjected to a Phase 3, open-label, randomized, double-arm study to evaluate the efficacy of GO in combination with conventional chemotherapy (NCT00372593). Fig. 2 illustrates the specific targeting of the CD33 antigen on leukemia cells by GO, highlighting its mechanism of inducing DNA damage, which ultimately triggers apoptosis. The chemotherapeutic regimen chosen to be combined with GO as one of the arms included asparaginase, cy-

tarabine, daunorubicin, etoposide, and mitoxantrone compared to the same chemotherapy regimen without GO in CD33-positive AML patients who were newly diagnosed [52]. A total of 1070 children and adults up to the age of 29 years were enrolled in this clinical study. Eligibility criteria excluded promyelocytic leukemia (PML), pregnancy, prior chemotherapy, RT, any anti-leukemic therapy, or secondary AML. A total of 48 participants failed to fulfil the eligibility criteria, and, thus, only 1022 patients were analyzed in this trial. The primary objectives of this study included measures of event-free survival (EFS) and OS at a time duration of 3 years. Participants were randomly assigned to one of two treatment arms: Arm A received conventional chemotherapy alone, while Arm B received conventional chemotherapy in combination with GO. In Arm B, GO was administered at a dose of 3 mg/m^2 on day 6 of the first induction course and day 7 of the second intensification course. This trial concluded that GO combined with chemotherapy yields improved EFS, and this combination can be brought into practice if the safety profile can be further enhanced.

A single-arm Phase II clinical trial (NCT02117297) was conducted to assess the impact of GO as a consolidation therapy following allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients with average-risk AML. The study included 26 patients under the age of 25 years who had achieved their first CR and underwent allo-HSCT from either a matched family donor or an unrelated donor. GO was administered intravenously at a dose of 9 mg/m^2 over a two-hour infusion, given twice between days 60 and 180 post-transplantation. Eligible participants had CD33 expression of $\geq$10% and were free from significant comorbid conditions involving cardiac, hepatic, pulmonary, or renal systems. The primary endpoints of the study included the incidence of graft failure (assessed on day 42 post-transplant), EFS, OS, and safety outcomes, which were evaluated at one year following treatment. Secondary endpoints included the analysis of minor histocompatibility antigens to detect cytotoxic T lymphocytes and the evaluation of donor chimerism to estimate the risk of relapse and the potential development of acute or chronic graft-versus-host disease (GVHD). The comprehensive assessment of these parameters aimed to determine the safety and therapeutic efficacy of GO as post-transplant consolidation (allo-HSCT). If the findings support a favorable risk-benefit profile, GO may serve as a valuable post-transplant therapy in AML. Additional clinical investigations involving GO are outlined in Table 3.

4.2 Vadastuximab Talirine

Vadastuximab talirine is an IgG1-kappa mAb that includes PBD dimer SGD-1882 as a payload, which is linked to the antibody through a (valine–alanine dipeptide) MC cleavable linker [48]. Vadastuximab talirine or SGN-CD33A, a CD33-targeting ADC, was analyzed in a non-randomized, open-label, Phase 1 clinical trial to determine its safety in AML patients when administered in combination with a standard 7+3 regimen (NCT02326584). The SGN-CD33A doses were escalated and administered in combination with the standard treatment [53]. A total of 116 participants were enrolled in this clinical trial. SGN-CD33A was administered either as a single dose (day 1) or in two doses, given on days 1 and 4 of the cycle, in combination with the standard 7+3 cytarabine and daunorubicin regimen. The study included patients with all subtypes of AML, aged 18 years or older, who had normal liver and kidney functions and an Eastern Cooperative Oncology Group (ECOG) status of 0 or 1. The patients who had received treatment for myelodysplastic syndrome (MDS) or myeloproliferative neoplasms (MPN) previously or those with impaired lung or cardiac function were excluded. The standard dose of administration for the induction phase was 100 mg/m^2/day of cytarabine on days 1–7 and 60 mg/m^2/day of daunorubicin on days 1–3. In the consolidation phase, high-dose cytarabine, 3 g/m^2, was administered on the first, third, and fifth day of every cycle. Primary endpoints included parameters such as incidence of dose-limiting toxicities (DLT), AEs, and laboratory abnormalities. Secondary outcomes such as OS, leukemia-free survival (LFS), and minimal residual disease (MRD) were measured at 3 years, and CR was measured at the end of induction. The study was conducted in the induction and consolidation phases in combination, wherein vadastuximab talirine was administered in a split dose. In the maintenance phase, SGN-CD33A was given alone. A second induction phase was then employed, in which a single dose of SGN-CD33A was administered. The results of the induction phase demonstrated a cohort of 42 patients who were administered a split dose of SGN-CD33A. A total of 4 of the 42 participants received 10 mcg/kg on days 1 and 4, while 38 participants received 20 mcg/kg on day 1 and 10 mcg/kg on day 4. A cohort of 25 patients was administered a single dose of vadastuximab talirine. A total of 14 participants received a dose of 30 mcg/kg, while 11 participants received a dose of 40 mcg/kg. Based on the results of this trial, we conclude that the combination of vadastuximab talirine with the standard 7+3 regimen is beneficial compared to the conventional 7+3 regimen practice in terms of efficacy; meanwhile, the safety portfolio of both arms did not differ remarkably.

A Phase-1, open-label, non-randomized, two-arm study was carried out to evaluate the safety profile of vadastuximab talirine, administered either alone or in combination with hypomethylating agents (HMAs), in patients diagnosed with AML (NCT01902329). A total of 195 participants aged 18 years or older were enrolled in this clinical trial [54]. The purpose of this study was to find the MTD of SGN-CD33A along with its pharmacokinetic profile [55]. Patients with CD33-positive AML, an ECOG status of 0 or 1, and adequate liver and kidney function, who had relapsed or had new cases of AML, were included in

the study. The exclusion criteria were patients with inadequate lung function, those who had received anti-leukemic chemotherapy within the previous 14 days, or those who had received allo-HSCT. AE-related incidences and laboratory abnormalities were the primary measured outcomes. The OS, CR, and duration of CR were the secondary outcomes. One arm was treated with a combination therapy of SGN-CD33A and HMA, while the other arm received SGN-CD33A monotherapy. The dose of HMA, such as azacitidine, was 75 mg/m^2 for 7 days, and that of decitabine was 20 mg/m^2 for 5 days. SGN-CD33A was administered intravenously either as a single dose on day 1 or split into two doses, administered on days 1 and 4 of the 3-week cycle. We may conclude from this study that the combination of HMAs with vadastuximab talirine augments the efficacy and RR, while close monitoring is needed to manage the hematological suppression.

4.3 Brentuximab Vedotin

BV, also assigned as SGN-35, is an ADC composed of the IgG1 kappa antibody that specifically targets the CD30 antigen by binding to the monomethyl auristatin E (MMAE) molecule via a protease-cleavable linker known as valine-citrulline [56]. A Phase I, open-label, single-arm study was conducted to evaluate the administration of BV in combination with the minimum effective concentration (MEC) regimen as a conventional re-induction chemotherapy, which included mitoxantrone, etoposide, and cytarabine (NCT01830777). According to several other research studies, BV is a drug that gradually slows the progression of malignancies, suggesting that BV may be associated with the expression of CD30. Since the AML tumor cells express the CD30 antigen, BV may help treat AML [56]. A total of 22 patients opted into the study, which included individuals aged 18 years or older. The participants were required to undergo all tests and procedures before commencing the study, including physical capability assessments, urine and blood tests, pregnancy tests, electrocardiograms (ECGs), and electroencephalograms (EEGs). Furthermore, only patients who had relapsed with AML after a 3-month remission and expressed the CD30 antigen were allowed to participate in the study. For this particular study, the treatment was divided into two phases: (A) Re-induction phase, the patients were administered a 3+3 dose escalation model of IV BV (0.9 mg/kg, 1.2 mg/kg, 1.8 mg/kg) on day 1 followed by IV MEC chemotherapeutic regimen (mitoxantrone: 10 mg/m^2 IV for 6–10 mins; etoposide: 100 mg/m^2 IV for 60 mins; cytarabine: 1000 mg/m^2 IV for 60 mins) from day 3–7. All patients at this stage received different doses of the study drug to assess the maximum tolerable and safe dose of BV. The patients were allocated to the next phase if this treatment did not result in a reduction in the number of leukemic cells. (B) Maintenance phase—patients were administered a single IV BV every 21 days for 12 months and were continuously assessed. This study primarily aimed to determine the MTD of BV that is safe for administration with the MEC regimen and contributes to the minimal number of side effects in relapsed AML with CD30 expression for at least 2 years. Secondarily, this study also focused on DLTs and governing RR, OS, and disease-free survival rate. This study proved the safety of BV with MEC in CD30-expressing relapsed AML. Furthermore, investigations focusing on its efficacy parameters are needed in addition to an extensive exploration of safety aspects in a larger population.

