Current attempts to implement siRNA-based RNAi in leukemia models

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Leukemias arise from genetic alterations in normal hematopoietic stem or progenitor cells, leading to abnormal blood population with transformed cells. With the advent of RNAi and its pharmacological mediator siRNA, it has become possible to downregulate specific drivers causing leukemias. In this review, we present unique aspects of RNAi-mediated therapy and delivery technologies. Recent updates on molecular targets and delivery systems are discussed emanating from *in vitro* cell models and preclinical animal models. We conclude with a view on the future of RNAi in leukemia therapy, emphasizing possible measures to achieve higher efficacy and improved safety.

Introduction

Leukemia arises from genetic alterations in normal hematopoietic stem or progenitor cells, leading to impaired regulation of cell fate and abnormal accumulation of ill-differentiated cells in blood. Approximately 350,000 people are diagnosed with leukemia annually in the world, leading to ~250,000 deaths each year. An overall 5-year relative survival rate of 56% (between 2003 and 2009) was estimated for various leukemias [1]. Resistance development over the course of therapy is a significant concern with the front-line chemotherapy [2,3]. In chronic myeloid leukemia (CML), for example, development of resistance to the tyrosine kinase inhibitor imatinib and failure to reach a complete cytogenetic response occurred in 24% of patients within 18 months [4,5]. Broad activities of conventional drugs also lead to nonspecificity in drug action, so that agents that target leukemia-specific aberrations are urgently needed to establish the foundation of next-generation therapies. To this end, exploiting RNAi to silence the aberrant proteins responsible for the disease is highly promising [6,7]. The RNAi targets a particular mRNA for destruction (or translational blockage) by employing agents that bind to specific regions in the mRNA. Unlike point mutations that can abolish drug activity, silencing with RNAi should be less prone to resistance development [8] and, owing to control over mRNA pairing, offers higher specificity and adaptability. RNAi for leukemia has reached clinical trials in CML; a nonviral Bcr-Abl siRNA formulation was used to treat a Philadelphia chromosome ($Ph1^+$) CML patient intravenously (10–30 μg/kg) and intratumorally (300 μg) at CML nodules; some evidence of Bcr-Abl silencing was noted after the first intravenous treatment but not afterwards [9]. Recent work in preclinical leukemia models identifies new targets and generates an effective delivery system, creating opportunities for wider application of RNAi in leukemia therapy.

This review summarizes recent efforts to employ RNAi for leukemia therapy, focusing on developments in the past two years. We focus our analysis on (i) molecular targets emanating from patient-derived cells and (ii) functional delivery systems used in leukemic cells. Nonviral delivery of RNAi agents (primarily siRNA) and factors affecting therapeutic efficacy have been emphasized, given the likelihood of this type of delivery methodology to reach clinical trials first. We conclude with a perspective on the future of RNAi and its clinical deployment in leukemic disease management.

Crucial considerations in nonviral delivery of RNAi reagents

Therapeutic RNAi can be applied by several approaches, including a plasmid encoding for short hairpin RNA (shRNA), double-stranded

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siRNA or antisense oligonucleotides consisting of DNA sequences complementary to an mRNA. The anionic polynucleotides cannot cross cellular membranes on their own and they have to rely on carrier molecules for intracellular delivery (Fig. 1). Cationic biomolecules as carrier molecules offer the safety of nonpermanent interactions with genomic materials and make them more likely for clinical deployment. Broadly speaking, the carriers include: (i) formulations of multiple lipids along with polynucleotides to form liposomal or solid lipid nanoparticles (NPs); (ii) polycationic carriers (single or a cocktail of species) where the polynucleotides are condensed into polyionic NP networks; (iii) chemical entities suitable for derivatization of polynucleotides to make them plasma-membrane permeable (e.g. with cholesterol, cell-penetrating peptides, etc.); and (iv) functional carriers composed of multiple domains, including cationic, lipophilic, hydrophilic and targeting (e.g. antibody-derivatized) moieties (Fig. 1). Carriers under development are usually tested in individual cell models, and little information is available about the specificities of carriers in different leukemic cells and, more importantly, in other cells derived from normal tissues such as fibroblasts or epithelial cells. Antibody (Ab)-mediated targeting to leukemic cells is promising to reduce exposure to nonleukemic cells and concentrate the agents on leukemic cells; this approach requires unique or significantly elevated levels of receptors on leukemic cells against which humanized Abs can be generated. Endocytosing receptors are beneficial in this regard [10]. Certain types of leukemias, however, can obviate targeted delivery. A case in point is in CML, where the aberrant Bcr-Abl oncogene is expressed only in transformed cells and specific RNAi agents targeting the fusion protein mRNA should be ineffective in normal cells. Not all leukemias present this opportunity, but leukemias with unique mutations or amplifications can serve as early candidates for clinical entry.

