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REVIEW

Research advances in nanomedicine, immunotherapy, and combination therapy for leukemia

Zhuoya Wan[1] | Runzi Sun[2] | Pearl Moharil[3,4] | Jing Chen[1,4] | Yuzhe Liu[5] | Xu Song[1] | Qiang Ao[1]

[1]Institute of Regulatory Science for Medical Device, National Engineering Research Center for Biomaterials, Sichuan University, Chengdu, China

[2]Department of Immunology, School of Medicine, University of Pittsburgh, Pennsylvania, USA

[3]Department of Cell Biology, Harvard Medical School, Harvard University, Massachusetts, USA

[4]Department of Pharmaceutical Science, School of Pharmacy, University of Pittsburgh, Pennsylvania, USA

[5]Department of Materials Engineering, Purdue University, Indiana, USA

Correspondence
Qiang Ao, Institute of Regulatory Science for Medical Device, National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, China.
Email: aoqiang@tsinghua.edu.cn

Abstract

In the past decade, clinical and laboratory studies have led to important new insights into the biology of leukemia and its treatment. This review describes the progress of leukemia research in the United States in recent years. Whereas the traditional method of treatment is chemotherapy, it is nonselective and could induce systemic toxicities. Thus, in parallel with research on new chemotherapies, great emphasis has been placed on developing immunotherapies. Here, we will review the current immunotherapies available in research and development that overcome current challenges, specifically looking in the field of chimeric antigen receptor T-cell (CAR-T) therapies, checkpoint inhibitors, and antibody-drug conjugates. With about 100 clinical trials for CAR-T therapies and 30 in checkpoint inhibitors for leukemia treatment, scientists are trying to make these technologies cheaper, faster, and more feasible. Further describing the delivery of these therapeutics, we look at the current progress, clinical, and preclinical status of nano-based medicines such as liposomes, polymeric micelles, and metal nanoparticles. Taking advantage of their physicochemical and biologic properties, nanoparticles have been shown to increase the efficacy of commonly administered chemotherapies with reduced adverse effects.

KEYWORDS
nanotechnology, immunotherapy, cancer, hematology

1 | INTRODUCTION

Leukemia is a cancer that starts in the developing blood cells of the bone marrow. When a bone marrow cell becomes cancerous, it no longer matures the way it should and becomes a leukemia cell.[1-4] In 2020 alone, around 60,530 new cases of leukemia and 23,100 deaths have been estimated by the American Cancer Society for Leukemia in the United States. Depending on whether the disease is acute or chronic, and whether it develops in myeloid cells or lymphoid cells, leukemia could be generally divided into four groups: acute myeloid (or myelogenous) leukemia (AML), chronic myeloid (or myelogenous) leukemia (CML), acute lymphocytic (or lymphoblastic) leukemia (ALL), and chronic lymphocytic leukemia (CLL). Different types of leukemia have different treatment options and outlooks. Based on patients' classification and leukemia subtype, patients are classified in different risk groups and receive appropriate treatments. Whereas high-risk patients are treated with monotherapies such as chlorambucil, non-"high-risk" patients are administered a combination of fludarabine, cyclophosphamide, and rituximab (FCR) as a standard.[5] Whereas this combination therapy is associated with decent complete remission (CR) and overall survival (OS), high-risk patients still deal with a high mortality rate (with a 5 yr OS of 10–20%).[6-8] In this review, we summarize the recent advances and current status of leukemia treatment including chemotherapy, immunotherapy, and formulation strategies for improved therapy (Fig. 1). We majorly focus on important clinical trials that are currently ongoing or clinical data that has been published for leukemia treatment. We also summarize important results from preclinical studies that have demonstrated great potential for clinical transition. Nanomedicine based strategies are also included in this review and will provide more insights about how to use formulation strategies to address the potential issues raised in other strategies.

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2 | CHEMOTHERAPY AND COMBINATION THERAPEUTIC APPROACHES FOR LEUKEMIA TREATMENT

Chemotherapy is the most commonly employed approach for leukemia treatment.[1-4] Due to the distinctive morphology and genetic abnormality in different types of leukemia, stratification of patients helps caregivers identify appropriate chemotherapies.[4]

In the case of CLL, patients are classified into "fit or go-go," "unfit or slow go," and "high risk" depending upon their renal function and level of comorbidity. For patients who fall into the category of "fir or go-go" (normal renal function and none of less comorbidities), combined chemoimmunotherapy of FCR is used as standard.[5] In contrast, patients with damaged renal function and high comorbidity cases in Europe are usually treated with monotherapy of alkylating agent chlorambucil.[4] Other parameters, such as platelet count and serum albumin levels can help evaluate the risk. Additionally, genetic abnormality plays a vital role in stratifying patients. Cases in AML consistently exhibit unique patterns in genetic mutation, which, according to a two-hit model of leukemogenesis, can be classified into activation of pro-proliferation pathways and dysfunction of normal hematopoietic differentiation.[6-8] These genetic mutations also help to stratify patients by prognostic factors, into favorable, intermediate, and adverse risk groups.[9] Different risk groups tend to respond drastically different to the same induction therapy. An induction therapy typically contains a "7+3" regimen: 7 d of continuous cytarabine with subsequent 3 d of anthracycline. This is recommended for the favorable and intermediate risk group as a well-established treatment.[10] However, this induction therapy must be reinforced with consolidation to eradicate residual diseases and prevent relapses for the best outcome.

