Expert Opinion

Drug delivery in acute myeloid leukemia

Johannes Kohlschütter, Stefan Michelfelder & Martin Trepel†
†*University Medical Center Hamburg-Eppendorf, Department of Oncology and Hematology, Martinistrasse 52, D-20246 Hamburg, Germany*

Background: Acute myeloid leukemia was among the first malignancies to be cured by drug therapy alone, but overall survival rates remain unsatisfactory and have changed little over the past 20 years. Conventional chemotherapeutic regimens, which almost invariably include cytarabine and anthracyclines, are untargeted, and more specific therapies are needed. *Objective*: We have chosen acute myeloid leukemia as a disease prototype to review established and novel targeted approaches in leukemia treatment. *Methods*: Our selection of the reviewed literature focused on drug delivery aspects. *Conclusion*: While the toxicity profile of chemotherapeutics has been improved by liposomal formulations and antibody conjugation for leukemia-directed uptake, their efficacy has probably not changed significantly. Drugs with an alternative mode of action, including kinase inhibitors, hold great promise. Further improvements may result from the characterization of novel acute myeloid leukemia (AML) cell surface receptors and of leukemic stem cells, as well as from the design of leukemia-targeted gene therapy vectors.

Keywords: AAV-display, acute myeloid leukemia, chemotherapy, drug delivery, drug targeting, gene delivery, kinase inhibitors, phage display

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1. Introduction

The term leukemia covers a heterogeneous group of diseases characterized by the malignant clonal proliferation of blood progenitor cells. These cells primarily grow and expand in the bone marrow, and from there spread to the entire body via the blood circulation. Thus there is an accumulation of abnormal, often immature leukemic cells in the bone marrow, peripheral blood and other tissues. The expansion of the malignant clone within the bone marrow results in a reduced number of normal red blood cells, platelets and neutrophils. This causes a variety of systemic symptoms and signs, the most important of which are anemia, bleeding and an increased risk of life-threatening infections. The latter is the most frequent cause of death in leukemia.

Based on the kinetics of disease onset and course, as well as the differentiation of the malignant clone, leukemias are divided into acute and chronic, and myeloid and lymphocytic, respectively. While acute leukemias usually have a rapid course and, if untreated, invariably lead to a fatal outcome within weeks or months after the initial presentation, chronic leukemias tend to have a longer course of years or even decades.

In terms of drug delivery, leukemia has unique features. Most importantly, leukemia is by definition a systemic disease, and therefore drug delivery will always have to use a systemic route. Most of the currently available therapeutic agents, both established and experimental, are applied intravenously, but an increasing number of newer drugs are applicable orally or subcutaneously.

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This review focuses on acute myeloid leukemia (AML). Drug therapy and delivery have been studied extensively in this form of leukemia and we can only summarize some of the many new aspects in drug therapy that have evolved for this disease during the last decade. The term AML comprises several subgroups of leukemias that share the acute course and the myeloid marker profile, but vary in differentiation, genetic aberrations, response to treatment and prognosis. Yet, except for acute promyelocytic leukemia, the therapeutic approach for most AMLs has been similar, something that may change over the next decade based on the availability of targeted drugs and tailored treatment strategies.

AML evolves based on a series of genetic changes in a hematopoietic precursor cell, altering normal hematopoietic growth and differentiation and finally resulting in expansion of the malignant clone in the bone marrow and peripheral blood. These cells apparently have unlimited proliferation potential, but usually do not maturate into regular blood cells such as erythrocytes, platelets, or neutrophils. Like in other malignancies, the genetic alterations in AML result in both the activation of oncogenes and the dysfunction of tumor suppressor genes. Unlike most solid tumors, however, many hematologic malignancies, including AML, are associated with a single characteristic cytogenetic abnormality such as the translocation t(15;17) in acute promyelocytic leukemia.

Current treatment strategies are mainly based on high dose chemotherapy regimens using anthracyclines and cytarabine as backbone drugs [1]. After having achieved complete remission, allogeneic stem cell transplantation plays an increasing role, especially in high risk patients with unfavorable cytogenetic profiles, other risk factors, or relapsed disease. The toxicity of these current treatment regimens is considerable, preventing their use especially in elderly patients [1].