A non-randomized, open-label, single-arm study was conducted, divided into three phases: a pilot phase, Phase I, and Phase II (NCT02096042). All three phases were organized with respect to their specific goals. The pilot phase aimed to evaluate the safety of BV in patients with AML. Phase I aimed to assess the safety of BV in combination with azacytidine, while Phase II evaluated the efficacy of BV combined with azacytidine in treating AML. Three groups were planned for the pilot phase and Phase I, each comprising 3–6 participants. Phase II was to enroll 25 participants. A total of 61 patients were estimated to participate in the study, but only one participant was enrolled. Due to the extremely low participation rate, the study was terminated prematurely. The individuals would have been chosen based on the following criteria: age 18 years or older, biopsy-confirmed diagnosis of AML, expression of the CD30 antigen, and normal blood and biochemical parameters. In the pilot phase, patients were to receive an IV infusion of BV 1.2 mg/kg for 30 mins on days 1, 8, and 15 of the 28-day cycle. In Phase I, IV infusion of BV, 1 mg/kg for 30 mins on days 1, 8, and 15 of the 28-day cycle, along with 75 mg/m^2/day of 5-azacytidine IV or subcutaneous (SC) on days 1–7 of the 28-day cycle, was provided to the patients. In Phase II, the considered dose of BV was the MTD assessed in the pilot phase and Phase I, while the dose of azacytidine remained unchanged from Phase I. After the completion of the 28-day cycle, the study aimed to assess the MTD of BV with azacytidine in addition to minimal DLTs. Moreover, the study tried to evaluate the objective response rate (ORR), CR, CR with incomplete platelet recovery (CRp), CR with incomplete hematological recovery (Cri), and partial remission (PR) after four 28-day cycles up to 120 days. Due to the early termination of the study, there was no analysis of the results and their associated AEs.

4.4 Pivekimab Sunirine

Pirvekimab sunirine (PS), also referred to as IMGN632 or MGN 632, is a humanized anti-CD123 monoclonal antibody (G4723A) conjugated to a DNA mono-alkylating agent from the indolinobenzodiazepine pseudodimer (IGN) class. The cytotoxic payload is linked via a cleavable linker, specifically maleimidocaproyl-valyl-citrullinyl-p-aminobenzyloxycarbonyl (mc-val-cit-PABC). Each antibody can bind to two payload molecules [57]. An open-label, multicenter, Phase Ib/II trial aimed to estimate

the safety and acceptability of IMGN632 and evaluate the agonistic action toward leukemia when administered as a monotherapy or in combination with azacitidine and/or venetoclax in relapsed and CD123-positive AML patients (NCT04086264). A parallel type of interventional study was conducted where 242 adult patients aged 18 years or older were allocated in a non-randomized manner. Patients who were categorized in the group of experimental regimen A were subjected to receive IV IMGN632 in a dose of 0.015 mg/kg, 0.045 mg/kg, or 0.09 mg/kg on the seventh day of a 28-day cycle, along with daily administration of azacitidine. The SC or IV administration was conducted using a dose of 75 mg/m^2 from the first to the seventh day of a 28-day cycle. Patients enrolled in the experimental regimen B group received intravenous IMGN632 at doses of 0.015 mg/kg, 0.045 mg/kg, or 0.09 mg/kg on day 7 of a 21-day treatment cycle. This was administered in combination with oral venetoclax, starting at 100 mg on day 1, escalating to 200 mg on day 2, and then to 400 mg from day 3 onward, continued daily through day 21 of the same cycle. Patients who were designated in the arm of experimental regimen C were directed to receive a triple therapy of IMGN632, azacitidine, and venetoclax where IMGN632 was administered IV on day 7 of a 28-day cycle at doses of 0.015 mg/kg or 0.045 mg/kg in conjugation with daily administration of azacitidine, SC, or IV in doses of 35–75 mg/m^2 from days 1–7 of a 28-day cycle and orally administered venetoclax in a dose of 100 mg on day 1, 200 mg day 2, and 400 mg on day 3, which continued daily up to day 28 of the 28-day cycle. Patients who were assigned to the experimental regimen D arm were managed using a monotherapy of IMGN632 administered IV at a dose of 0.045 mg/kg on day 1 of a 21-day cycle in fit and unfit MRD+ patients. The primary outcome measures, including safety and tolerability, preliminary anti-leukemic activity, and MRD levels, were evaluated over approximately 7 months, 20 months, and 18 months, respectively. The secondary outcome measures monitored in patients within 1 year of treatment included TEAEs, dose escalation and expansion studies, and anti-drug and antibody concentration.

A Phase 1 trial is to be conducted to estimate the most supportable and efficacious dose of IMGN632 in newly diagnosed AML patients (NCT06034470). Thirty adult patients aged 18 or older received IMGN632 IV in combination with fludarabine IV, high-dose cytarabine IV, granulocyte colony-stimulating factor (G-CSF) SC, and idarubicin IV (FLAG-Ida) regimen. Patients undergone specific procedures essential for assessing drug efficacy, including biospecimen collection, bone marrow aspiration and biopsy, as well as multi-gated acquisition scanning. After completion of study treatment, patients followed up every 3 months for the forthcoming 2 years. The primary outcome measures monitored include the incidences of DLTs after completion of 1 cycle (42 days). The DLTs were subcategorized as: (a) Any grade of $\geq$4 organ toxicity; (b) continued severe myelosuppression, as defined by absolute neutrophil count (ANC) $<$500/µL and platelet count $<$25,000/µL, for $>$42 days after treatment of idarubicin and IMGN632. The secondary outcome assessed for 2 years of the study include incidence of AEs, MRD rates, MRD status, relapse-free survival, CR rates, and duration of cytopenia, along with the proportion of patients receiving allo-HSCT. Results of this trial may be retrieved post-completion, which is estimated to conclude after December 2027. The determination of the safety and efficacy aspects of IMGN632 in newly diagnosed AML patients may raise the possibility of whether this drug can be employed in a neoadjuvant setting.

4.5 AVE9633

AVE9633, also known as IMGN-633, is an ADC comprising a humanized anti-CD33 IgG1 mAb-conjugated via a cleavable disulfide linker to the cytotoxic maytansinoid DM4 (N2′-deacetyl-N2′-(4-mercapto-4-methyl-1-oxopentyl)-maytansine), which induces cell death through tubulin depolymerization [48]. A phase I, non-randomized, open-label, single-arm interventional study was conducted to assess the maximum safe dose and related pharmacokinetic parameters of AVE9633 as a sole agent in the treatment of CD33-positive relapsed/refractory AML (NCT00543972). Since the study did not provide enough validation regarding the therapeutic action of the molecule at lethal doses, it was terminated within a year. A summary of the one-year methodology employed in the study is provided below. A total of 25 individuals aged 18 years or older were estimated to be enrolled for the trial, but only 12 were actually enrolled and recruited. Only those individuals were allowed to participate who had been suffering from CD33-positive relapsed AML even after the administration of a standard therapy regimen, as if no other remedial option was available. Additionally, their ECOG performance status/score was required to be between 0 and 2. Participants who were undergoing GO therapy or had undergone allo-HSCT within 6 months before the trial were readily excluded from the study. Participants who were administered any type of chemotherapy, RT, targeted therapy at least 3 weeks prior to the trial commencing, or any previous exposure to AVE9633 were not permitted. The study involved the administration of an IV infusion of AVE9633 on days 1, 4, and 7 of the 4-week cycle in patients with CD33-positive, refractory AML. The study primarily aimed to evaluate the MTD of AVE9633 and appraise the incidence of DLTs at particular dose levels within the decided study period. The study also focused on determining the clinical activity, safety profile, and pharmacokinetic parameters such as CR, time and duration of CR, CRp, PR, peripheral blast clearance, and certain AEs correlated to it. The study might have yielded better outcomes if the administration of AVE9633, instead of a single exposure, had been combined with another therapy

that could work synergistically in terms of therapeutic efficacy and safety, or if its dose had been adjusted appropriately.