Currently established chemotherapy provides decent CR and OS rates to certain patient populations. However, as mentioned earlier, the high-risk complex cases face a higher treatment-related mortality.[4] Moreover, there is no first-line chemotherapy available for elderly patients with AML and CLL.[11] To improve such outcomes, novel strategies are now aiming to be more efficient by reducing dosing, and ameliorating the adverse toxicity of therapies.

3 | IMMUNOTHERAPEUTIC APPROACHES FOR LEUKEMIA TREATMENT

Although chemotherapies have been the mainstream therapeutic strategy for leukemia treatment, this approach is nonselective on the cellular level and could induce many undesirable side effects. The advent of novel immunotherapies has revolutionized cancer treatment over the past decade, both in oncology and hematology.[12,13] Instead of nonselectively targeting tumor cells that are actively growing, immunotherapy can target those cancer cells by harnessing body's own immune system. Some leukemia cancers that are particularly difficult to treat can now be effectively eradicated using the power of such approach.

In this review, we highlight some of the important recent advances in the field of immunotherapies for leukemia, including antibody-drug conjugates (ADC), immune checkpoint inhibitors (ICIs), and chimeric antigen receptor (CAR) T-cell therapy. As published data from clinical trials is still scarce for the majority of immunotherapies, we will integrate currently running clinical trials and preclinical studies to point out upcoming directions in this field. The different immunotherapeutic strategies that have been considered for treating leukemia are described in the following sections.

3.1 | CAR-T cells for immunotherapy

CAR-T cell therapy has revolutionized the treatment of leukemia over the past decade and has achieved great success in clinical studies[14-16] (Fig. 2). In brief, CAR-T cells are autologous T cells derived from patients and have been engineered and modified to recognize antigens expressed on the surface of cancer cells.[17,18] At first, T cells are collected from a patient through apheresis process and then modified ex vivo by introducing a gene that codes for an antigen recognition receptor, often a single-chain variable fragment from an antibody, that is fused to T cell costimulatory domains.[19,20] Finally, these genetically modified T cells are transfused back into the patient for targeting the leukemia cells.[17,21] In the following section, we summarize the CAR-T therapies that have already been investigated in clinical trials in a variety of leukemia cancer types (Table 1).

To date, there are a couple of clinical trials for CAR-T cell therapies, with a majority of them focusing on B-cell malignancies.[22-24] As CD19 is primarily expressed on B malignant cells and barely expressed on hematopoietic stem cells, it has been considered to be an effective and safe therapeutic target for leukemia therapy, mostly in the early clinical development for CAR-T therapy.[25-27]

The year 2017 was definitely a landmark year in CAR-T therapy with two CAR-T therapeutics, based on targeting CD19 antigen approved by the FDA.[22,28-31] In August 2017, "Tisagenlecleucel-T" (Kymriah; Novartis) became the first FDA-approved drug for pediatric and under 25-yr-old young adults with refractory or relapse (R/R) B-cell malignancies and ALL.[32,33] It is worthwhile to mention that the progression of this approval was granted less than 6 mo after the FDA accepted Novartis's biologic license application and grants priority review. This was based on a phase II (ELIANA is a single-arm phase II clinical Trial (NCT02435849) PMID: 29385370) single-arm trial, which achieved great success. Statistically, CR or CR with incomplete blood count recovery (CRi) was observed in 83% of patients within 3 mo of infusion. This therapy is currently under review in Europe for R/R B-cell ALL and is being assessed in CLL treatment. Two months later, "Axicabtagene Ciloleucel" (Yescarta; Kite Pharma/Gilead Sciences) was approved by the FDA and became the second-to-market CAR-T therapy.[34,35] This approval was based on a single-arm phase II (ZUMA-1 is a phase 1/2 multicenter clinical study with number NCT02348216; PMID: 29226797) trial with an overall response rate (ORR) around 82% and a CR rate of 58%. Again, this date (October 2017) was more than a month ahead of its target review date. This therapy demonstrated highly promising data in adults with R/R B cell ALL. These two