A number of factors predicting poor outcome have been described for AML, including poor performance status, advanced age, karyotype and other molecular changes [2]. Overall, the prognosis of patients suffering from AML remains poor, despite significant therapeutic advances over the last two decades. Less than 40% of AML patients under 60 years of age can be cured [1,3-5]. In older adults, accounting for the majority of AML patients, long-term disease-free survival is rare and the available treatment options are limited [3,5,6]. These discouraging facts have spurred major efforts in the development of novel targeted therapies in the treatment of AML. Many of such new therapies targeted to specific molecular features of AML are currently under clinical evaluation and some of them are discussed below.

This review focuses on which drugs are available to be delivered to AML cells, what delivery routes they take and what their potentials and their limitations are. Delivery has to take into account the general approach, which is always systemic in leukemia, the route of administration, the interaction of the drug with the cell membrane (active internalization, passive diffusion) and intracellular trafficking.

2. Drugs and drug delivery for acute myeloid leukemia

2.1 Classical cytostatic drugs

Standard chemotherapeutic regimens for AML treatment are based on a combination of anthracycline and cytarabine.

2.1.1 Anthracyclines

Anthracycline development began in the 1960s [7]. Most of these agents have to be administered intravenously, except for idarubicin for which an oral formulation is available. Anthracyclines are taken up by the target cell via passive diffusion and, once inside the nucleus, intercalate with DNA. Furthermore, they inhibit strand re-ligation by topoisomerase II, causing DNA double-strand breaks [8]. After hepatic metabolization, anthracyclines are eliminated by biliary excretion. Daunorubicin is the anthracycline most often used for AML treatment. Its lipophilic analog idarubicin and its active metabolite 13-hydroxyidarubicin have a longer half-life than daunorubicin. Despite preclinical evidence suggesting otherwise, clinical trials have failed to prove a substantial advantage of idarubicin over daunorubicin in terms of efficacy and toxicity [9]. Mitoxantrone is a synthetic anthracycline analog used in combination with cytarabine for AML with at least comparable and maybe superior efficacy in upfront and reinduction regimens [10,11].

2.1.2 Cytarabine

Cytarabine was approved by the FDA almost 40 years ago. The drug is administered parenterally, for induction regimens usually intravenously, and has a short half-life requiring high-dosed short time or medium-dosed continuous infusions [12,13]. Inside the cell, the phosphorylated drug enters the nucleus and is incorporated into DNA in place of cytosine, blocking DNA replication. Cytarabine is metabolized by cytidine deaminases and is eliminated by renal clearance. Like other chemotherapeutics, its action is cell cycle-dependent, and therefore its therapeutic effects are focused on rapidly dividing cells like cancer cells, despite its unspecific biodistribution.

2.1.3 Standard treatment for patients in good physical condition

The most common chemotherapy regimen to induce remission in AML is daunorubicin as a 15 min intravenous injection daily for 3 days plus cytarabine given by continuous intravenous infusion for 7 days (so-called '3 + 7' regimen). With this regimen, 60 – 80% of patients, depending on age and other risk factors, achieve a complete remission [1,14]. This response rate has not been improved to a clinically

relevant extent by changing the dose of either of the two agents or by adding an additional drug. The cytostatic agents used for remission induction confer substantial toxicity including myelosuppression, mucositis, diarrhea and cardiotoxicity.

2.2 Novel therapeutic agents

In view of the high remission rates achieved in AML patients using the standard chemotherapeutic regimens, novel agents would have to meet high standards of efficacy to replace these regimens [15]. However, relapse rates and toxicity, as well as the limited treatment options in elderly patients, highlight the urgent need for novel agents that improve disease-free survival and do not add substantial toxicity. While conventional chemotherapy may remain the backbone of treatment, novel agents could be added to improve outcome. Within the last few years, many such novel agents have been introduced. Some of them have started to gain the status of a standard treatment option in certain settings, such as liposomal or antibody-conjugated chemotherapy. Others are currently at a more experimental stage, including farnesyltransferase inhibitors [16], histone deacetylase inhibitors [17], proteasome inhibitors [18], and anti-angiogenic agents such as bevacizumab [19]. Yet many challenges remain; these are addressed at the end of this article.