4.6 SGN-CD123A

SGN-CD123A consists of a humanized anti-CD123 antibody conjugated to two molecules of a highly potent DNA cross-linking agent called a PBD dimer, via a stable, protease-cleavable dipeptide linker [58]. A phase 1, open-label trial was conducted to determine the safety, efficacy, and anti-leukemic activity of SGN-CD123A (NCT02848248). The study was conducted in two parts: part A was the dose escalation stage, and part B was the dose expansion phase [59]. Patients received up to two induction cycles, each lasting 3 weeks, followed by two cycles of the dose expansion stage, also lasting 3 weeks. A total of 17 participants were enrolled in this study. Patients aged 18–74 years, with normal baseline renal and hepatic function, an ECOG score of 0 or 1, and detectable CD123 leukemia, were included in this trial. Participants who had received prior allo-HSCT, a history of cardio or cerebral events within 6 months, or had PML were excluded. Participants received an IV infusion of SGN-CD123A every 3 weeks. The primary outcomes that were measured included the type and severity of the AEs, laboratory abnormalities, and DLT, along with measurement of secondary outcomes such as CR, RR, LFS, and OS. The results demonstrate the high activity of SGN-CD123A against leukemic cells regardless of drug resistance. Despite achieving tremendous anti-tumor results, the study was terminated due to undisclosed reasons. Further investigations into the utilization of SGN-CD123A in AML may reveal a more promising scenario in anti-leukemic therapy.

4.7 Zilovertamab Vedotin

VLA-101, also known as zilovertamab vedotin or MK-2140, is assembled by a humanized mAb that binds to the MMAE payload, which is an anti-microtubule cytotoxin, through a cleavable linker mc-val-cit-PABC [60]. An open-label, Phase 1 dose-escalation basket trial was conducted to assess the numerous characteristics of ADC zilovertamab vedotin, including its pharmacokinetic profile and efficacy in eliciting immunological responses, as well as safety and efficacy in patients previously treated for hematological malignancies (NCT03833180). Zilovertamab vedotin was administered intravenously as part of a non-randomized allocation to 330 patients aged 18 years or older with normal renal and hepatic profiles. Patients included in the study were found to have resolved acute toxic effects that occurred from previously administered chemotherapeutic drugs used for treatment. Patients who had a spread of malignancy in the central nervous system and had any other cardiovascular, renal, or hepatic complications were excluded from the study. Groups of 3 to 6 subjects were sequentially recruited at gradually increasing dose levels within each dosing schedule, where zilovertamab vedotin was administered in a 3+3 dose-escalation pattern. The primary outcome measures, such as the MTD of zilovertamab vedotin and recommended dosing regimens, were determined. Other outcomes, such as OS, ORR, progression-free survival (PFS), and CR without measurable residual disease (CRMRD), time to response (TTR), and duration of response (DOR), along with pharmacokinetic parameters, were also recorded. Data for participants who faced AEs were investigated. Details of participants who discontinued the study due to an AE were also noted. As part of the currently ongoing study, the primary and secondary outcomes for ADC administration in participants allocated to schedule 1 have been published. The ADC, zilovertamab vedotin, demonstrated positive outcomes in terms of safety and efficacy when administered to patients with recurrent non-Hodgkin lymphoma (NHL) [61]. These results on the effectiveness of ADCs in NHL assume that ADCs will also yield constructive results when administered for the treatment of relapsed/recurrent AL of myeloid or lymphoid origin.

4.8 DCLL9718S

DCLL9718S is an ADC consisting of an IgG1 anti-CLL1 antibody conjugated to two PBD dimers through a cleavable disulfide linker, designed to target C-type lectin-1 (CLL-1) [10]. CLL-1 is expressed on the surface of AML blast cells but not on hematopoietic stem cells. A Phase 1, open-label, non-randomized, multicenter, dose-escalation study was conducted to evaluate the efficacy, safety, and tolerability of DCLL9718S as a single agent and in combination with azacitidine (NCT03298516). This study comprised two parallel arms, with each arm undergoing two stages: a dose-escalation stage followed by a dose-expansion stage [62]. Participants in arm A received escalating doses of DCLL9718S in a 21-day cycle to determine the MTD and recommended phase 2 dose (RP2D), followed by administration of DCLL9718S at the RP2D in the dose expansion stage. Similarly, participants in arm B received DCLL9718S in the same pattern as that of arm A, along with azacitidine 75 mg/m^2 SC or IV on days 1–7 to determine the MTD and RP2D, followed by DCLL9718S at the RP2D in combination with the same dose of azacitidine. Nineteen participants aged $\geq$18 years were enrolled in this study who had a diagnosis of AML as per the WHO criteria, an ECOG score of $\leq$2, and adequate end-organ function. Participants who had undergone prior allo-HSCT or solid organ transplantation, who had active CNS involvement, or who had a family history of long QT interval were excluded. The primary outcome measures included a percentage of participants with AEs and DLTs, MTD, and the RP2D of DCLL9718S. The secondary outcome measures were the pharmacokinetics of DCLL9718S, OS, EFS, and PFS. Based on the safety and efficacy data, the study was stopped in the dose escalation phase due to toxicities for

which the PBD payload of DCLL9718S was accountable in an uncertain manner. CLL-1 remains a workable target for AML, although the potential toxicities pose a definite risk, particularly at doses that may exhibit anti-leukemic effects.

4.9 Miscellaneous ADCs

The ADCs that have been elucidated in the preceding sections are, by any means, considered as the first and foremost ADCs that should be utilized as a targeted therapy for AML. However, in addition to those ADCs, several other immunoconjugates are still being studied and reported in relation to AML treatment, including IMGN779, LOP628, and AGS67E. IMGN779, as an anti-CD33 immunoconjugate, comprises the mAb Z4681A that is attached to a drug DGN462, which is a DNA alkylating agent and consists of a dimer known as indolino-benzodiazepine (a mono-imine moiety) via a disulfide linker (sulfo-SPDB) [63]. The next is LOP628, a humanized anti-c-kit ADC that particularly targets the CD117 proto-oncogene. LOP628 is composed of the mAb, IgG1/kappa, which is conjugated with a maytansine payload through a non-cleavable linker [64]. One additional ADC is AGS67E, a human IgG2 mAb designed to target the CD37 antigen present on the surface of malignant cells. The protease-cleavable linker facilitates the binding of this antibody to MMAE, which serves as a payload/drug and is a microtubule-disrupting agent [65].

As all the previous ADCs have been previously mentioned with respect to their completed, ongoing, or terminated trials, in a similar manner, certain trials have also been conducted for these ADCs. The trial for the ADC, IMGN779, has been issued under (NCT02674763). Similarly, the study conducted on LOP628 can be explored under (NCT02221505). Lastly, the research carried out for the ADC AGS67E can be referenced from (NCT02610062). Thus, the ADCs presented here are among those that can also be used in the treatment of AML, but further study is required.

5. Challenges Associated With ADCs

Navigating the landscape of ADCs in cancer therapy presents a set of formidable challenges that warrant scrutiny. Despite their targeted precision, ADCs encounter hurdles such as off-target toxicities, limited payload delivery, potential immunogenicity, and several other challenges. This section aims to address the challenges associated with ADC development and implementation, providing insights into the ongoing quest for solutions in the pursuit of refined cancer therapeutics. Critical challenges in the utilization of ADCs are comprehensively presented in Fig. 3, emphasizing factors such as off-target toxicity, cost considerations, immunogenicity, intricacies in linker chemistry, premature payload release, linker stability, and complexities in antibody engineering. Moreover, Fig. 3 highlights the diverse challenges that hinder the seamless utilization of ADCs, including off-target toxicity, cost implications, immunogenic responses, complexities in linker chemistry, premature payload release, concerns over linker stability, and intricacies in antibody engineering.

5.1 Resistance Mechanisms

One of the major challenges hampering the delivery of the efficient therapeutic potential of ADCs is the development of resistance. Conventional treatment options for AML have also failed due to drug efflux by the adenosine-triphosphate (ATP)-binding cassette (ABC) protein superfamily [66]. Of all efflux pumps, the P-glycoprotein (P-gp) is identified as pumping out several anti-leukemic agents from cells, including but not limited to calicheamicin derivatives. Thus, since calicheamicin forms part of the ADC assembly of GO, the calicheamicin derivatives detach from GO in lysosomes and are subsequently pumped out. The underlying mechanism responsible for this phenomenon is the size and structural similarity of calicheamicin with that of identified substrates of multidrug resistance (MDR) P-gp [67]. Such as, *in vitro* studies have demonstrated the mediation of resistance to GO in cell lines expressing P-gp. This substantiation was further supported by observations showing that the sensitivity of GO increased *in vitro* in the presence of cyclosporine A, an MDR inhibitor. These findings suggest a modulating effect of P-gp on cytotoxicity, highlighting the potential use of MDR inhibitors in enhancing the efficacy of anti-leukemic agents both *in vitro* and in clinical settings [68].