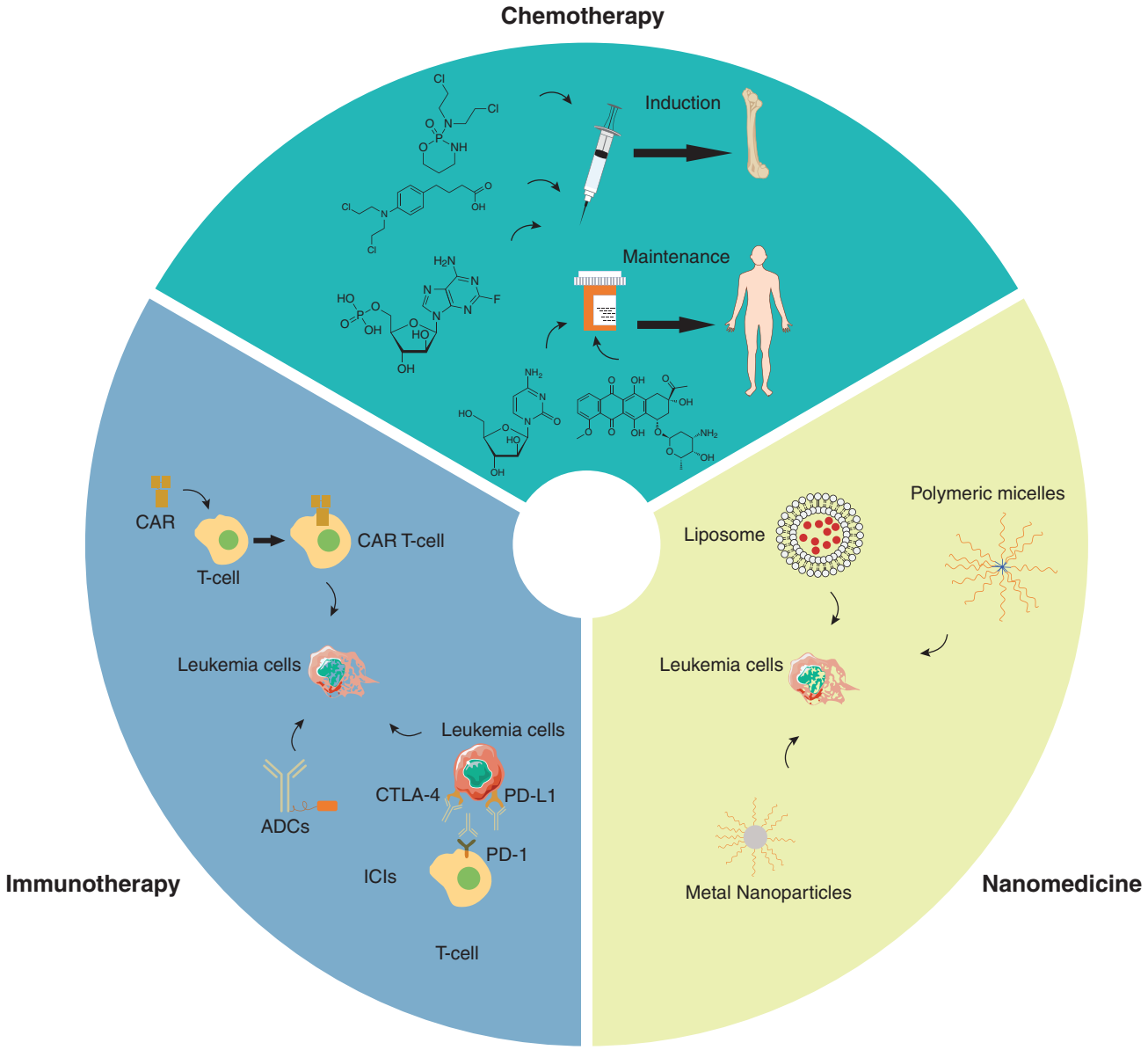

FIGURE 1 Current strategies for leukemia treatment

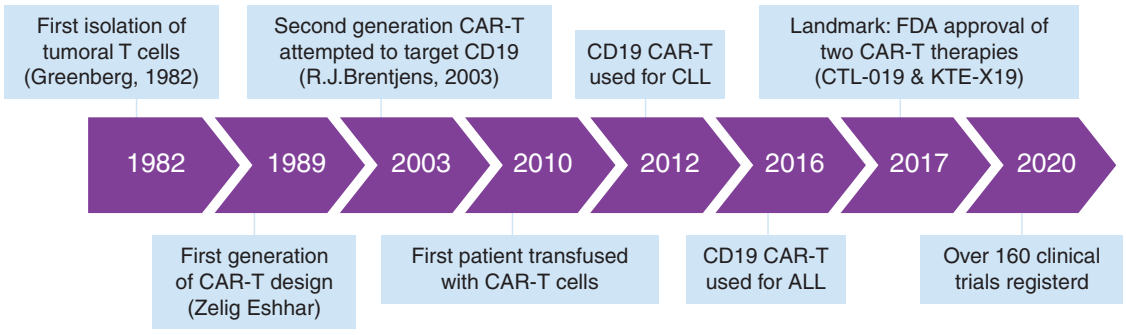

FIGURE 2 Progression of CAR-T therapeutics for leukemia over the years

TABLE 1 Selective chimeric antigen receptor T cell therapies in clinical trials

Study	Institution	Targets	Indication(s)	No. of patients	Outcomes
Kochenderfer et al. (2010, 2012)	NCI	CD19	NHL (4) and CLL (4)	8	ORR = 80% (1 CR, 5 PR)
Brentjens et al. (2011)	MSKCC	CD19	CLL (8) and ALL (8)	10	CLL (1 PR, 2 SD), ALL (1 durable B cell aplasia
Brentjens et al. and Davilla et al. (2013, 2014)	MSKCC	CD19	ALL	16	CR = 88%
Grupp et al, and Maude et al. (2013, 2014)	Upenn	CD19	ALL	30	CR = 90%
Porter et al. (2015)	Upenn	CD19	CLL	14	ORR = 57% (4CR, 4 PR)
Turtle et al. (2016)	FHCRC	CD19	ALL	29	CR = 93%
Stein et al. (2018)	Memorial Sloan-Kettering Cancer Center (New York, NY, USA)	CD33	AML	240	CR + CRi = 28%
Montesinos et al. (2020)	Hospital Universitari i Politècnic La Fe (Valencia, Spain)	CD123	AML	326	CR = 11%

ORR = overall response rate; PR = partial regressions; CLL = chronic lymphocytic leukemia; ALL = acute lymphocytic leukemia; CR = complete remission; CRi = incomplete blood count recovery; and NHL = non hodgkin's lymphoma.