2.3 Liposomal delivery of chemotherapeutic drugs

Anthracyclines are one of the two standard chemotherapeutic drugs used in AML. However, their toxicity is of concern. Above all, cardiotoxicity is dose-limiting and cumulative dose-dependent, which often prevents anthracycline retreatment in relapsed AML or even upfront treatment in patients with cardiac disease.

To increase the therapeutic index, liposomal formulations were proposed as carriers for cancer therapeutics several decades ago [20]. Liposomes encapsulate an aqueous solution containing the drug inside a hydrophobic membrane. Liposomal encapsulation results in reduced anthracycline uptake by normal, non-neoplastic tissues. In contrast, delivery to tumor tissue and to the bone marrow is enhanced due to the passage of liposomes through fenestrations of the vascular endothelium, which are characteristic for these but not for other tissues [21,22]. Liposomes are believed to be taken up by membrane fusion rather than endocytosis unless they are modified specifically to trigger this event [23]. Liposomal formulations are characterized by slower pharmacokinetics compared to non-encapsulated administration of a given drug. They may therefore be the agents of choice when the objective is to maintain a defined plasma concentration with little change over time, rather than high but quickly decaying peak levels.

Liposomal formulations of doxorubicin and daunorubicin are currently available for clinical use. The application of liposomal daunorubicin in AML has been extensively reviewed elsewhere [24]. Briefly, compared to conventional daunorubicin application, liposomal daunorubicin results in reduced conversion into its toxic metabolite daunorubicinol and a reduction in toxic side effects such as cardiotoxicity, alopecia, nausea, or myelosuppression. In addition, various *in vitro* studies suggest that liposomes may help to overcome P-glycoprotein-mediated efflux of anthracyclines, a mechanism believed to contribute substantially to anthracycline resistance in AML and other tumor cells [25,26]. Liposomal daunorubicin combined with cytarabine or alone yielded a complete remission rate of approximately 30 – 45% in patients with refractory or recurrent AML [27,28].

Liposomes can be targeted by the incorporation of homing molecules into their hydrophobic surface. For instance, the attachment of folate molecules to liposomes [29] via a PEG anchor was used to target cells expressing the folate receptor, a common property of malignant cells in general [30] and of AML cells in particular [31,32]. The efficiency of such targeting approaches could possibly be increased if the expression of a receptor of interest can be stimulated, such as is possible with all-trans retinoic acid that induces an upregulation of the folate receptor in AML cells *in vitro* [33].

Efficient liposomal delivery may require sophisticated strategies, depending on the drug of interest. For arsenic trioxide, a procedure for the formation of nickel (II) arsenite complexes in liposomes that release the active drug under acidic pH conditions as present in lysosomes has recently been suggested [34]. Increasing particle stability is an important issue in improving liposomal therapy, but it may be achieved at the cost of impaired drug release. A recently described approach using lipase may overcome this problem [35].

2.4 Novel drugs interacting with intracellular targets

The tremendous success of the BCR-ABL tyrosine kinase inhibitor imatinib mesilate in chronic myeloid leukemia has stimulated the exploration of novel agents targeting various pathways in cancer. For AML, our increasing knowledge about intracellular signaling cascades involved in this disease has revealed a number of promising targets for inhibitory therapy by small molecules. They are usually applied orally and do not depend on receptors for cellular uptake.

One therapeutic approach is directed towards the RAS protein, which is frequently mutated and therefore dysregulated in AML and other malignancies [36]. Attachment of RAS and other regulatory molecules to the plasma membrane is crucial for their functionality. Small molecule farnesyl transferase inhibitors such as tipifarnib and lonafarnib [37], after passively diffusing into the cell, inhibit RAS membrane anchoring. Tipifarnib has achieved clinical responses in patients with refractory and relapsed poor-risk AML [16] and is currently being evaluated in Phase III trials [38,39].

Another novel therapeutic approach targets the FMS-like tyrosine kinase 3 (FLT3). Mutations in the *FLT3* gene

producing internal transmembrane duplications (FLT3/ITD) are common in AML and result in constitutive FLT3 activation [40,41]. A number of small molecule inhibitors of FLT3 have been evaluated in clinical trials lately, including tandutinib (MLN518), lestaurtinib (CEP-701) [42] and PKC412, and evidence of anti-leukemic activity has been seen [42-44]. Like other kinase inhibitors, these agents are orally applicable and their delivery to AML cells is receptor-independent.