Recent advances in molecular targets for leukemia therapy

Many potential targets have been identified for RNAi with various mechanisms of action and they have been functionally classified based on: (i) decreasing expansion of leukemic cells (i.e. decreased proliferation, increased apoptosis or increased differentiation); (ii) sensitizing leukemic cells to chemotherapy; (iii) modulating leukemic cell mobility and homing to protective bone marrow niche (i.e. better retention of malignant cells in circulation leading to better drug response); and (iv) eradicating leukemic stem cells (LSCs) [11]. The promising targets are continuing to be expanded (Fig. 2 and Table 1); hypoxia inhibitory factor 1α (HIF-1α) was recently shown to be upregulated in bone marrow cells of CML patients. HIF-1α has also been linked to LSCs in acute myeloid leukemia (AML) [12]. Silencing was not explored in primary cells but HIF-1α silencing in a CML model (K562 cells) with Lipofectamine® 2000 led to reduced colony formation with the K562 cells [12]. Growth-factor-independent 1B (GFI-1B) was another upregulated intermediate from bone marrow aspirates of CML patients, which was effectively silenced in primary cells and the K562 model with a liposomal carrier [13]. An enhanced inhibition of cell growth was seen with co-silencing GFI-1B along with Bcr-Abl in that study. Specific histone deacetylase (HDAC) isoform expressions were also associated with negative outcomes in acute lymphoblastic leukemia (ALL) patients; siRNA-mediated silencing in T cells by electroporation in an ALL model led to sensitization of cells to chemotherapy [14]. Heat shock protein 27 (Hsp27) was another target upregulated in bone marrow cells of pediatric AML patients and silencing in an AML (THP-1) cell model with a lipidic carrier (X-tremeGENE™ siRNA reagent) led to chemosensitization [15]. Nucleoplasmin was another recently explored target in the K562 cells; a relatively high concentration of siRNA (200 nM) was capable of reducing cell growth and inducing apoptosis in this model [16]. Another class of pro-survival proteins, ID (DNA-binding family of helix–loop–helix) proteins were targeted in primary chronic lymphocytic leukemia cells using siRNA and a liposomal HiPerFect™ carrier. siRNA treatment against ID2 and ID3 alone reduced cell survival in three out of four different patient cells, and sensitized the drug response in one out of four patient cells. A large heterogeneity was noted in expression of ID2/ID3 genes among cells, possibly indicating a variable effect of antileukemic effects when these proteins were targeted with siRNAs [17]. The relationship between intracellular levels of target mRNA and efficiency of RNAi-mediated silencing remains an important issue to investigate, considering its obvious clinical impact.

Another unique target recently explored for siRNA therapy was CD22 with deleted exon 12 (CD22ΔE12). A liposomal [18] and a cationic peptide [19] formulation of CD22ΔE12 siRNAs were functional in xenografts derived from precursor B cell lymphoblastic leukemia (PBL) patient cells. The rate of leukemia formation was significantly reduced in patient cells treated with liposomal formulation of CD22ΔE12 siRNA [18]. Because CD22ΔE12 is normally missing in normal B cells, siRNA against this target might serve as a specific reagent without affecting normal B cell development. MAX dimerization protein 3 (MDX3) was another intermediate identified in PBL, which seemed to function as an antiapoptotic protein and can be silenced in at least in one patient's cells *in vitro* with increased incidence of apoptosis and chemosensitization [20]. Hsp32, in addition to its importance in AML, was also considered a crucial mediator in ALL primary cells and cell lines, serving as a survival factor in mutated cells and, hence, a target for RNAi intervention [21].

A cell-surface protein recently targeted with siRNA was the C-X-C chemokine receptor type 4 (CXCR-4; CD184), which was implicated in homing of leukemic cells to the bone marrow niche especially after drug therapy [22], and was upregulated in drug-resistant CML (K562) cells [23]. siRNA-mediated silencing CXCR4 with lipopolyplexes resulted in retarded growth in the latter model in culture. We recently extended this observation to an AML model (THP-1), and AML primary cells, where two out of five AML patient cells were responsive to siRNA silencing and displayed reduced growth [24]. More importantly, the siRNA-mediated silencing was equally effective whether the THP-1 cells were cultured in the presence or absence of bone marrow stromal cells. This is a distinct advantage for RNAi agents over conventional drugs, because conventional drugs typically exhibit a reduced efficacy when leukemic cells are in contact with other bone marrow cells. The hyaluronic acid receptor CD44 was also successfully targeted with similar lipopolyplex formulations of siRNA in $CD34^+$ primary cells from AML patients, lowering the adhesion of leukemic cells to bone marrow stromal cells [25]. Even the KG-1a cells, which represent the more immature $CD38^-$ leukemic cell fraction, were responsive to CD44 siRNA treatment. However, the extent of CD44 silencing was