Another resistance mechanism is provided by multidrug resistance protein 1 (MRP1), which is overexpressed in 10–30% of AML patients, with a higher frequency noted in relapse cases compared to the initial diagnosis. MRP1 can reduce GO-induced cytotoxicity in both cell lines and certain primary AML samples [69]. In addition, the involvement of anti-apoptotic proteins Bcl-2 and Bcl-x is also noted to contribute to GO resistance. Studies have demonstrated the heightened effect of GO by the Bcl-2 antisense oligonucleotide, while a diminished GO response has been observed in the presence of overexpressed Bcl-2 and bcl-x. GO induces proapoptotic activation of BAK and BAX, along with stress-activated protein kinase, in sensitive AML cells, whereas it does not affect resistant ones. This suggests a potential role for these factors in GO resistance. Additionally, the activation of survival signaling pathways, such as PI3K/AKT, MEK/ERK, and JAK/STAT, is associated with resistance to GO in AML cells. An AKT inhibitor, MK-2206, has been shown to restore the sensitivity of resistant AML cells to GO and calicheamicin [68,70].

The pharmacological efficacy of GO is anticipated to improve following effective delivery to the bone marrow. However, elevated CD33 tumor burden in the peripheral circulation may bind to the antibody component of the GO–ADC complex, thereby reducing its bioavailability and impeding efficient delivery to the bone marrow blast cells. This sequestration contributes to drug re-

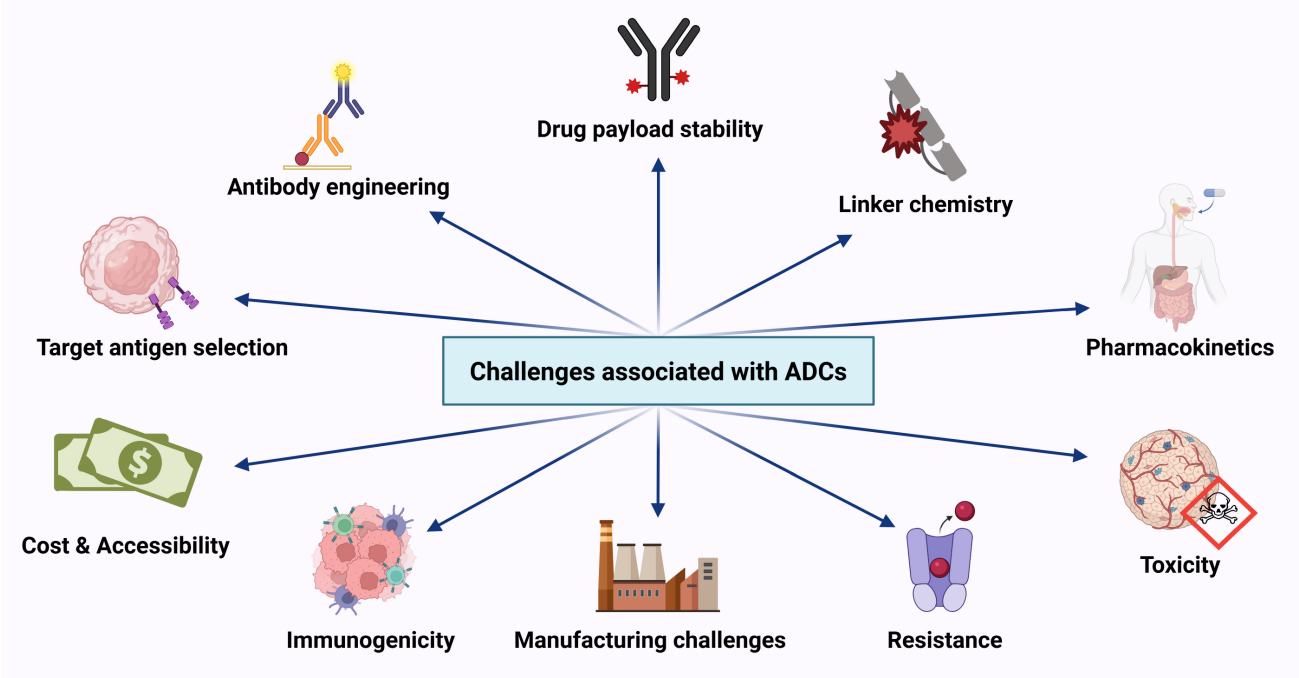

Fig. 3. Challenges associated with the use of ADCs. This figure delineates the multifaceted challenges associated with the application of ADCs in therapeutic contexts. Key challenges include off-target toxicity, cost considerations, immunogenicity, intricacies of linker chemistry, premature payload release, concerns over linker stability, and the complexities of antibody engineering. Understanding and addressing these challenges are crucial for the successful development and implementation of ADCs in clinical settings.

sistance, resulting in a diminished clinical response due to reduced GO uptake at the intended target site [71]. In addition to this mechanism, the cell cycle status also plays a role in resistance. Resting or non-proliferative cells exhibit reduced sensitivity to calicheamicin-induced cytotoxicity and show minimal uptake of GO, even when CD33 is expressed. Interestingly, when these quiescent cells are activated, their sensitivity to GO increased. *In vitro* studies have demonstrated that the combination of GO with granulocyte colony-stimulating factor (G-CSF), a known MDR modifier, can enhance therapeutic efficacy. This is attributed to G-CSF-mediated promotion of cell cycle progression into the G2/M and hypodiploid phases, thereby increasing GO susceptibility [68]. Furthermore, valproic acid, a histone deacetylase (HDAC) inhibitor, has also been reported to augment GO activity. Despite these promising *in vitro* findings, the synergistic effects of combining GO with agents such as G-CSF or valproic acid remain insufficiently characterized in clinical trials [72].

5.2 Drug Release and Stability

The architecture of the ADCs is designed to ensure stability in systemic circulation while allowing for the selective release of the cytotoxic drug at the tumor site. However, preserving this stability poses several challenges. Premature release of the drug, often due to linker degradation or cleavage, can result in systemic toxicity and suboptimal therapeutic outcomes. Factors within the systemic circulation, such as circulating proteolytic enzymes, can compromise the integrity of linkers. Additionally, the heterogeneous nature of the TME, including variations in pH, enzymatic activity, and oxygen levels, further influences the rate and efficiency of payload release. A disruption in the controlled release mechanisms may result in subtherapeutic plasma concentrations, thereby weakening the intended pharmacological effect while increasing the risk of off-target toxicity [73,74].

5.3 Immunogenicity

Immunogenicity refers to the ability of a drug or any molecule to induce an immune response in the body. Both the mAb and payload component of the ADCs can elicit immune responses as a consequence of the formation of anti-drug antibodies. This significantly affects the development and clinical use of ADCs, presenting a barrier to achieving an optimal pharmacological response. The mAb component of ADCs is often engineered from non-human sources, which can trigger immune cascades. The immune system may further recognize the foreign antibody as non-self, leading to the production of antibodies against it. Considering cytotoxic drug payloads, small molecules act as haptens, sometimes binding with endogenous proteins and promoting the production of neoantigens. This may trigger immune responses, bringing forth challenges such as adverse reactions, reduced efficacy, neutralization of drug efficacy, and altered pharmacokinetics [74].

Table 1. Summary of key clinical trials evaluating ADCs in various treatment settings of AML.

Sr No	ADCs name	Clinical trial (Name or NCT no.)	Phase	Primary outcomes	Secondary outcomes	Result
1.	Gemtuzumab ozogamicin	NCT00372593	III	EFS and OS	Remission induction rate, PFS, mortality, time to marrow recovery, and toxicities, including infection complications	Febrile neutropenia: 52.25% vs. 54.79%; EFS: 53.1% vs. 46.9%; OS: 69.4% vs. 65.4%; AEs varied.
2.	Vadastuximab talirine	NCT02326584	Ib	Incidence of AEs, incidence of laboratory abnormalities, incidence of DLT	CR, DFS, OS, blood concentrations of ADC and metabolites, incidence of ATA, and rate of MRD clearance	Induction and consolidation Phase: MTD: 20 mcg/kg (day 1), 10 mcg/kg (day 4); AEs in 25%; CR: 60%, CRc: 76%, MRD-negative: 73%. Maintenance Phase: DLTs in 4/25; CR: 50%, CRc: 75%, MRD-negative: 89%; mortality day 30: 1%, day 60: 7%.
3.	Vadastuximab talirine	NCT01902329	I	Incidence of AE and incidence of laboratory abnormalities	Blood concentrations of ADC and metabolites, Incidence of ATA, rate of CR, duration of CR, relapse-free survival, and OS	Monotherapy: CR: 6%, CRc: 19%, mortality: 8%/27%; combination: CR: 43%, CRc: 70%, mortality: 2%/8%; TEAEs: 20%.
4.	Brentuximab vedotin (BV)	NCT01830777	I	MTD + MEC	Incidence of DLT, RR, OS, CD30 status, assessment of pharmacodynamic effects of CD30, and DFS	MTD of BV: 1.8 mg/kg; RR at highest dose: 42%; median OS: 9.5 months; DFS: 6.8 months; AEs similar.
5.	Brentuximab vedotin (BV)	NCT02096042	I/II	MTD and ORR	—	The trial was terminated due to low enrollment of participants.
6.	AVE9633	NCT00543972	I	Incidence of DLT	CR, CRp, PR, and incidence of AEs	The trial was terminated because the study did not display enough validation regarding the therapeutic action of the molecule up to lethal doses.
7.	SGN-CD123A	NCT02848248	I	Type, incidence, and severity of AEs, laboratory abnormalities, and incidence of DLT	Blood concentration of ADC and metabolites, incidence of ATA, rate of remission, duration of CR, DFS, and OS	The trial was terminated with an undeclared reason for termination.