While the oral application of small inhibitory molecules simplifies their use in an out-patient setting, this may not always be the preferred route of administration given the poor oral intake and nausea experienced by many cancer patients under treatment [45]. In addition, target specificity remains an issue in kinase inhibitor therapy. Under some conditions, inhibitors with multiple targets may have beneficial effects, as shown recently for the multi-kinase inhibitor sorafenib in a xenograft model of FLT-driven leukemia [46]. Yet the lack of specificity of some kinase inhibitors may account for limited anti-leukemic activity and side effects. The latter are usually considered mild compared to those associated with conventional cytostatic drugs, but can occasionally be quite severe, for example in heart tissue, as described for imatinib and other agents [47].

In terms of specificity, agents such as monoclonal antibodies or peptides targeting cell surface molecules may therefore be superior to small molecules.

2.5 Receptor-targeted drug delivery in AML

Targeting cell surface molecules in cancer is a paramount issue in drug delivery, affecting both efficacy and specificity (and therefore toxicity) of an anti-neoplastic drug. By specific homing after systemic administration, compounds are directed to the cell type or tissue of interest. This prevents their action in non-target tissues, thereby increasing therapeutic efficiency while decreasing adverse effects. Thus, as for other malignancies, drug-conjugated ligands targeting unique surface receptors have been developed for AML treatment.

2.5.1 Anti-CD33 monoclonal antibodies

During the last decade, targeted monoclonal antibodies have revolutionized cancer therapy. In AML, the CD33 antigen is a promising target since it is ubiquitously expressed on myeloid blasts in most patients, but neither on healthy pluripotent hematopoietic stem cells nor most non-hematopoietic cell types. CD33 is a member of the sialic acid binding Ig-like lectin (Siglec) family and has two cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs). CD33 is involved in cell–cell interactions and signaling in the hematopoietic system and may have regulatory functions in the immune system and in cell proliferation [48,49]. The first targeted compound successfully used in AML treatment was Gemtuzumab ozogamicin (GO), a monoclonal anti-CD33 antibody linked to the cytotoxic agent calicheamicin. The conjugate is usually given as a 2-h intravenous infusion. Following systemic administration, GO is efficiently and specifically directed to CD33-positive cells. Upon binding to CD33, the GO–CD33 complex is rapidly internalized. The uptake is boosted by new CD33 molecules replacing the internalized ones [50]. Lysosomal release of calicheamicin and translocation to the nucleus cause DNA double-strand breaks and cell death. The efficacy of the drug is influenced both by CD33 expression level and P-glycoprotein activity [51]. Consequently, the therapeutic efficacy of GO may be potentiated by *in vivo* stimulation of CD33 surface expression on AML blasts in patients with G-CSF [52], or by reducing the calicheamicin efflux of malignant cells by P-glycoprotein inhibitors [53].

GO treatment in patients with relapsed AML can result in remission rates as high as almost 30% [4,39,48,54-56]. As CD33 is also expressed by benign myeloid precursor cells, Kupffer and sinusoidal liver cells, myelosuppression and hepatotoxicity are common GO side effects [48]. In addition, anaphylactic reactions and veno-occlusive disease have been described as life-threatening side effects in a low but significant number of patients. Other toxicities of GO include fever, hypotension and abnormal liver function tests, all of which are usually transient [57].

Anti-CD33 antibodies have shown effects against leukemic cells *in vitro* even without the attachment of a cytotoxic drug [58]. However, the unconjugated humanized anti-CD33 monoclonal antibody lintuzumab failed to elicit anti-leukemic effects when added to conventional chemotherapy in a Phase III trial [59]. Nevertheless, the promising studies using GO reveal the potential of targeted drug delivery in AML treatment.