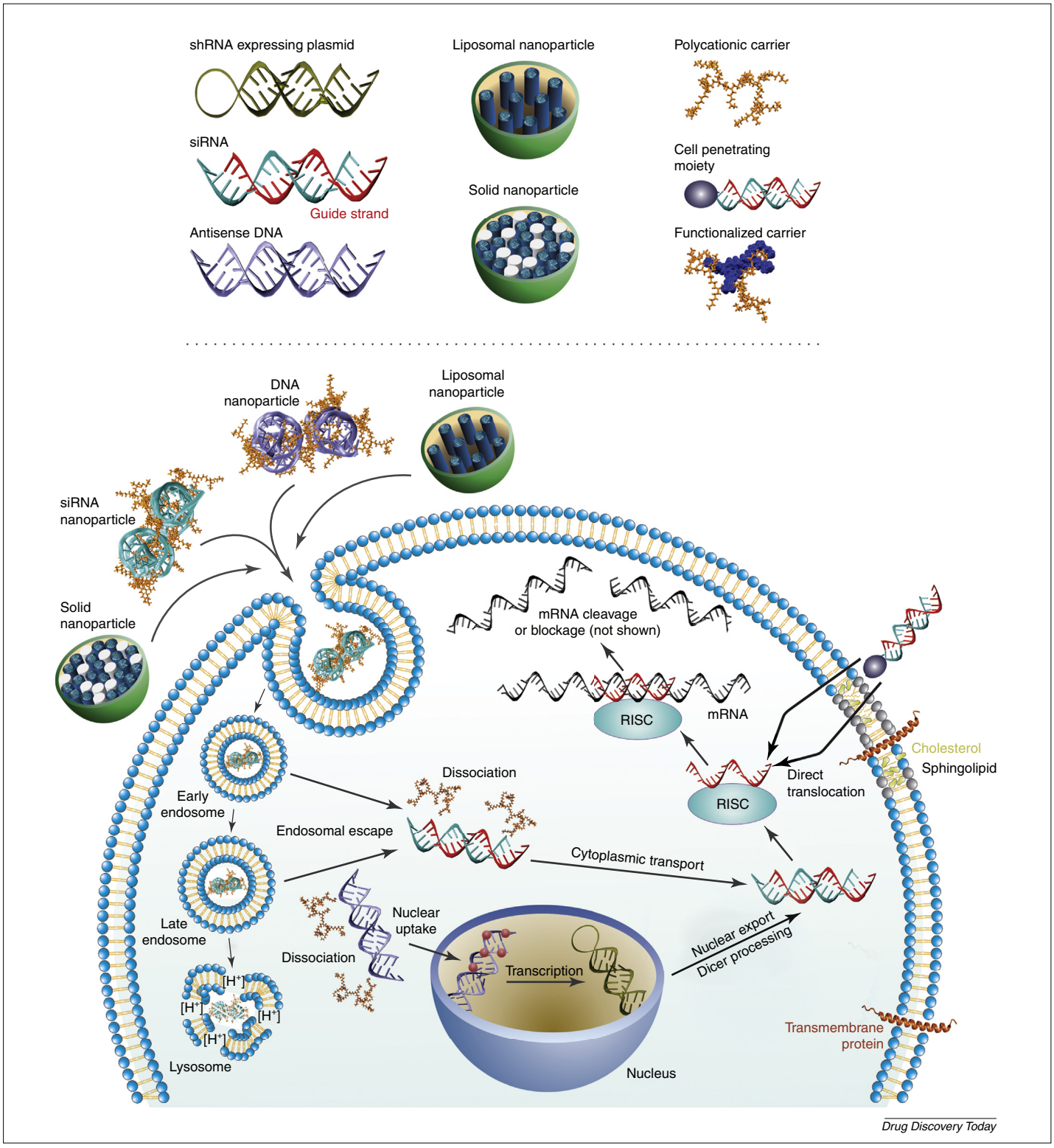

FIGURE 1

Main steps involved in nonviral delivery of RNAi reagents (siRNA and plasmid DNA encoding for shRNA) into a cell. Nanoparticulate formulations with siRNA/DNA or chemical modification of siRNA are needed for passage through the cell membrane.

lower than that reported for solid tumor cells using lipid-based gene silencing [26–28]. The slower growth of $CD34^+$ AML cells (vs adherent tumor cells), a reduced cell surface area and a lower endocytic activity [10] might have all contributed to the relatively low silencing efficiency in leukemic cells.