Table 1. Continued.

Sr No	ADCs name	Clinical trial (Name or NCT no.)	Phase	Primary outcomes	Secondary outcomes	Result
8.	Zilovertamab Vedotin	NCT03833180	I	MTD and RDR	Average number of molecule infusions administered, number of participants with TEAE, DLT, SAE, AESI, number of participants that discontinued the treatment due to AEs, number of participants that used supportive care or concomitant medication, plasma concentration of ADC, plasma concentration of total UC961 antibody, plasma concentration of MMAE, Cmax of ADC, Tmax of ADC, AUC of ADC, Vd of ADC, clearance of ADC, half-life of plasma concentration of ADC, Cmax of total UC961 antibody, Tmax of total UC961 antibody, AUC of total UC961 antibody, Vd of total UC961 antibody, clearance of total UC961 antibody, half-life of plasma concentration of total UC961 antibody, Cmax of MMAE, Tmax of MMAE, AUC of MMAE, Vd of MMAE, clearance of MMAE, half-life of plasma concentration of MMAE, number of participants with ADC reactive antibodies, OR, CRMRD, % change from baseline in tumor dimension, TTR, DOR, PFS, TTF, and OS	51 patients in schedule 1; doses: 0.5–2.5 mg/kg; MTD: 2.5 mg/kg; ADC showed safety, efficacy in recurrent NHL.
9.	DCLL9718S	NCT03298516	I	% of participants with AEs, % of participants with DLT, MTD, and RP2D	Serum concentration of ADC, plasma concentration of ADC, AUC of ADC, Cmax of ADC, total clearance of ADC, half-life of ADC, Vss OF ADC, % of participants with CR, CRi, CRp, and OR, DOR, OS, EFS, PFS, and change from baseline in ADA	67% had grade $\geq$3 AEs; febrile neutropenia: 33%, pneumonia: 28%; grade 4 toxicity in two; no CR/PR; MTD undetermined.

Abbreviations: ADA, anti-drug antibody; ADC, antibody–drug conjugates; AE, adverse event; AESI, adverse event of special interest; ATA, anti-therapeutic antibodies; AUC, area under curve; Cmax, maximum plasma concentration; CR, complete remission; CRc, composite complete remission; CRi, CR with incomplete blood count recovery; CRMRD, complete response without measurable residual disease; CRp, CR with incomplete platelet count recovery; DFS, disease-free survival; DLT, dose-limiting toxicity; DOR, duration of response; EFS, event-free survival; MEC, minimum effective concentration; MMAE, monomethyl aurastatin E; MRD, minimum residual disease; MTD, maximum tolerated dose; OR, overall response; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PR, partial remission; RP 2D, recommended Phase II dose; RR, response rate; SAE, serious adverse event; TEAE, treatment emergent adverse event; Tmax, time to Cmax; TTF, time to treatment failure; TTR, time to response; Vd, volume of distribution; Vss, volume of distribution at steady state.

Table 2. Preclinical studies evaluating the safety and efficacy of GO in AML.

Sr no	Animal model	Material and method	Indication	Target	Results	Conclusion
1.	Rats, monkeys	HL-60 human PML cell line, xenograft tumor model	AML	CD33+ target cells	GO was not lethal to rats up to 7.2 mg/m^2 and in monkeys up to 22 mg/m^2 [50].	Differences in tissue examination of liver, kidney, and testes were observed. Significant myelotoxicity was also noticed.
2.	Mammalian cells	Strongly CD33 positive myeloid cell line HL-60, weakly CD33 positive myeloid cell line K-562	AML	CD33+ target cells	The results are given for antibody labeling, cell binding of ^{211}At anti-CD33, DNA damage, caspase activation 24 hour after antibody treatment, apoptosis and necrosis induction 24 hours post-treatment with antibody bound and free ^{211}At, cell survival experiments, caspase activation and necrosis initiation monitored 72 hours post therapy [51].	The retrieved data suggests that ^{211}At-anti-CD33 leads to sufficient DNA DSBs to overcome the resistance of anti-cancer drugs.

Abbreviations: AML, acute myeloid leukemia; ^{211}At, Astatine-211; CD, cluster of differentiation; DSB, double-stranded breaks; GO, gemtuzumab ozogamicin; HL, human myeloid leukemia cell line; PML, promyelocytic leukemia.

Table 3. Clinical trials evaluating GO in various settings of AML.

NCT number	Title	Setting of AML	Primary outcomes	Secondary outcomes
NCT04337138	A Study of Treatment Patterns and Clinical Outcomes in Patients Diagnosed with Acute Myeloid Leukemia Who Received Mylotarg in the Real-World.	Relapsed or refractory AML.	rwEFS, rwRFS, rwOS, number of participants with first positive response.	Duration of therapy.
NCT02221310	Immunochemotherapy and AlloSCT in Patients with High Risk CD33+ AML/MDS.	High-risk AML or MDS	RR.	-
NCT00882102	Decitabine and Gemtuzumab Ozogamicin in Acute Myelogenous Leukemia (AML) and High-Risk Myelodysplastic Syndrome (H-R MDS).	AML, MDS, or MF.	Number of participants with CR.	-
NCT02182596	DNR and AraC Combined to Fractionated Mylotarg® in Patients with First Relapse of AML (MYLOFRANCE2).	Relapsed AML patients >50 and <70 years.	DLT.	Secondary endpoint.
NCT00968071	Decitabine and Gemtuzumab Ozogamicin in Acute Myelogenous Leukemia and High-risk Myelodysplastic Syndrome.	AML or high-risk MDS.	Number of participants with CR.	-
NCT00476541	NOPHO-AML 2004 Study for Children with Acute Myeloid Leukemia.	Newly diagnosed AML.	EFS.	OS.
NCT03839446	Phase II Study of the Combination of Mitoxantrone, Etoposide and Gemtuzumab Ozogamicin (MEGO) for Patients with Acute Myeloid Leukemia Refractory to Initial Standard Induction Therapy.	Combination therapy of GO, mitoxantrone and etoposide in patients who did not respond to first line induction therapy.	CR rate.	PFS, OS, cytogenetic status, and blast percentage.
NCT00037583	Study Evaluating the Safety and Efficacy of Gemtuzumab Ozogamicin in Patients with Acute Myeloid Leukemia.	In younger *de novo* patients with AML.	-	-
NCT04173585	TEAM-Trial: Targeting Epigenetic Therapy Resistance in AML With Bortezomib (TEAM).	Refractory/relapsed AML.	CR and CRi rate.	-
NCT00044733	Study Evaluating Gemtuzumab Ozogamicin in Acute Myelogenous Leukemia After Stem Cell Transplant.	Relapsed CD33-positive AML patients.	-	-
NCT00037596	Study Evaluating Gemtuzumab Ozogamicin in Acute Myeloid Leukemia.	Relapsed or refractory patients and Alder *de novo* patients with AML.	-	-
NCT00233909	A Trial of Gemtuzumab Ozogamicin (GO) in Combination with Zosuquidar in Patients with CD33 Positive Acute Myeloid Leukemia.	CD33 + AML.	-	-
NCT00766116	Combination 5-azacitidine and Gemtuzumab Ozogamicin Therapy for Treatment of Relapsed Acute Myeloid Leukemia (AML).	Relapsed AML.	Number of participants with DLTs.	Number of participants who responded to the combination treatment.

Table 3. Continued.