FDA-approved therapies exhibited remarkable clinical efficacy in treating certain blood cancers and herald a clinical paradigm shift in leukemia treatment.

In comparison to B cell malignancies, CAR-T therapy development for AML treatment is much more challenging. To date, only a few CAR-T therapies have been explored in clinical trials in the context of AML and there are no licensing authorities approved for CAR-T therapy in AML patients. This progression has been delayed by the lack of suitable AML targetable surface antigen.[36,37] A variety of targets have been explored in the preclinical and clinical development of CAR-T cell AML therapy, including CD33, NKG2D, FLT3 (CD135), CD7, CD123, CD133, LeY, CLL-1, and FRβ.[38-45]

CAR-T therapies have already been used in the clinic with many encouraging results for a variety of leukemia cancer types. However, there are many more challenges that need to be overcome, opening new avenues for the optimization of current CAR-T therapies and translation of this therapy from the clinical setting to the real world. Current uncertainties lie with respect to the long-term sequelae and the best practices for follow-up and management. First of all, the main concern about CAR-T therapy is the long-term safety profile. Therefore, FDA ensures postmarketing studies to monitor long-term safety as well as the risk of secondary malignancies.[46] The two severe and life-threatening toxicities associated with CAR-T therapy are cytokine-release syndrome (CRS) and neurotoxicity, also named as CAR-T cell-related encephalopathy syndrome (CRES).[47-50] CRS is the most common side effect, which is triggered by activation and proliferation of T cells and the release of cytokines such as IL-2, IL-6, and IFN-r. Based on their severity, these range from low-grade basic symptoms to high-grade syndromes that could lead to multi-organ dysfunction. Severe CRS can also evolve into fulminant hemophagocytic lymphohistiocytisis in some rare cases. The second adverse event, CRES, occurs concurrently with or after CRS.[20] Management of CRS and CRES depends on the severity and grade of the syndromes.[14,49] However, the details of clinical indications and treatment associated with CRS and CRES are beyond the scope of this review. Furthermore, a high price tag for CAR-T therapy has also hindered their broad application. However, this issue can be addressed properly as more and more companies are pioneering the development of CAR-T therapy and soon faster and cheaper alternatives will arrive in the market.[51]

3.2 | Checkpoint inhibitors for immunotherapy of leukemia

Immune evasion is a very important hallmark for the progression and survival of cancer cells. Monoclonal antibodies targeting immune checkpoint proteins have achieved remarkable success in the clinic by reversing immunosuppression for a variety of solid tumors, including lung and skin cancer.[12] In most recent decades, immune evasion has also been regarded as one of the key mechanisms of aberrant proliferation of progenitor cells, which finally progresses into hematologic malignancies.[52] These results have prompted us to find their ways into hematology, particularly in the field of leukemia.[53,54] To date, although there are still no approved ICIs, and over 30 clinical trials are currently ongoing to evaluate the efficacy of ICIs as monotherapies, or as a part of combination strategies for leukemia patients.[55] The role of ICIs in the leukemia treatment will become clearer when these results are available. It is worthwhile to mention that anti-PD-1 has shown great success in Hodgkin's lymphoma and is being tested in a variety of non-Hodgkin lymphomas. In addition, there is growing evidence from preclinical studies both in vitro and in vivo, proving their great potential in treating leukemia. In the following section, we summarize the ICI therapies that have already been investigated in clinical trials in a variety of leukemia cancer types (Table 2). In addition, some interesting preclinical results have also been discussed.

TABLE 2 Current Immune checkpoint inhibitors (ICIs) in clinical development

Agent	Target	Indication(s)	No. of patients	Combination	Outcomes/references
Pidilizumab	PD-1	AML	8	Monotherapy	ORR = 12.5%
Nivolumab	PD-1	R/R AML	70	Azacytidine	ORR = 33% (CR/CRi = 15, PR = 1, HI = 1)
		R/R AML	14	Azacytidine, Ipilimumab	ORR = 43%
		Elderly AML	10	Azacytidine	ORR = 60%
		CLL	138	Ibrutinib	Active, ongoing
Pembrolizumab	PD-1	R/R AML	26	HiDAC	ORR = 42% (CR/CRi = 9, PR = 1, MLFS = 1)
		R/R AML	10	Decitabine	ORR = 20%
		CLL. NHL	25	Monotherapy	ORR = 44% (RT patients) ORR = 0% (advanced CLL)
Ipilimumab	CTLA-4	R/R AML after allo-SCT	12	Monotherapy	ORR = 42%

ORR = overall response rate; PR = partial regressions; CLL = chronic lymphocytic leukemia; ALL = acute lymphocytic leukemia; CR = complete remission; CRi = incomplete blood count recovery; AML = acute myeloid leukemia; HI = hematologic improvement; and MLFS = morphologic leukemia-free state.