Finally, it is important to highlight two recent studies that are yielding a new class of targets, namely long noncoding RNA (lncRNA), in leukemic cells [29]. Overall, 1050 endoribonuclease-prepared siRNAs (esiRNAs) were identified against lncRNAs in the CML K562 model. The noncoding transcripts, on average,

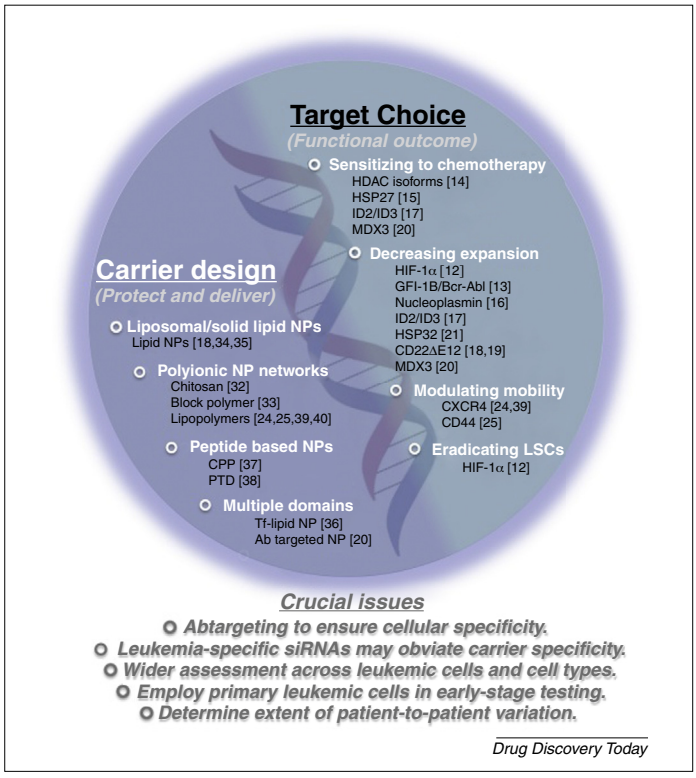

FIGURE 2

A summary of nanoparticle (NP) formulations and choice of molecular targets recently explored as antileukemic therapies. A summary of crucial issues for the next steps of exploring siRNA for leukemia therapy is also provided.

had lower expression than the protein-coding transcripts, and preliminary studies indicated the average silencing efficiency of esiRNAs against lncRNAs was slightly lower compared with protein-coding transcripts. Whether such a difference is functionally relevant for therapy remains to be explored in leukemia models. In an independent study, two micro-RNAs (miRs) were explored in the same CML model; hsa-miR-1245b-3p was the most expressed miR, whereas hsa-miR-2278 was the least expressed miR in drug-resistant K562 cells [30]. siRNA delivery was used to establish linkages between miR expression and mRNA downregulation, ultimately controlling K562 cell fate by leading to increased drug sensitivity. The hsa-miR-2278 miR was identified as a putative tumor suppressor, the upregulation of which was considered beneficial as a basis for therapy [30].

Functional delivery systems

It is our belief that NP systems will be the practical (effective) way to implement RNAi in clinical intervention (Fig. 3). Even if polynucleotides can be designed to penetrate the plasma membrane without a complexing carrier [31], they still need to be protected against physiological nucleases and it is not yet clear if nuclease-insensitive RNAi agents will be practical drugs. The individual capabilities of NP systems (e.g. specific cell binding, endosomal escape, nuclear targeting, etc.) can be modularly tailored and conveniently incorporated into a single formulation. Studies to identify new molecular targets have relied on electroporation, which cannot be translated to the clinical setting, and commercial carriers to minimize any uncertainties in delivery efficiency.