NCT number	Title	Setting of AML	Primary outcomes	Secondary outcomes
NCT00893399	Study of Chemotherapy in Combination with All-trans Retinoic Acid (ATRA) With or Without Gemtuzumab Ozogamicin in Patients with Acute Myeloid Leukemia (AML) and Mutant Nucleophosmin-1 (NPM1) Gene Mutation.	AML and NPM1 mutation.	OS.	Rates of CR after induction therapy, CIR and CID in CR, EFS, number of days in hospital during each cycle and during the whole intervention, kind, frequency, severity, timing and relatedness of AEs and laboratory deviations seen throughout the treatment cycles, incidence of infection and duration of neutropenia and thrombocytopenia post-induction and consolidation therapy, quality of life assessed by the EORTC QLQ-C30.
NCT00161668	Study Evaluating Mylotarg (Gemtuzumab Ozogamicin) in Usual Care.	Usual care.	-	-
NCT01041040	LAM07: Study to analyze the Efficacy of a Risk Adapted Treatment Strategy, Including Gemtuzumab Ozogamicin (GO) During Consolidation, for Patients with Acute Myeloid Leukemia (AML)	AML	To predict the prognosis of AML patients, post-induction therapy should be based on MRD, cytogenetics, and molecular findings. This assessment will evaluate the safety and effectiveness of a post-remission therapy plan that incorporates GO and enhances currently available treatment options, tailored to the prognosis of the patient.	To evaluate the numerous prognostic factors in AML, such as the karyotype and the molecular findings at diagnosis, and the MRD level at the conclusion of the induction.
NCT00049179	S0117 Gemtuzumab Ozogamicin Plus Cytarabine in Treating Patients with Relapsed Acute Myeloid Leukemia.	Relapsed AML.	CR.	-
NCT00089050	Gemtuzumab Ozogamicin and Cyclosporine in Treating Older Patients with Relapsed Acute Myeloid Leukemia.	Relapsed AML in adults aged >60 years	Efficacy in terms of CR rate, toxicity, and pharmacokinetics.	Correlate clinical response to laboratory studies of drug susceptibility.
NCT00962767	Comparison of Two Treatments in Intermediate and High-risk Acute Promyelocytic Leukemia (APL) Patients to Assess Efficacy in 1st Hematological Complete Remission and Molecular Remission.	Intermediate and high-risk adult patients with APL.	To assess efficacy in achieving first hematological CR and molecular remission.	Comparison of short and long-term toxicity of treatment, patient quality of life, and OS.
NCT00304447	Study Evaluating the Effect of Corticosteroids on Mylotarg® Infusion-Related Adverse Events in Patients With Leukemia.	AML.	Infusion-related AEs.	Mylotarg® induced CR and CRp.
NCT00003131	CMA-676 in Treating Patients with Acute Myeloid Leukemia in First Relapse.	AML in first relapse.	Not provided.	Not available.
NCT00195000	Mylotarg and Ara-C in Untreated Patients above 60 Years with AML and High-Risk MDS.	AML and advanced MDS.	CR.	Not available.
NCT00003673	CMA-676 in Treating Older Patients with Acute Myeloid Leukemia in First Relapse.	AML in first relapse.	Not provided.	Not available.
NCT00006265	Gemtuzumab Ozogamicin and High-Dose Cytarabine in Treating Patients With Relapsed or Refractory Acute Myeloid Leukemia.	Relapsed/refractory AML.	Rate of CR.	Toxicity.

Table 3. Continued.

NCT number	Title	Setting of AML	Primary outcomes	Secondary outcomes
NCT00860639	Efficacy of Gemtuzumab Ozogamycin for Patients Presenting an Acute Myeloid Leukemia (AML) With Intermediate Risk (LAM2006IR).	AML with intermediate risk.	EFS.	CR, OS, relapse rate, toxicity and tolerability, MRD.
NCT01698879	Prospective Study of Mylotarg and G-CSF in Acute Myeloid Leukemia Treatment.	AML.	CR.	Secondary toxicity, mortality at induction, capacity to obtain HPC for autotransplantation, relapse, and survival rate.
NCT03727750	Evaluating QTc, PK, Safety of Gemtuzumab Ozogamicin (GO) in Patients With CD33+ R/R AML.	CD33+ relapsed/refractory AML.	QTcF.	CL, volume of distribution, Cmax, Tmax, AUC, AEs, SAEs, ADA, NAb, CR, CRi, and OS.
NCT00017589	Oblimersen and Gemtuzumab Ozogamicin in Treating Older Patients With Relapsed Acute Myeloid Leukemia.	Relapsed AML.	Not provided.	Not available.
NCT00909168	Induction, Consolidation and Intensification Therapy for Patients Younger Than 66 Years With Previously Untreated CD33 Positive Acute Myeloid Leukemia (AML) (MYFLAI07).	Newly diagnosed AML.	Feasibility, efficacy (CR + PR rate), toxicity, RFS, DFS, and OS.	MRD, prognostic clinical relevance of biological features at onset, feasibility, and outcome of consolidation with BMT.
NCT00895934	Vorinostat, Azacitidine, and Gemtuzumab Ozogamicin for Older Patients with Relapsed or Refractory AML	Relapsed/refractory non-APL AML	DLT (phase I) and DLT after vorinostat dose	CR and relapse of disease.
NCT00143975	Gemtuzumab Ozogamicin in Combination with A-HAM in Refractory AML (GO-A-HAM).	Primary refractory AML.	Rate of CR.	Kind, incidence, severity, temporal sequence, and correlation of side effects of the study drugs, as well as the rates of VOD and OS.
NCT03848754	Pracinostat and Gemtuzumab Ozogamicin (PraGO) in Patients with Relapsed/Refractory Acute Myeloid Leukemia.	Relapsed/refractory AML.	DLTs.	Survival rate, PFS rate, CR, CRi, PR, and MLFS.
NCT00006122	Gemtuzumab Ozogamicin With or Without Chemotherapy in Treating Older Patients With Acute Myeloid Leukemia.	AML.	Not provided.	Not available.
NCT03531918	Gemtuzumab Ozogamicin With G-CSF, Cladribine, Cytarabine & Mitoxantrone for Untreated AML & High-Grade Myeloid Neoplasm.	AML or high-grade myeloid neoplasm.	MTD, EFS.	Mortality rate, relapse-free survival rate, OS, MRD + CR, MRD + CRi, MRD + CR/CRi, MRD + MLFS, MRD + alapasia.
NCT00121303	Cytarabine and Daunorubicin With or Without Gemtuzumab Ozogamicin in Treating Older Patients With Acute Myeloid Leukemia or Myelodysplastic Syndromes.	AML or MDS.	EFS, DFS.	CR, OS, relapse rate, and toxicity.
NCT00052299	Chemotherapy With or Without Gemtuzumab Ozogamicin in Treating Older Patients With Acute Myeloid Leukemia (AML-17).	AML.	OS.	CR, CRp, DFS, relapse rate, death incidence, EFS, and toxicity.
NCT00085709	S0106 Cytarabine and Daunorubicin w/ or w/o Gemtuzumab Followed By HD Cytarabine and Either Gemtuzumab or Nothing in de Novo AML.	*de novo* AML.	DFS, CR.	Toxicity.
NCT03390296	OX40, Venetoclax, Avelumab, Glasdegib, Gemtuzumab Ozogamicin, and Azacitidine in Relapsed or Refractory Acute Myeloid Leukemia.	Relapsed or refractory AML.	Number of participants with a response.	OS.
NCT01407757	Study of Gemtuzumab Ozogamicin Therapy in DNA Samples From Patients With Acute Myeloid Leukemia Treated on COG-AAML0531.	AML	Genetic changes in CD33 impact the outcome of GO-based therapy.	-

Table 3. Continued.