Since FMS-like tyrosine kinase 3 (FLT3) is expressed in approximately 90% of AML cells and plays a major role in survival and proliferation signaling in leukemia blasts, several FLT3 small inhibitor molecules have been demonstrated to show anti-leukemic activity, as outlined above. Nevertheless, the lack of specificity of these kinase inhibitors remains a significant problem as they also interact with several other cellular kinases [4]. Furthermore, cellular targets of most chemotherapeutic agents are located in the nucleus, therefore rapid internalization of drug–ligand conjugates is critical to maximize therapeutic efficacy while minimizing side effects. Towards this end, several FLT3-directed antibodies were isolated using a cell-based phage library screening protocol and two fully human antibodies with the capability to trigger efficient receptor internalization upon binding to FLT3 were generated [60]. Such anti-FLT3 antibodies may be promising therapeutic agents in FLT3-expressing AML for receptor blocking or for antibody-guided cytotoxic drug therapy.

For the further development of receptor-targeted cancer therapy, a comprehensive understanding of differential receptor expression is needed. So far, very little is known about receptors specifically expressed in AML cells and their

interaction during disease development and progression. Some knowledge about the unique receptor profiles of AML cells may be gained from microarray gene expression profiling [61,62]. Among the limitations of such approaches is the fact that the protein expression patterns do not necessarily correlate with the functional state and extracellular accessibility of the potential target molecule. Protein-based techniques may be of advantage here, as discussed in the following section.

2.6 Novel cell surface markers as potential therapeutic targets in AML

Phage display is a powerful tool to select for novel ligands targeting cell type-specific surface molecules, even if only the cell type of interest, rather than an exact target receptor, is known *a priori*. The receptors bound by such ligands can be subsequently identified in the majority of cases. Screening phage displayed human antibody libraries on primary AML blasts, Bakker *et al.* enriched a single chain Fv fragment strongly binding to myeloid cells. The antigen was identified to be the transmembrane glycoprotein C-type lectin-like molecule 1 (CLL-1). CLL-1 acts as a signaling receptor and is expressed in > 90% of AML samples. CLL-1 appears to be restricted to hematopoietic, particularly myeloid, cells. It is also weakly expressed in $CD34^+/CD38^+$ or $CD34^+/CD33^+$ progenitor cells. Of note, CCL-1 expression is absent in the $CD34^+/CD38^-$ or $CD34^+/CD33^-$ stem cell compartment [63] but may be found in $CD34^+/CD38^-$ leukemic stem cells [64]. Almost 70% of CD33-negative AMLs expressed CLL-1, indicating that CLL-1 complements CD33 as a therapeutic cell surface target for AML. Anti-CLL-1 antibodies may therefore have great potential for AML therapy and for the detection of AML stem cells. This may improve the efficacy of current therapeutics, especially when combined with CD33-directed therapy [63].

A non-biased approach to the identification of high affinity binding ligands is the screening of phage libraries displaying small random peptides. This strategy has been successful for a variety of cell types and tissues *in vitro* and *in vivo* [65,66]. Linked to cytotoxic agents, such peptide ligands can be exploited for targeting cytotoxic drugs or other therapeutic agents to the cell type of interest [67-71]. Furthermore, screening phage peptide libraries allows for the exploration of epitopes recognized by known antibodies or even the identification of novel molecular markers by fingerprinting of circulating antibodies in cancer patients [72-75].

In a recent study, we selected phage libraries on AML cell lines. We identified a peptide with the amino acid sequence CPLDIDFYC, which strongly and specifically binds to AML cells [76]. Binding correlated with the expression of the AML1/ETO fusion gene, which is a result of the chromosomal translocation t(8;21), the most frequent karyotype aberration in AML. We identified VLA-4 ($\alpha4\beta1$) integrin as a potential receptor for the leukemia cell-binding CPLDIDFYC peptide [76]. VLA-4 is involved in cell–cell and cell–extracellular matrix adhesion by interaction with the vascular cell adhesion molecule VCAM-1 and the extracellular matrix protein fibronectin. Attachment to fibronectin within the bone marrow stroma appears to mediate resistance to chemotherapeutic drugs in leukemia cells [77]. CPLDIDFYC and other VLA-4 antagonists such as the monoclonal anti-VLA-4 antibody natalizumab may therefore serve as future therapeutic agents in AML for receptor blocking or for cytotoxic drug delivery.