TABLE 1

A list of delivery agents, molecular targets, specific leukemia types and cell models used to explore recent (2013–2015) antileukemia therapies

Delivery system	Target(s)	Cancer type	Application	Refs
Lipids				
Lipofectamine® 2000	HIF-1α	CML	*In vitro* (K562 cells)	[12]
DOTAP	BCR-ABL/GFI1B	CML	*In vitro* (K562 cells)	[13]
X-TremeGENE™	Hsp27	ALL, AML, leukemia cell	*In vitro* (AML-M4/M5, OCI/AML-3)	[15]
HiPerFect™	Nucleoplasmin	CML	*In vitro* (K562 cells)	[16]
HiPerFect™	ID2/ID3	CML	*In vitro* (leukemia cells/MEC1)	[17]
DOTAP/DOPE	CD22ΔE12	PBL	*In vitro* (*in vivo* expanded ALL cells, RAJI)/*in vivo*	[18]
HiPerFect™	STAT5A	CML	*In vitro* (K562/IMA 3 μM)	[30,42]
DODAB/MO/PEG-ceramide	BCR-Abl/GFP	CML	*In vitro* (K562, H1299)	[34]
Tf-NP (DOTMA, DODMA or DCChol)/(EggPC/mPEG-DSPE)	RRM2	AML	*In vitro* (MV4–11)/*in vivo*	[36]
Lipofection	Hsp32	ALL	*In vitro* (ALL patient cells)	[21]
Polymers				
PVBLG	CD22ΔE12	PBL	*In vitro* (*in vivo* expanded ALL cells)	[19]
PEI-lipid	CXCR4	AML	*In vitro* (THP-1, AML patient cells)	[24,39]
PEI-lipid	CD44	AML	*In vitro* (KG-1 and KG-1a)	[25]
Chitosan	VEGF/Flt-1	Myelogenous leukemia	*In vitro* (U973 cells)	[32]
PNP-p(CL-DMAEMA)/p(CL-PEG)	Luciferase	AML	*In vitro* (SKNO cells)	[33]
PTD polymers	Notch-1		*In vitro* (Jurkat T cell, PBMC)	[38]
PEI-lipid (cholesterol)	BCR-Abl	CML	*In vitro* (K562 cells)	[40]
Nanoparticles				
Iron oxide/PEI NP	MXD3	ALL	*In vitro* (Jurkat: T cell ALL, Reh: PBL)	[20]
Others				
Cell-penetrating peptide	Luciferase	AML	*In vitro* (SKNO-1 cells)	[37]
Electroporation	HDAC	ALL	*In vitro*, human T cell ALL cell line (CCRF-CEM)	[14]
CpG-siRNA (no carrier)	STAT3	AML	*In vivo*	[41]