Leukemia has proven to be an immune responsive cancer with high expression of checkpoint proteins. For instance, PD-1/PD-L1 axis has been demonstrated in the preclinical studies as an important immune evasion mechanism. Consistent with this, Zhang et al. found significant up-regulation of PD-L1 on C1498 AML murine cancer cell in vivo, although the expression was barely found in vitro.[56] Genetically knocked-out PD-1 or antibody-mediated knockout has significantly augmented antitumor immune response along with a significant prolonged survival in AML murine mouse models. Methylation inhibitor 5-azacytidine (Aza) is the first-line agent for treating elder leukemia patients. Zhang et al. found a positive correlation of Aza concentration with the expression of PD-1, demonstrating a potential synergy between Aza and anti-PD-1 treatment.[57,58] In a phase II clinical trial, Daver et al. assessed the synergistic effect of nivolumab (anti-PD-1) and Aza in R/R AML patients, showing some encouraging results; an overall 33% AML patients responded to this therapy with 16 patients achieving CR/CRi and 7 of them reaching the standard of hematologic improvement.[58] Following this work, they observed the up-regulation of CTLA-4-expressing effector T cell population in only in nonresponders, compared to responders on the aforementioned combination therapy.[58] These findings prompted researchers to explore CTLA-4 up-regulation as a potential mechanism of anti-PD-1 resistance, which has now been reported in the context of solid tumors. Notably, anti-CTLA-4 as a single agent has shown particular benefits for AML patients.[59] More clinical trials have been initiated to test the efficacy of a combination of anti-PD-1 and anti-CTLA-4 in AML patients (NCT02397720 and NCT03600155) as shown in Table 2.

3.3 | Antibody-drug conjugates for immunotherapy of leukemia

ADCs therapies consist of three components[50]: (i) a tumor-specific targeting monoclonal antibody, (ii) a cytotoxic chemotherapeutic agent, and (iii) a specialized linker that covalently connects the aforementioned two components, successfully bridging innovative immunotherapy and traditional chemotherapies. The monoclonal antibody maintains the function of its targeting effect against specific tumor antigen expressed on leukemia cells. More specifically, the covalently linked therapeutic can be released in the acidic environment of the lysosomes and achieving its cytotoxic activity. Meanwhile, chemotherapies such as azacitidine and decitabine can induce cell death by breaking the DNA double strand and arresting the cell cycle. The ADC complex is internalized upon binding to the corresponding antigen on the surface of leukemia cells along with the linked cytotoxic molecule, leading to cytotoxicity and cell death after its release. This strategy significantly improves the efficacy and reduces systemic toxicity anticancer drugs (Table 3).

BESPONSA® (inotuzumab ozogamicin) is the first and only FDA-approved CD22-directed ADC indicated for the treatment of adults with relapsed or refractory B-cell precursor ALL. The CD33 antigen is primarily expressed on the surface of blast cells in >80% of AML patients and elevated levels of CD33 have been correlated with poor prognosis.[50] Regarded as a promising therapeutic target for AML treatment, IMGN779, a Novel CD33-Targeting Antibody-Drug Conjugate With DNA-Alkylating Activity has been studied by some researchers as a CD33-targeted ADC utilizing DGN462, a potent DNA-alkylating agent, a novel DNA-alkylating agent consisting of an indolino-benzodiazepine dimer containing a mono-imine moiety. IMGN779 has shown great targeted activity against AML cell lines in vitro (IC_{50} ranging in pM), and in vivo against xenograft models in SCID mice. A recent study shows high targeting efficiency of this ADC against CD33 with complete tumor regressions (CR) and partial regressions (PR) in all animals at a single dose of 0.6 mg/kg.[60] Following this, other studies hypothesized that combination treatment of AML cells with the poly (ADP-ribose) polymerase (PARP) inhibitor, olaparib, would further enhance the antileukemic activity of IMGN779 in preclinical human AML models. They found the combination indices for IMGN779 and olaparib therapy ranged from 0.7 to 0.9, consistent with synergistic effects. There was an increase in cellular apoptosis, and a significant reduction in CFU growth of progenitor cells established from bone marrow samples of patients with AML.[61]

TABLE 3 Current ADCs and their specific targets as leukemia therapies

Agent	Sponsor	Target	Indication(s)	Payload
Alemtuzumab (Campath)	Genzyme	CD52	CLL	DM1 (N2'-Deacetyl-N2'-(3-mercapto-1-oxopropyl) maytansine)
Denintuzumab Mafodotin (SGN-CD19A /SGN-19A)	Seattle Genetics	CD19	ALL	Monomethyl auristatin F (MMAF)
Gemtuzumab ozogamicin (Mylo Targ)	PFIZER	CD33	AML	Calicheamicin
Inotuzumab ozogamicin (Besponsa)	PFIZER	CD22	ALL	Ozogamicin
Coltuximab ravtansine (SAR3419)	Sanofi	CD19	DLBCL	Ravtansine (DM4)
Loncastuximab Tesirine (ADCT-402)	ADC Therapeutics	CD19	B-cell ALL	Pyrrolobenzodiazepines (PBD) dimer
Brentuximab vedotin (Adcetrix)	Seattle Genetics	CD30	CTCL	Monomethyl auristatin E (MMAE)

CLL = chronic lymphocytic leukemia; ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia; DLBCL = Diffuse large B-cell lymphoma; and CTCL = Cutaneous T-cell lymphoma.