2.7 Leukemic stem cells as potential therapeutic targets in AML

Acute leukemia most likely develops from a single transformed hematopoietic progenitor cell. A substantial amount of evidence suggests that, once this cancer has evolved, a subpopulation of leukemia cells with the stem cell-like characteristics of asymmetric division and self-renewal capacity drives the course of the disease. The characterization of these leukemic stem cells (LSCs) has therefore gained tremendous interest during the last decade. LSCs may withstand cytotoxic chemotherapy as they are often in a quiescent state, unlike their rapidly proliferating progeny [78]. LSCs are therefore considered to be responsible for the recurrence of leukemia even after initial treatment success. LSCs have been characterized by the presence or the absence of various sets of surface markers, but are widely recognized to be part of the $CD34^+/CD38^-$ cell compartment [79,80].

LSCs may be distinguished from non-malignant hematopoietic cells by the presence of the interleukin-3 receptor α chain (CD123) [81]. This finding has made CD123 a potential therapeutic target. A diphtheria toxin-interleukin-3 fusion protein has shown toxicity against LCSs while sparing normal progenitors *in vitro* [82,83], and such treatment prolonged survival in a mouse model [84]. The compound was recently evaluated in a Phase I study [85].

While markers exclusively expressed on LSCs appear particularly attractive for the purpose of targeting LSCs, there is evidence that certain receptors can be promising therapeutic targets even if they are expressed on other cell types as well. The adhesion molecule CD44 – although expressed ubiquitously – is thought to be crucial to the malignant properties of AML LSCs, and an activating anti-CD44 antibody reduced engraftment of AML cells in a mouse model [86].

2.8 Gene delivery

Despite many hurdles, gene therapy might be a future option for AML treatment. The spectrum of therapeutic transgenes mediating killing of malignant cells includes genes encoding toxic, pro-apoptotic, antiproliferative proteins or classical suicide genes such as the herpes simplex virus thymidine kinase gene. Alternatively, immune system-mediated cancer cell elimination may be

achieved by delivery of genes encoding costimulatory molecules, for example IL-2, IL-7, IL-12 [87-89], or immunomodulatory molecules such as CD40, CD80 [90-92], or interferon β [93].

One of the major unsolved issues in gene therapy is vector application and delivery to the cells or tissue of interest. Development of efficient and specific vectors for gene transfer is just as crucial to therapeutic success as is the choice of the transgene itself. At present, viral vectors remain the most effective means for therapeutic gene delivery, although substantial progress in non-viral transduction of hematopoietic cells has been achieved, including electroporation, nucleofection and particle bombardment techniques [94]. Initial *in vitro* experiments have suggested lentiviral [92], retroviral, or adenoviral vectors as suitable delivery vehicles for leukemic cells [95]. However, unintended integration of retroviral vectors into the genome or adverse immune reactions elicited by adenovirus administration are serious safety issues to be considered in choosing vectors for clinical application. Over the past few years, vectors derived from adeno-associated virus (AAV) have emerged as efficient tools to achieve long-term gene expression in a wide range of cell types. The low frequency of random integration into the genome [96], as well as the absence of a substantial cellular immune response, make AAV vectors promising tools in terms of biological safety [97,98].

Various approaches have been taken to make the binding of therapeutic vectors to target cells more efficient and specific. Bispecific conjugates such as antibodies that bind to both a vector and a target cell are one strategy [99]. However, such complexes may be unstable or immunogenic, compromising efficiency and safety. This issue may be overcome by covalent vector modifications. Towards this end, AAV offers various opportunities for targeting. The natural tropism of AAV capsids may be changed by exploiting the diversity of natural serotypes [100]. Alternatively or in addition, peptides mediating binding to the cell type of interest can be identified by random phage display library screening and subsequently be introduced into an AAV capsid region critical for receptor binding [101-106]. However, the success rate of this approach is variable. Our own experience has been that only a minority of selected peptide ligands function in modified vector capsids such as adenovirus or AAV equally well as they do in targeted phage particles. This may be attributable to the fact that the phage-derived peptides were selected only for cell or receptor binding but not for subsequent post-targeting cell entry that is required for gene transfer. Furthermore, the structural context is probably crucial. The binding property of a ligand peptide may change unpredictably when it is incorporated into a virus capsid protein subjecting it to structural constraints not present in the phage capsid that was used for selection of the ligand from the random library. Taking these limitations into account, we and others have developed random peptide-display libraries based on the gene therapy vector capsid itself for AAV [107,108] and later for retroviruses [109-112]. Thus, peptide ligands binding to a cell type of interest within the specific viral capsid protein context can be selected. Using this approach, vectors were isolated that specifically and efficiently transduce the cell types they have been selected for [107,108,113].