NCT number	Title	Setting of AML	Primary outcomes	Secondary outcomes
NCT00927498	A Randomized Study of Gemtuzumab Ozogamicin (GO) With Daunorubicine and Cytarabine in Untreated Acute Myeloid Leukemia (AML) Aged of 50–70 Years Old.	Not previously treated AML.	EFS.	CR, OS, incidence of relapse, and possible predictors of response to mylotarg.
NCT00028899	Monoclonal Antibody Plus Chemotherapy in Treating Young Patients With Relapsed or Refractory Acute Myeloid Leukemia or Myelodysplastic Syndromes.	Relapsed or refractory AML	EFS	Toxicity, RR, and prognostic factor analysis.
NCT00372593	Combination Chemotherapy With or Without Gemtuzumab in Treating Young Patients With Newly Diagnosed Acute Myeloid Leukemia.	Young adults with *de novo* AML.	EFS and OS at 3 years.	DFS, mortality, time to marrow recovery, and toxicity.
NCT01020539	Allogeneic Stem Cell Transplantation Followed By Targeted Immune Therapy In Average Risk Leukemia (AML/MDS/JMML).	AML.	MTD.	MRD, OS, EFS, and incidence of MHA.
NCT01723657	Risk Adapted Treatment for Primary Acute Myeloid Leukemia (AML).	Primary AML.	CRR, DFS.	Toxicity, MRD, and feasibility of post-remission treatment.
NCT00005962	Comparison of Three Treatment Regimens in Treating Patients With Relapsed or Refractory Acute Myelogenous Leukemia.	Relapsed or refractor AML.	-	-
NCT00038831	Allo Transplantation With Mylotarg, Fludarabine and Melphalan for AML, CML and MDS.	High risk AML.	MTD, DLT.	Patient response.
NCT01139320	Biomarkers in DNA Samples From Younger Patients With Newly Diagnosed Acute Myeloid Leukemia Receiving Gemtuzumab Ozogamicin.	Newly diagnosed AML in young patients.	Relationship between clinical response to GO and coding polymorphism in CD33.	-
NCT00136084	Treatment of Patients With Newly Diagnosed Acute Myeloid Leukemia or Myelodysplasia.	Newly diagnosed AML.	MRD.	EFS, MRD, toxicity, and clinical response.
NCT00049517	Combination Chemotherapy With or Without Monoclonal Antibody Therapy in Treating Patients With AML Leukemia.	AML.	OS, DFS.	OS.
NCT00070174	Gemtuzumab Ozogamicin in Treating Young Patients With Newly Diagnosed Acute Myeloid Leukemia Undergoing Remission Induction and Intensification Therapy.	Newly diagnosed AML.	Safety, CRR.	Feasibility and the effect of karyotypic abnormalities.
NCT01503541	S9031-9333-0106-0112-A Study of Biomarkers in Samples From Patients With Acute Myeloid Leukemia Treated With Standard Chemotherapy With or Without Gemtuzumab Ozogamicin.	AML.	Biomarker assays in highly purified populations of AML.	-
NCT00008151	Gemtuzumab Ozogamicin, Fludarabine, and Total-body Irradiation Followed by Peripheral Stem Cell or Bone Marrow Transplantation in Treating Patients With Advanced Acute Myeloid Leukemia or Myelodysplastic Syndrome.	Advanced AML.	RR, DFS, blast clearance, safety.	-
NCT00454480	Combination Chemotherapy With or Without Gemtuzumab Ozogamicin or Tipifarnib in Treating Patients With Acute Myeloid Leukemia or High-Risk Myelodysplastic Syndromes.	AML.	OS, CR, toxicity, DOR,	-

Table 3. Continued.

NCT number	Title	Setting of AML	Primary outcomes	Secondary outcomes
NCT00077116	Idarubicin, Cytarabine, and Gemtuzumab Ozogamicin in Treating Patients With Previously Untreated High-Risk Myelodysplastic Syndrome or Acute Myeloid Leukemia Secondary to Myelodysplastic Syndrome.	AML secondary to MDS.	CR, severity of toxicity.	DFS, OS, and toxicity.
NCT01019317	Fludarabine and Cytarabine in Acute Myelogenous Leukemia (AML) and High-Risk Myelodysplastic Syndrome (MDS).	AML.	CR.	-

Abbreviations: ADA, anti-drug antibody; AEs, adverse events; AML, acute myelogenous leukemia; APL, acute promyelocytic leukemia; AUC, area under the curve; BMT, bone marrow transplant; Cmax, maximum plasma concentration; CD, cluster of differentiation; CID, cumulative incidences of death; CIR, cumulative incidences of relapse; CL, clearance; CR, complete response; CRi, complete remission with incomplete hematologic recovery; CRp, complete remission with incomplete recovery of platelet count; CRR, complete remission rate; DFS, disease-free survival; DLT, dose-limiting toxicity; DOR, duration of response; EFS, event-free survival; EORTC, European Organization for Research and Treatment of Cancer; GO, gemtuzumab ozogamicin; GVHD, graft vs. host disease; HPC, hematopoietic progenitor cells; MDS, myelodysplastic syndrome; MF, myelofibrosis; MHA, minor histocompatibility antigen; MLFS, morphologic leukemia-free state; MRD, minimal residual disease; MTD, maximum tolerated dose; NAb, neutralizing antibodies; OS, overall survival; PFS, progression-free survival; PR, partial remission; QLQ-C30, Quality of Life Core Questionnaire; QTcF, QT interval using Fridericia's formula; RFS, recurrence-free survival; RR, response rate; rwEFS, real-world event-free survival; rwOS, real-world overall survival; rwRFS, real-world relapse-free survival; SAEs, serious adverse events; Tmax, time to reach maximum plasma concentration; VOD, veno-occlusive disease.

5.4 Imaging and Diagnostics

Developing competent imaging methods for monitoring the ADC distribution, assessing target engagement, and evaluating therapeutic response is vitally important. Meanwhile, tracking the course of ADCs in the body after administration allows for the early detection of any challenges in therapy and the optimization of the strategies involved in ADC administration. The lack of real-time imaging tools hinders the optimization of ADC safety and efficacy-related parameters [75].

5.5 Cost and Regulatory Approval

The procedures involved in the development of ADCs and the supply chains contributing to ADC retrieval both incur significantly high expenses, leading to challenges in affordability and equitable patient access. This factor prevents patients with financial constraints from receiving ADC regimens, negatively impacting patient outcomes. Additionally, rigorous regulatory scrutiny is involved in the approval of these ADCs for further prescription. Any delays in regulatory approval or reimbursement issues can limit patient access to these novel therapies [76]. These few challenges significantly impact the safety, efficacy, and patient access to ADCs, and addressing these obstacles requires extensive investigations, technological advancements, and collaborative efforts between manufacturers, researchers, clinicians, and regulatory bodies. Thus, forming a multi-disciplinary team may optimize the aspects involved in ADC monitoring and administration for improved cancer treatment outcomes.

6. Advancements in ADC Technology

ADCs, as a rousing technique, have fortunately revolutionized the prospects of targeted cancer therapy in contrast to standard chemotherapy. A significant increase in the development of ADCs has been observed over the past few years, particularly between 2019 and 2022, when eight new ADCs received FDA approval. Additionally, nearly 57 new ADCs entered Phase I trials in 2022, representing a 90% increase compared to 2021 [77]. Fine-tuning and advancements in the technology of each component of ADC, whether it be an antibody, linker, payload, or its conjugation design, somehow resolve the complications such as heterogeneous expression of target antigens and tumor heterogeneity, low drug loading and tumor uptake, as well as poor circulation, which have led to the buildup of new and updated immunoconjugates [78]. Subsequent explorations in the area of improving antigen–antibody binding have accelerated the utilization of bispecific mAbs that conjugate with two non-overlapping antigenic determinants, as well as the other biparatopic mAbs that recognize two different gene epitopes on the same antigen [79]. Furthermore, to enhance the therapeutic index, limit the activity of Fab paratopes in healthy and normal tissues, and elevate the specificity of ADCs toward the target antigen, a modified tumor-specific mAb, identified as Probody, has recently been tested and analyzed [80]. Considering the payloads that are combined with mAbs to form ADCs, they are also paving the way for advanced developments, taking into account properties such as adequate efficacy, resolution of acquired drug resistance, high stability, and reduced toxicity [81]. Several first- and second-generation ADCs mostly use potent tubulin inhibitors (maytansinoids, auristatin, eribulin, tubulysin) as payloads; however, since these lacked effectiveness against static tumors, the third-generation ADCs adopted DNA-damaging agents (enediyne, topoisomerase-1-inhibitors, PBDs, duocarmycin) as cytotoxic payloads that can wholly damage the cell cycle [32]. Despite the usage of these agents throughout the three generations of ADCs, these agents are still lagging in terms of developing severe side effects and drug resistance. Hence, novel payloads, such as RNA inhibitors (thailanstatin A, amatoxins), Bcl-xL inhibitors, NAMPT inhibitors, and carmaphycins, are required to overcome the limitations of traditional payloads. Currently, greater attention is being given to immune-based ADC payloads that include toll-like receptor agonists, stimulator of interferon gene (STING) agonists, and glucocorticoid receptor modulators (GRMs), as they significantly contribute to tumor immunotherapy [82]. Active strategies are still emerging, including ADCs with dual payloads, ADCs with proteolysis targeting chimera (PROTAC) molecules, ADCs with near-infrared photoimmunotherapy (NIR-PIT), and peptide drug conjugates (PDCs). In addition to the role of mAbs and payloads, the linkers also share an equivalent importance in the structure and design of ADCs. Hence, continuous progress and renewal are necessary in the linkers and their conjugation techniques. There are currently two types of known linkers: cleavable (hydrazone, cathepsin, disulfide, and pyrophosphate diester) linkers and non-cleavable linkers, which are presently used with ADCs to bind the antibody to the payload and promote the specific release of the payload to the target antigen. However, these non-cleavable linkers still led to certain instabilities and heterogeneity of ADCs, as well as off-target toxicities, which provided insight into the requirement for overcoming these deficiencies [83]. Thus, novel chemical triggers, linker–antibody conjugates, and linker–payload conjugates have emerged as key components in the development of new linkers. The chemical triggers include acid-cleavable, GSH-cleavable, phosphatase and sulfatase cleavable, glycosidase cleavable, photo-responsive, and biorthogonal cleavable triggers (break the intracellular release of drugs in traditional ADCs). Linker–antibody conjugates include maleimide attachment (improves the stability of ADCs), bis-piperazine attachment (produces highly homogenous ADCs), Pt (II) based attachment, and linker–payload conjugates contain quaternary ammonium attachment (connects all the payloads with tertiary amine) [33]. Hence, this can lead to the conclusion that progressive, developable antibodies, ad-