CD123 has also emerged as an attractive AML target due to its elevated expression on AML cells as compared to normal bone marrow cells. However, reports of severe myelosuppression and myeloablation have been presented in preclinical studies for some CD123-targeted therapies. In a study published in *Blood*, a novel humanized anti-CD123 antibody was generated with two engineered cysteines for conjugation of indolino-benzodiazepine dimers as therapeutic agents. These were cytotoxic to AML cells in a dose-dependent manner and did not appear to affect the viability of monocytes, lymphocytes, and multipotential progenitors, cell populations with consistent low CD123 levels.[62]

4 | NANOMEDICINE STRATEGIES FOR LEUKEMIA TREATMENT

Because conventional cancer treatments can be toxic with long-term toxicities, nanomedicine has become a very powerful approach to improve cancer treatment, by designing nanoscale devices for the delivery of drugs, cancer diagnosis, or therapeutics.[63,64] However, these nano-based strategies have mostly been focused on solid tumors due to their advantageous enhanced permeability and retention effect.[65-67] In the past decade, organic nanoparticles (polymeric, lipidic, and carbon-based nanomaterials), and inorganic nanoparticles (metal nanoparticles, mostly noble or inert metals) have generated a lot of interest in the field of drug delivery.[68-70] These particles can be chemically manipulated with respect to their composition, size, and shape to effectively cross biologic barriers and deliver therapeutics. Specific molecules, such as antibodies, proteins, aptamers, and peptides can be used as efficient targeting agents or ligands toward cancer cells. After successful targeting of desired cells, the payload is released in the cancer microenvironment.

4.1 | Liposomes

Liposomes are one of the most well-established nano-formulations for drug delivery. They are known to prolong circulation time of drugs in blood and, therefore, improve therapeutic outcome.[68-70] One of the first demonstrations of using liposomes in the field of delivery of ALL drugs was done by Kobayashi et al. in 1975, where they encapsulated cytosine arabinoside resulting in significantly increased survival of leukemia bearing mice. Later, a vincristine sulfate liposome system was developed in the 1990s and was approved by FDA under the name Marqibo in 2012 as an injected nano-drug used against relapsed Ph-ALL patients. CPX-351 (Vyxeos), FDA approved in August 2017 for adult AML, is a liposomal formulation has been designed for the co-delivery of cytarabine and daunorubicin at a fixed synergistic dosage (cytarabine/daunorubicin molar ratio = 5:1).[71-73] In the treatment of AML, CPX-351 demonstrated superior overall median survival (>3.61 mo), event-free survival (>1.22 mo), and remission rate (>14.4%) without increasing toxicities and mortalities in comparison to 7 + 3 based "golden therapy." Following the success of CPX-351 for AML, many more groups have been investigating liposomes as a tool to improve the overall efficacy of antileukemic therapies and to reduce the toxicities presented by these agents of interest.[74-76]

In 2014, Tan developed a liposomal formulation to co-deliver safingol and C2-ceramide, which are known bio-active sphingolipids with antileukemia efficacy. This novel formulation significantly reduced the sphingolipids-associated toxicities observed in the free combination and extended the median survival from 24 to 37 d in comparison to single drug C2-ceramide loaded liposome.[77] Other liposome formulations have also been developed to evaluate their efficacy against AML cells, such as daunorubicin-emetine (a protein synthesis inhibitor) liposomes, which demonstrated a significantly enhanced efficacy against MOLM-13 cells in vitro. Similarly, GTI-2040 (ribonucleotide reductase-targeting inhibitor) liposomal formulation also showed superior antitumor efficacy in comparison to their free drugs.[78,79]

Alvocidib (Flavopiridol) is a flavonoid alkaloid CDK9 kinase inhibitor under clinical development for the treatment of AML (Tolero Pharmaceuticals, Inc., Utah, United States).[80,81] However, the application of alvocidib is largely hindered by its solubility, high-protein binding affinity, and severe side effects including

TABLE 4 Summary of various NPs and targeting strategies in development for treatment of leukemia

Formulation	Targeting ligand	Type	Agents	Mechanism of action	Progress
Lipid nanoparticle	CD33 Peptide	AML	GTI-2040[79]	An antisense oligonucleotide (ASO) against the R2 subunit of ribonucleotide reductase (RNR)	Preclinical
lanthanide-doped nanoparticles (LDNp)	CD33 Antibody	AML	p53-activating dode-cameric peptide termed PMI[108] (TSFAEY-WALLSP)	Intracellular PMI kills AML cells by antagonizing MDM2 and/or MDMX e the two functional inhibitors of the tumor suppressor protein p53	Preclinical
Gold nanoparticles (AuNPs) with adsorbed high-density lipoprotein (HDL)	Passive targeting	AML	BMS309403 (BMS)	Selectively inhibits AML-promoting factor fatty acid-binding protein 4[98]	Preclinical
Poly (maleic anhydride-co-vinyl acetate) (MAVA) copolymer	Ara-C prodrug for glioma treatment	Glioma (C6 Cell line) In vitro study	Cytarabine (Ara-C)[109]	Antimetabolite, antiviral, and immunosuppressive agent	Preclinical
Bio-conjugate (HA-Ara-C) Amphiphilic small molecular prodrug of Ara-C	Folate receptor (FR)	CML K562 In vitro study	Cytarabine[110]	Antimetabolite, antiviral, and immunosuppressive agent	Preclinical
Ara-C prodrug DTA-Ara by conjugating 2-decyltetradecanoic acid (DTA), a double-chained fatty acid with 24 carbons with Ara-C	Passive targeting	CML K562 cell line AML HL-60 cell line	Cytarabine[111]	Antimetabolite, antiviral, and immunosuppressive agent	Preclinical
Gold nanoparticles (AuNPs)	A novel nuclear localization signal peptide	AML	Anti-221 and AS1411[97]	NCL/miR-221/NF-kB/DNMT1 axis as a new molecular pathway promoting aggressive acute myeloid leukemia (AML) leukemogenesis.	Preclinical
Chitosan nanoparticles	Passive targeting	Leukemia	Cytarabine[112]	Antimetabolite, antiviral, and immunosuppressive agent	Preclinical
Dual drug liposome	Passive targeting	AML	Cytarabine Daunorubicin[74-76]	Antimetabolite, antiviral, and immunosuppressive agent & Topoisomerase Inhibitor	FDA approved
PEGlayted mitoxantrone liposome (PLM-60)	Passive targeting	Leukemia	Mitoxantrone	Type II topoisomerase inhibitor; disrupts DNA synthesis and DNA repair by intercalation between DNA bases	Phase I/II clinical trial
Alvocidib (flavopiridol) liposome	Passive targeting	Leukemia	Alvocidib[82]	Flavonoid alkaloid CDK9 kinase inhibitor	Preclinical