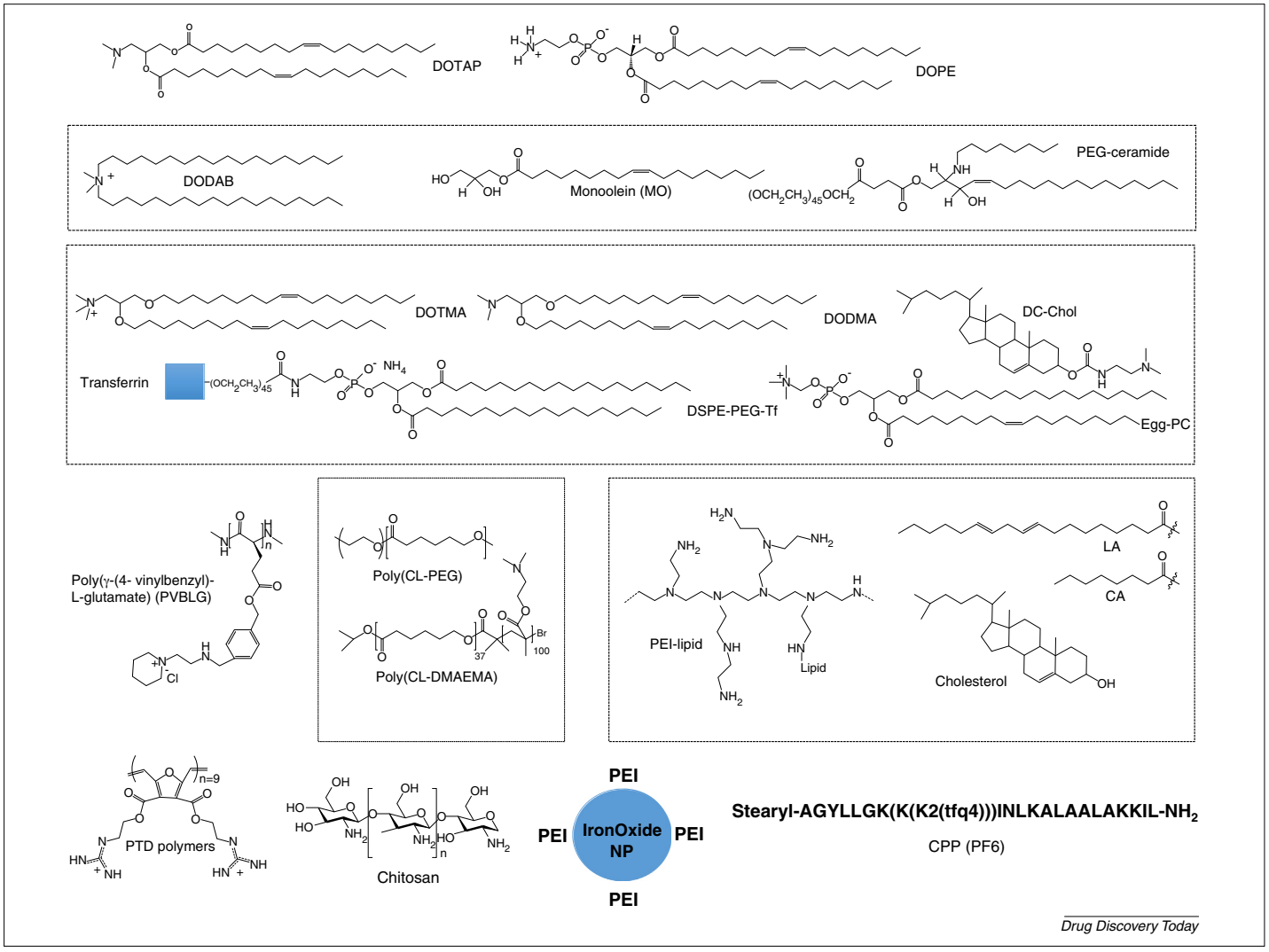

FIGURE 3

Components of drug delivery systems (where available) used to deliver RNAi reagents in leukemic models.

Commercial carriers are presumably not limiting target discovery because of inefficient delivery (this is an assumption inherent in such studies and not often tested). The most common commercial carriers, Lipofectamine® (a liposomal formulation probably of cationic lipids) and HiPerFect™ (a mixture of cationic and neutral lipids) are derived form lipidic systems. The Bcr-abl siRNA that was used in one CML patient was delivered with a dispersed anionic lipid formulation [9]. It is likely that this is down to convenience (i.e. availability) of commercial agents and a longer history of lipid formulations in drug development, and not because of specific activity of lipidic systems in leukemic cells. Indeed, a polymeric chitosan NP was shown to function at an equivalent level to that of Lipofectamine® 2000 in leukemic U937 cells [32]; and polymeric NPs, created from a blend of poly(caprolactone)-poly((dimethylamino)ethylene methacrylate) and poly(caprolactone)-PEG (degradable-hydrophilic block) [p(CL-DMAEMA)/p(CL-PEG)], were functional in an AML cell (SKNO-1) model [33].

Specific liposomal systems continue to be formulated for functional silencing in leukemic cells. A dioctadecyldimethylammonium bromide (DODAB) and monoolein (MO) lipoplex formulation of siRNA was reported for Bcr-Abl silencing in K562 cells [34]; PEGylation by a post-encapsulation approach was found to be the most effective in preventing leaching of siRNA from lipoplexes and the resultant lipoplexes were more effective in silencing the oncogene and inhibiting cell growth. A practical concentration for siRNA was used for this purpose (50 nM), but the presence of PEG was detrimental (to some extent) to the resulting silencing activity despite optimization [34]. Others have demonstrated effective delivery of antileukemic siRNAs in a xenograft model with conventional liposomes [from cationic lipid 2,3-dioleoyloxypropyltrimethylammonium chloride (DOTAP) and neutral lipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE)] bearing no PEGs [18]. The lipoplex had a plasma half-life of 5.5 hours and prolonged the lifespan of NOD/SCID mice bearing PBL cells. The simplicity and previous use of this formulation bodes well for its clinical translation. It will be important to determine if further improvements could be implemented with PEG or other lipids shown to be effective in leukemic cells [10]. Even DOTAP on its own seems to be effective in primary CML cells [35], where 35–45% (on average) silencing of the Bcr-Abl oncogene was realized in cells derived from four patients.