We have recently screened random AAV-displayed peptide libraries on several AML cell lines, enriching the leukemia targeting peptide motif NQVGSWS [114]. Vectors displaying such peptides transduced several hematopoietic cancer cell lines but not a panel of control cells. Consequently, such targeted AAV mutants can be used for therapeutic suicide gene transfer, achieving cell type-specific killing in AML cells [114].

3. Conclusion

Despite all efforts to optimize drug therapy during the last two decades, acute myeloid leukemia remains a devastating disease with a dismal prognosis, especially in the elderly. Chemotherapy based on anthracyclines and cytarabine, in some cases combined with stem cell transplantation, offers the only chance of a cure. However, such therapy and curative outcome is usually limited to the minority of patients, that is the young, fit patients with few or no risk factors.

Drug delivery to leukemia has to take into account the need for systemic drug administration and the need for prevention of collateral damage caused by the toxicity of current therapy regimens. In terms of drugs and drug delivery, recent progress comprises the liposomal formulation and antibody-guided application of classical chemotherapeutic agents, as well as the identification of novel drug targets for intracellular kinase inhibitors. These concepts have begun to prove their value in clinical studies and some of them will likely gain status as established leukemia therapeutics in the near future. More experimental approaches that are likely to translate into therapeutic concepts within the next 10 years include the targeting of leukemic stem cells and the design of gene therapy vectors specifically and efficiently targeting leukemia cells.

4. Expert opinion

Acute myeloid leukemia is a systemic disease. As such, it used to be the hallmark of success of modern cancer drug therapy. When the classical cytostatics were introduced in the treatment of cancer several decades ago, acute leukemias were among the few malignancies in which consequent improvement of cytostatic drug development and treatment protocols actually resulted in cure of some of these patients who, before that, invariably died of their disease. Ever since, however, progress has been slow and the gain in survival

rates has been slight. Most of this progress has been unrelated to drug development or drug delivery, but rather to advances in supportive care (including anti-infectants and optimized transfusion indications) and allogeneic stem cell transplantation, which is now associated with significantly less toxicity and is amenable even to the elderly beyond 70 years of age.

But what are the advances in terms of novel drugs or novel drug delivery mechanisms? In fact, substantial progress has been made in this area, even though it may not yet have translated into improved survival rates. The development and optimization of liposomal packaging of key drugs in AML treatment, such as daunorubicin, reduce toxicity and therefore improve therapeutic indices. Whether their theoretical advantage in efficacy translates into a clinically meaningful one has yet to be proven. We believe that if there is one, it is probably small, for the reasons discussed below. Along a similar line, the conjugation of cytostatic drugs to antibodies that target AML cell surface receptors such as CD33 must be considered as a significant advancement, even though the most significant toxicity profile of classical unconjugated cytostatics – the suppression of hematopoiesis – occurs with anti-CD33 conjugates as well. This is because CD33 is not a truly AML-specific antigen. Other side effects, however, are less severe than in conventional chemotherapy and therefore both liposomal and antibody-conjugated targeted drugs may replace conventional drug formulations within the next 10 years.

While such advances in drug delivery reduce or change the profile of side effects, they seem not to have an impact, or at least not a major one, on relapse rates compared to conventional drugs. Thus it seems that the issue of leukemia cell resistance to therapy is an issue of the molecular mechanism of drug action rather than an issue of drug delivery. It is therefore mandatory to identify novel therapeutic targets both inside and outside of the leukemia cells to develop drugs with no cross-resistance to those that are already available. In this regard, as in cancer therapy in general, enormous efforts have been dedicated both by academic research as well as by the industry, to translate our ever-increasing knowledge in cancer biology into therapeutic strategies.

For AML, the most relevant drug developments have been kinase inhibitors blocking RAS membrane anchoring or FLT3 activity, both of which play a major role in AML pathobiology. Many more such small molecule drugs are currently being tested in clinical trials and we consider it very likely that some of them will have an enduring place in the arsenal of weaponry for the combat against AML. Interestingly, unlike for solid tumors, antibody therapies (other than for targeted drug delivery) have played a small, if any, role in the new generation AML drugs so far. This may change in the coming years. In fact, early studies suggest that the anti-angiogenic antibody bevacizumab may have anti-leukemic activity [19]. Beyond the understanding that unconjugated antibodies may be of therapeutic value in AML, such findings draw our attention to the micro-environment of AML cells, rather than the cancer cells as such as a promising therapeutic target in the future.