ALL = acute lymphocytic leukemia and CML = chronic myeloid leukemia.

nausea/vomiting, fatigue, diarrhea, and neutropenia.[70] To address these issues, alvocidib was encapsulated by a pH-gradient mechanism into liposomes, which consisted of different lipids such as Phospholipid hydrogenated soy phosphatidylcholine (HSPC)/Chol, HSPC/Chol/Tween-80, and HSPC/Chol/1,2-Distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE)-poly-(ethylene glycol) (PEG)2000.[82] Among them, HSPC/Chol/DSPE-PEG2000 liposome (~120.7 nm) exhibited a high entrapment efficiency (~70.4%) and was selected for pharmacokinetic studies in vivo where it increased half-life, area under the curve (AUC), and reduced clearance rate of the drug.

Mitoxantrone is a classic chemotherapy for AML, which significantly improves the survival rate of children suffering from ALL relapse,[38] but presents adverse effects including neutropenia, cardio-toxicity, and bone marrow suppression.[83] The first liposomal formulation of mitoxantrone was generated in the 1990s by Schwendener on the basis of electrostatic interactions between the cationic drug loaded into liposome composed of soy phosphatidyl choline, cholesterol (Chol), and D, L-α-tocopherol. This formulation showed improved efficacy and reduced toxicity in comparison to free drug in a variety of tumor models including murine ALL model L1210.[84] However, liposomal mitoxantrone was cleared rapidly from the blood circulation, which limited its application. To overcome this, a series of follow-up studies have been done to improve the pharmacokinetic profile. In a particular preclinical study, a novel

mitoxantrone soy phosphatidyl choline/Chol liposome system was developed by modifying the structure with 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(methoxy[polyethylene glycol]-2000) (DPPE-PEG2000) through a pH-gradient-mediated process. This formulation demonstrated superior pharmacokinetic properties with a 40-fold increase in the AUC.[85] Many similar studies have been conducted to optimize the mitoxantrone release profile in regard to its formulation. By formulating mitoxantrone into the conventional 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC)/Chol liposomes and sterically stabilized DSPC/Chol/DPPE-PEG2000 liposomes, a significantly extended the survival outcome was observed in a lymphocytic leukemia L1210 mouse model.[86] This system also showed an increase in drug release rate from DMPC/Chol liposomes (1.7 μg/μg lipid/h) in comparison to DSPC/Chol liposomes (< 0.0257 μg/μg lipid/h).[87,88] In the 2000s, research on mitoxantrone encapsulation continued with the use of 1,2-dioleoyl-sn-glycero-3-phosphocholine/Chol/cardiolipin, which was based on the electrostatic interaction between the cationic agent and the ionic cardiolipin.[89] The formulation was named liposome-entrapped mitoxantrone easy-to-use and evaluated in a phase I clinical trial (NeoPharm).[90] Another PEGylated liposome indicated that a formulation with hydrogenated soy phosphatidyl choline (HSPC)/Chol/1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(methoxy [polyethylene glycol]- 2000) (DSPE-PEG2000) and high Poly - ethylene glycol (PEG) density achieved a particle-size dependent efficacy.[91] It has been systematically studied that small-sized liposomes around ~60 nm exhibited faster release rate along with maximized efficacy and least toxicity.[92] The small-size formulation, termed PLM-60, was translated into a phase I clinical trial in patients with non-Hodgkin's lymphoma and found to maintain longer circulation time, exhibit less toxicity, and more efficacy in comparison to unencapsulated mitoxantrone. In 2018, a randomized phase I/II clinical trial (NCT03553914) had been initiated to evaluate PLM-60 in terms of their toxicity and overall response in patients with peripheral T cell lymphoma.[93]