To realize active targeting in leukemic cells, transferrin (Tf)-conjugated lipid NPs were recently synthesized by using a microfluidic device and tested in an AML model (MV4-11 cells) [36]. The NPs were a complex formulation of lipids from 3β-[*N*-(*N*,*N*-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol), 1,2-dioleyloxy-3-trimethylammonium propane (DOTMA) or 1,2-dioleyloxy-*N*,*N*-dimethyl-3-aminopropane (DODMA), formulated with egg phosphatidylcholine (EggPC) and methoxy-PEG distearoyl phosphatidylethanolamine (mPEG-DSPE), into which a Tf conjugate was inserted post NP fabrication. The NPs displayed dual plasma half-lives of 0.18 and 10.2 hours after intravenous injection, and effectively silenced a target (R2 subunit of ribonucleotide reductase) in subcutaneous MV4-11 xenografts. No growth inhibition, however, was explored in this study. Another cell-specific NP was designed with super paramagnetic iron oxide (SPIO) with a CD22 Ab and MXD3 siRNA for treatment of PBL [20]. The NPs were able to differentiate $CD22^{+}$ cells (B cells) from $CD22^{-}$ cells (T cells) *in vitro*. Because such targeting will seek normal and malignant B cells, it is important to target the right mRNA that is preferentially expressed in the latter.

Another class of carriers specifically explored in leukemic cells is short cationic cell penetrating peptides (CPPs), the efficacy of which was improved with stearyl (C18) grafting [37]; there was a lack of correlation between uptake and silencing efficiency with these CPPs, suggesting that functional activity was related to other processing events after internalization. A similar class of carriers, namely protein transduction domain (PTD)-mimicking polymers, were recently designed to replicate poly(arginine) and amphiphilic peptides functionally [38]. Peripheral blood mononuclear cells (PBMCs; three samples) were used to demonstrate the effectiveness of the carriers to downregulate *NOTCH1* expression, whereas a T cell model (Jurkat T cells) was used to show its superior delivery efficiency compared with cationic lipid formulations: HiFect™, Lipofectamine® 2000 and FuGENE® HD. Some inhibition of uptake was evident in the presence of serum, which could adversely affect the translation to animal models. The author's group continues to engineer amphiphilic (cationic and lipophilic) polymers to undertake siRNA delivery in leukemic cells. Polyethylenimine (PEI) substituted with caprylic and linoleic acids has emerged as a selection of effective carriers to undertake CXCR4 silencing in an AML model (THP-1 cells) and mononuclear cells obtained from AML patients [24,39]. Higher molecular weight (MW) amphiphilic PEIs were more effective in this model. A relatively uniform siRNA delivery was obtained among patient samples, but the patient cells with the highest cell volume also displayed a proportional increase in the uptake of the lipopolyplexes. Unlike the THP-1 model, linoleic-acid-substituted PEI was more effective in delivery to patient cells [24], suggesting that the carriers optimized in cell lines might not be ideal for primary cells. This class of polymers was also effective in the CML K562 cells [40]. It appeared that proper lipophilic:polycationic character of the polymeric backbone had to be optimized for different types of leukemic cells. The molecular basis of these observations is presently not known; it is likely that differences in serum protein interactions, plasma membrane composition and/or endocytic activities of cells could illuminate the differences in carrier efficiencies in different types of leukemic cells.

One unique RNAi agent in leukemia therapy might be a CpG–siRNA conjugate [41], which acts as a ligand for Toll-like receptor 9 (TLR9). The conjugate was shown to be internalized in AML cells *in vitro* without the need for a carrier [41], albeit at relatively high (250–500 nM) concentrations compared with NP systems (~50 nM). Systemic CpG–siRNA was internalized by grafted AML cells in a mouse model as well as normal myeloid immune cells in different organs. In a naive mouse model, no siRNA uptake was detected in hematopoietic stem cells, providing encouraging outcomes for the specificity of the agent. A CpG–siRNA against signal transducer and activator of transcription 3 (STAT3) reduced AML tumor burden and increased lifespan in the mouse model. The therapeutic activity, however, resulted from effects on AML cells (i.e. STAT3 inhibition) as well as the host immune system, involving secretions of pro- and anti-inflammatory cytokines.

One must note that these studies employed 'conventional' siRNAs, and employing modified siRNAs could prove more effective for suppressing leukemic targets. Kaymaz *et al.* observed that 5′-cholesterol-modified or fluorouridine-substituted (2′-ribose) siRNAs led to longer silencing (vs unmodified siRNA of the same sequence) in CML K562 cells when STAT3 and STAT5A/B were targeted *in vitro* using the HiPerFect™ carrier [42]. More-effective silencing at early points with cholesterol-modified siRNA indicated a more favorable uptake.