Further progress in antibody therapy will likely depend on the discovery of novel AML cell surface markers such as has been achieved with CCL-1, VLA-4 or FLT3. Selected ligands may be suitable to target cytotoxic drugs to AML cells as long as the ligands are internalized upon binding. Moreover, receptor-targeted peptides or antibodies might have the capability to induce further biological features in malignant cells as inhibition of cell proliferation or induction of cell death by blocking natural receptor ligand interactions or activation of complement-mediated cytotoxicity. Further, the combination of ligands covering multiple AML-specific receptors could be useful to increase the specificity and efficacy of targeted therapies and we consider it mandatory to explore such concepts in the clinical setting with the newly developed agents in the years to come.

A pertinent question is whether the characterization of leukemic stem cells (LSC) may result in novel treatment options for AML. We consider this to be very likely, even though it is still a novel concept. One explanation for treatment failure in AML might be the resistance of LSCs to currently used chemotherapeutic agents. Therefore, ligand-directed delivery of conventional drugs to LSCs may not solve all the therapeutic challenges associated with the functional LSC concept. We will need both further validation of LSC-specific markers allowing for LSC-directed drug delivery, as well as drugs that interfere with LSC activity and viability. Such drugs could enforce quiescence in LSC as long as they are applied. This would make AML a chronic disease requiring long-term drug treatment, as with imatinib in chronic myeloid leukemia. It is preferable, however, that drugs are found that kill LSC much more efficiently than those currently in use.

At present there are established treatment protocols which cure some and induce remission in most AML patients. This may be perceived to be an impediment to the clinical evaluation of novel candidate drugs, which is therefore mostly carried out in patients who are not eligible for standard therapy because of their age or frailty or because they relapsed after a preceding treatment. These patients possibly constitute a subgroup of AML cases that is particularly resistant to treatment, which may bias clinical results obtained for novel drugs. Viewed from a different perspective, however, this may be a good thing, as it is this patient population that most urgently requires novel drugs with improved efficacy and less toxicity.

While the evaluation of novel drugs as single agents in young AML patients without prior conventional therapy is currently not ethically feasible, it is promising to evaluate the effects of upfront combined application of standard antiproliferative therapy and target-specific novel agents.

In this setting, beneficial effects could possibly be detected even for candidate substances that have not shown considerable efficacy in previous studies. One problem is that AML may be considered as an 'orphan disease', since it is much less frequent than many solid tumors, more difficult to treat and therefore a 'market' not perceived as attractive as other cancers by the pharmaceutical industry.

How will the future AML treatment look like, for example, 10 years from now? AML therapy will likely be determined by the introduction of additional targeted drugs. In contrast, the next significant step after this will be the characterization of each individual patient as to which cocktail of conventional or novel targeted drugs he or she will benefit from. This is commonly referred to as 'tailored' rather than (but not substitutive to) 'targeted' therapy, such as it has been done for karyotypic profiling in AML during the last decade. While targeted drugs are in the process of implementation as standard therapies for AML, the molecular profiles allowing for tailored therapy remain to be determined in future trials, once the novel generation of drugs is evaluated in larger patient cohorts.

Declaration of interest

The authors declare no conflict of interest and have received no payment for the preparation of this manuscript.

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Affiliation

Johannes Kohlschütter*[1] Dipl. Mol. Med.,
Stefan Michelfelder*[2] Dipl. Biol. &
Martin Trepel[†1,2] MD PhD
[†]Author for correspondence
[†1]University Medical Center Hamburg-Eppendorf,
Department of Oncology and Hematology,
Martinistrasse 52,
D-20246 Hamburg, Germany
Tel: +49 40 42803 1980; Fax: +49 40 42803 7187;
E-mail: m.trepel@uke.de
[2]University of Freiburg Medical Center,
Department of Hematology and Oncology,
Hugstetter Strasse 55,
D-79106 Freiburg, Germany
*Both authors contributed equally to this work