4.2 | Polymeric nanoparticles

In addition to liposomal formations, polymeric nuclear proteins (NPs) delivery systems have also been investigated for leukemia treatment. This delivery system is majorly formed through the assembly of copolymers.[94] Zong et al. developed a mPEG-polylactic acid micelles for delivery of parthenolide (PTL), which was encapsulated in a protective degradable porous silicon and coated with E-selectin thioaptamer to direct this multifunctional vector toward bone marrow. PTL is a preclinical agent against resistant AML stem cells and PTL-loaded micelles system successfully inhibited AML burden by delivering PTL to bone marrow in patients-derived AML xenografts.[95] Additionally, Simon et al. developed a poly-D, L-lactadie-co-glycolide based polymeric nanoparticle for delivery of all-trans retinoic acid (a form of chemotherapy) for treating AML. Varshosaz et al. developed a newly synthesized folate and retinoic acid grafted/dextran (FA-RA/DEX) polymer to develop polymeric micelles for targeted delivery of doxorubicin (Dox) for AML treatment. This Dox-loaded micelle system exhibited enhanced in vitro cytotoxicity against KG-1 cells in comparison to free Dox. Certain dendrimer-based formulations have also been investigated for treating leukemia. Szulc et al. developed cytarabine-complexed dendrimers for formulating cytarabine triphosphate, which significantly enhanced the cytotoxicity against 1301 cells (a T cell leukemia cell line). For further improving targeting efficiency, co-polymeric NPs functionalized with CD19 antibodies have also been developed to delivery of Dox against ALL. Conjugation of such antibodies enhances the internalization of NPs via a receptor-mediated endocytosis in ALL cells. Such systems exhibit significantly higher therapeutic efficacy along with reduced systemic toxicity in comparison to free drug in ALL-bearing mice.[96] Therefore, polymeric nanoparticles are of great potential for leukemia treatment.

4.3 | Metal nanoparticle

Noble metal nanoparticles (gold and silver, in particular) are widely employed in biomedical applications mostly because of their unique optical properties and higher sensitivity to detect cancer cells. AuNPs exhibit various advantages such as low toxicity, greater biocompatibility, biodegradability, and high volume-to-surface ratio. AuNPs offer protection against degradation by RNases, thus increasing circulating times and subsequent increase of the payload of drug delivered to cells In 2018, Rong Deng et al. developed a novel a nuclear localization signal peptide-targeted gold nanoparticles co-delivery of anti-221 and AS1411 (NPsN-AS1411/a221) for targeting Nucleolin (NCL)/miR-221/NF-kB/DNA methyltransferases (DNMT1) signaling pathway, which has been demonstrated as a new molecular pathway for promoting AML progression. NPsN-AS1411/a221 remarkably inhibits leukemia proliferation in vitro and in vivo.[97] Shen et al. developed an AuNP-based delivery system (HDL-AuNPs-BMS) for AML by delivering BMS309403 (BMS), a small molecule that can selectively inhibit AML-promoting factor fatty acid-binding protein 4, which significantly increased antileukemia activity both in vitro and in vivo.[98] Gossai et al. modified AuNPs with dsDNA oligonucleotides with a sequence of BIRC5 gene, which is overexpressed in CML cell lines. These functionalized AuNPs were further loaded with dasatinib against CML (K562), which has demonstrated great antileukemia efficacy both in vitro and in vivo.[99] Many other in vitro and in vivo studies have also been pursued for delivering drug-loaded AuNPs.[100-102] For example, Vinhas et al. combined the silencing potential of oligonucleotide modified AuNPs with imatinib[103-107] Taken together, metal nanoparticles are of great potential for enhancing leukemia treatment.

5 | CONCLUSION

In this review, we look at chemotherapies currently used in the treatment of various types of leukemia. Although these drugs provide decent CR and OS, different risk groups respond differently to such treatments with high-risk patients facing a greater mortality rate. For such reasons, researchers are coming up with new strategies to treat the disease more effectively while reducing toxicity (Table 4).

We can now use the patients' own immune system to generate specific CAR-T cells engineered to specifically attack the body's cancer cells. This approach has met with a lot of success over the past decade and currently there are more than a hundred clinical trials registered for CAR-T therapies. Because leukemia is an immune-responsive cancer, another immunotherapeutic approach is the use of checkpoint inhibitors such as the anti-PD-1 and anti-CTLA-4 based therapies. There are currently more than 30 clinical trials evaluating the efficacy of such ICIs and some have shown positive results in patients with high CR/CRi. Elevated expressions of certain proteins and markers have also been studied to engineer ADCs against ALL in vitro and in vivo. Nano-based systems, such as liposomes, micelles, dendrimer, and metal nanoparticles have also emerged in the past decade, to further increase the efficacy of anticancer therapeutics and reduce their systemic toxicity. We discussed how various formulations have been developed and optimized by researchers in the past couple of years and is being evaluated in patients in many phase I/II clinical trials. Polymeric and metal based-nanoparticles have also been exploited in various leukemia cancer cell types in vitro and in vivo, to increase the efficacy and delivery of anticancer patients.

In summary, there are many exciting advancements in the specific therapeutic targeting of leukemia and its subtypes. As we discover more about the disease, researchers continue to learn more and more about the success and challenges faced by the nano- and immunotherapy and other novel approaches to successfully treat leukemia.

AUTHORSHIP

Z.W., R.S. and P.M. Contributed equally to this manuscript. Wan conceived of the concept for this review and created an outline, then all authors contributed sections and provided input and feedback on each other's text.

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ORCID

Qiang Ao https://orcid.org/0000-0001-6747-7100

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