vanced payloads, and innovative linkers have cleared the path for ADCs in the oncology sphere. In a few years, these may be heading toward non-oncological zones [84].

6.1 Nanoparticles and Nanostructures in the Treatment of Myeloid Leukemia

Unsatisfactory outcomes in AML pose a major problem regardless of the many advances in chemotherapy and available treatments. Nanotechnology offers numerous advantages, including personalized medicine, targeted drug delivery, the ability to overcome drug resistance, and controlled release, which may help improve the effectiveness of AML treatment [85].

6.1.1 Nanoparticles Targeting Leukemia Stem Cells

Leukemia stem cells (LSCs), due to their resistance to therapies, have been thought to be responsible for disease relapse. Traditional therapies may also fail to target these stem cells. Nanoparticles may be specifically designed to target and eliminate these stem cells. For example, a significant reduction in LSCs and improved efficacy can be obtained in AML xenograft models by effective delivery of multi-stage nanoparticles containing anti-LSC agents to the bone marrow [86].

6.1.2 Targeted Therapies Using Nanostructures

Some of the drawbacks of traditional chemotherapy include systemic toxicity and inefficacy due to inadequate accumulation of the drug at the target site. Nanoparticles and nanostructures can be engineered to deliver drugs to the target site, thereby reducing unwanted side effects and enhancing the efficacy of the treatment. ADCs encapsulating nanoparticles may reduce off-target effects, allowing the drug to reach the target site in the desired amount. One such application of targeted therapy involves coating nanoparticles with the membrane of leukemic cells [87]. This approach helps target leukemic cells by leveraging the innate homing characteristics of these membranes.

6.1.3 Erythrocyte Membrane-Coated Nanoparticles

Erythrocyte membrane-coated nanoparticles (EMCNPs) are also a part of nanotechnology, possessing the advantages of prolonged circulation time and evading immune detection. These EMCNPs mimic red cells and can be loaded with chemotherapeutic agents. Clinical trials are ongoing for EMCNPs [88].

6.1.4 Nanoparticle-Mediated Immunotherapy

Immunotherapy is one of the main treatment options for cancer. Nanoparticles can be utilized in immunotherapy to enhance the delivery of immune-modulating agents. CD33/CD3 bispecific T cell engagers (nanoTCEs) target AML cells that express CD33 on their cell surface. These nanoparticles effectively activate T cells, leading to the targeted elimination of AML cells [89].

6.1.5 Magnetic Nanoparticles for Hyperthermia Therapy

Magnetic nanoparticles have been employed in AML, wherein localized heating may cause cell death. These nanoparticles can be directed to the desired site of action by applying an external magnetic field. Upon exposure, the nanoparticles generate heat that selectively kills tumor cells, thereby allowing localized drug delivery. Iron oxide nanoparticles targeting epithelial cell adhesion molecules have been utilized in the treatment of leukemia [90].

6.1.6 Liposomal Formulations

Chemotherapeutic agents may be encapsulated in liposomal formulations to improve the efficacy and safety of these agents [91]. Despite the promising potential of nanotechnology in treating AML, numerous challenges persist in its application. Therefore, the long-term safety of nanoparticles must be assessed to identify and prevent their adverse effects. The manufacturing of nanoparticles is another significant challenge that needs to be addressed. The consistent quality of the manufacturing process of nanoparticles is important for large-scale applications. A detailed report on pre-clinical and clinical data is necessary for regulatory approval in the treatment of myeloid leukemia. Further consideration of the use of nanoparticles and nanostructures necessitates extensive research, with a focus on personalization and the combination of different therapies. Personalization of nanomedicine may help tailor therapies based on individual molecular characteristics and patient profiles. Nanomedicine can be combined with therapies such as immunotherapy and targeted therapy to improve its therapeutic profile and efficacy. The utilization of nanoparticles and nanostructures in the treatment of AML may provide a pathway toward improved patient outcomes by offering targeted drug delivery, overcoming resistance mechanisms, and enhancing the efficacy of therapy [92].

7. Future Research

As previously outlined, an immunoconjugate typically comprises a mAb, a linker, and a payload. Therefore, alterations in any of these elements may significantly affect the effectiveness of the ADC. Recent literature has showcased that the clinical activity of ADC is majorly dependent on ADC incorporation and its transport pathway within the cell [27]. The utilization of size-reduced antibodies as an alternative to large molecular size antibodies facilitates improved permeation and antigen-binding capacity for the ADC. However, the pharmacokinetic parameters should be concomitantly assessed when implementing these small-sized antibodies [42]. Presently, the major focus is on the development of immune-based payloads and ADCs with dual payloads, PROTAC molecules, NIR-PIT, and PDC [81]. Recently, research has been conducted on linkers that may release the payload upon contact with certain chemicals that may be released from the tumor. The development of chemically simplified linkers may help in

resolving complications associated with the therapeutic application of linkers and is an emerging research topic [33]. Additionally, to acquire target specificity and controlled release of drugs, ADCs can be combined with nanoparticles to form antibody-conjugate nanoparticles (ACNPs) [42]. Meanwhile, recognizing biomarkers and pinpointing evidence are necessary to administer appropriate ADCs in combination or as monotherapy to achieve the maximum possible efficacy [93].

8. Conclusion

The development of targeted therapies has surged rapidly in recent years, owing to the remarkable safety and efficacy profiles of novel treatments. Considering the potential of GO, ADCs have demonstrated enhanced clinical activity when administered as monotherapy. However, the FDA approval process for GO experienced several obstacles due to excessive toxicity and limited clinical efficacy; nonetheless, GO is now employed as a standard compound for newly diagnosed AML. Combination regimens of ADC alongside other therapeutic agents have also been reported to have superior pharmacological activity when prescribed as first-line therapies. Numerous ADCs are being explored for their application in AML across multiple settings, including neoadjuvant, adjuvant, post-surgery, consolidation, metastatic, and others. Nevertheless, a few ADC trials mentioned previously have been terminated due to high-grade toxicities and suboptimal clinical response. Ongoing technological advancements and investigations are poised to enhance the therapeutic window, with a strategic focus on refining payloads and linkers.

Challenges associated with immunogenicity may be resolved through co-administration of immunosuppressive agents or the use of humanized mAbs; meanwhile, those related to resistance may be tackled by a comprehensive understanding of TME to tailor ADCs as per specific cancer types, while ensuring optimal drug release kinetics. Considering the positive angle, many novel drugs have received approval over the past 2 years, and several others are on the horizon for approval. This influx of new therapeutic options holds the promise of increasing precision in AML prescriptions. Moreover, this influx may further favor individualized therapy if optimization of the GO dose, frequency, and combination with other drugs based on the patient population is practiced. To summarize, the evolving landscape of ADCs in AML, marked by breakthroughs and challenges, positions personalized and combination therapies as key elements in shaping the future of AML treatment.

Author Contributions

ACS – Manuscript original first draft preparation and subsequent editing, Literature and data survey; SGS, JDT, SHM – Figures and diagram designing, Manuscript Editing, Tables creation; RP – Writing original draft, Data curation, Reviewing and Editing; MRC, KD, BGP – Review topic conception, Design of content and skeleton, Manuscript draft review, editing and approval; Figures and Tables conception, Overall monitoring and co-ordination. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. Rajanikant Patel is from Granules Pharmaceuticals Inc. The content of this review article reflects the authors' own research, analysis, and interpretations, and no part of the study was influenced by the Granules Pharmaceuticals Inc.

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