Concluding remarks and future perspectives

Whereas numerous studies are exploring primary cells to pinpoint molecular changes leading to leukemic transformations, cell lines rather than primary cells are usually employed to assess the functional outcome of silencing the identified targets [21,43,44]. Employing primary cells in the latter studies will greatly enhance our understanding of siRNA-mediated therapeutic possibilities in patient cells. It is likely that commercial reagents are not ideal to deliver the RNAi agents to primary cells, and/or limited sample size does not allow a thorough evaluation in patient cells. Extent of silencing might simply not be significant, because some studies preferred lentiviruses for primary cells [45], which are generally considered to be more effective on primary cells. We also observed such a low silencing efficiency in some of our studies. For example, CD44 silencing was relatively low in patient cells (~20%, even though that was sufficient to reduce binding to bone marrow stromal cells *in vitro*) and CXCR4 silencing was achieved in two out of five patient samples [24]. This calls for more focus to undertake carrier improvements in primary cells. Patient-to-patient heterogeneity is expected when it comes to implementing RNAi, and whether this is caused by carrier efficiencies or intracellular physiology of targeted molecules needs to be revealed. The absolute levels and turnover rate of target mRNA, as well as surface characteristics of primary cells (such as surface proteoglycans and presence or absence of specific cell-surface receptors), could contribute to this variability. The efficiency of the carriers needs to be correlated to these features for a significant leap forward. In a study that employed electroporation, decreased cell survival was found in ten out of 30 leukemia patients with a kinase siRNA library (i.e. one-third responsiveness), so that patient-to-patient variations in target biology seem to be an issue even with a relatively homogeneous delivery system such as electroporation [46].

Identifying new targets responsible for leukemic transformations and effective drug response is worthwhile; one cannot always predict therapeutic responses in silencing a particular target and the more targets we have at our disposal the more likely it is that we can generate effective therapies. The new targets might also provide new possibilities to downregulate leukemic survival networks in a coordinated way by targeting multiple crucial mediators. More than conventional drugs, the RNAi approach, because of ease in generating effective agents against a particular target, might be ideal for discovering and subsequently delivering multiple agents to control leukemic growth. However, there are already established targets for RNAi implementation: (i) Bcr-Abl in CML is a proven oncogene for therapeutic intervention (although CML LSC might not be fully dependent on Bcr-Abl activity for survival [13,47]); (ii) differentially expressed CD44 isoforms can distinguish leukemic from normal cells [48]; and (iii) the most frequently mutated gene in AML, Fms-like tyrosine kinase 3 (FLT3) [49], is a cytokine receptor that leads to oncogenic activation. It is the availability of an effective delivery system that prevents the entry of RNAi into clinical testing in these cases. One can see the advantage of simple formulations (such as conventional liposomes) for clinical entry but more-sophisticated systems, with their ability to display cellular targeting and intracellular stimuli-responsiveness, will probably be required for superior efficacy, at the expense of more-extensive preclinical testing to fully understand their safety and efficacy profiles.

The efficiency of carriers has mostly been investigated experimentally, whereas the molecular details governing the performance are overlooked at times. Computational tools, such as molecular dynamics (MD) simulations, can be useful to explore molecular details and give valuable insights for the design of efficient formulations. The influence of a carrier's chemical nature and its modification on (i) the mechanism of complexation with nucleic acids, (ii) functional properties of complexes, such as surface change density and hydrophobicity, (iii) cellular interactions and uptake of complexes and (iv) intracellular liberation of nucleic acids can be predicted with MD simulations. We recently probed the substitution of a short (3C) hydrophobic moiety, propionic acid (PrA), onto MW PEI, and tested its performance in delivering siRNA to CML K562 cells [50]. A non-monotonic trend was observed in uptake and resulting silencing efficiency of complexes (Fig. 4a), where intermediate substitution levels yielded the best performance. A new assembly mechanism for abundant PrA substitution was revealed from atomistic MD simulations (Fig. 4b) – migration of hydrophobic PrA moieties to complex core to minimize their interactions with the aqueous phase. Surface cationic charge density, as well as surface hydrophobicity, was deleteriously affected, which possibly impeded the performance of the polymer in delivering the siRNA to the CML cells. MD simulations were vital to shed light on the molecular details responsible for the carrier performance, which could not be obtained from the experimental tools at our disposal.

Finally, RNAi has been touted as an ideal means to undertake specific molecular interventions, but recent evidence indicates significant changes in intracellular networks as a result of silencing individual targets. Using BCL11A siRNA in the diffuse large B cell lymphoma SUDHL6 cells, hundreds of differentially expressed genes were identified using global gene analysis by microarray

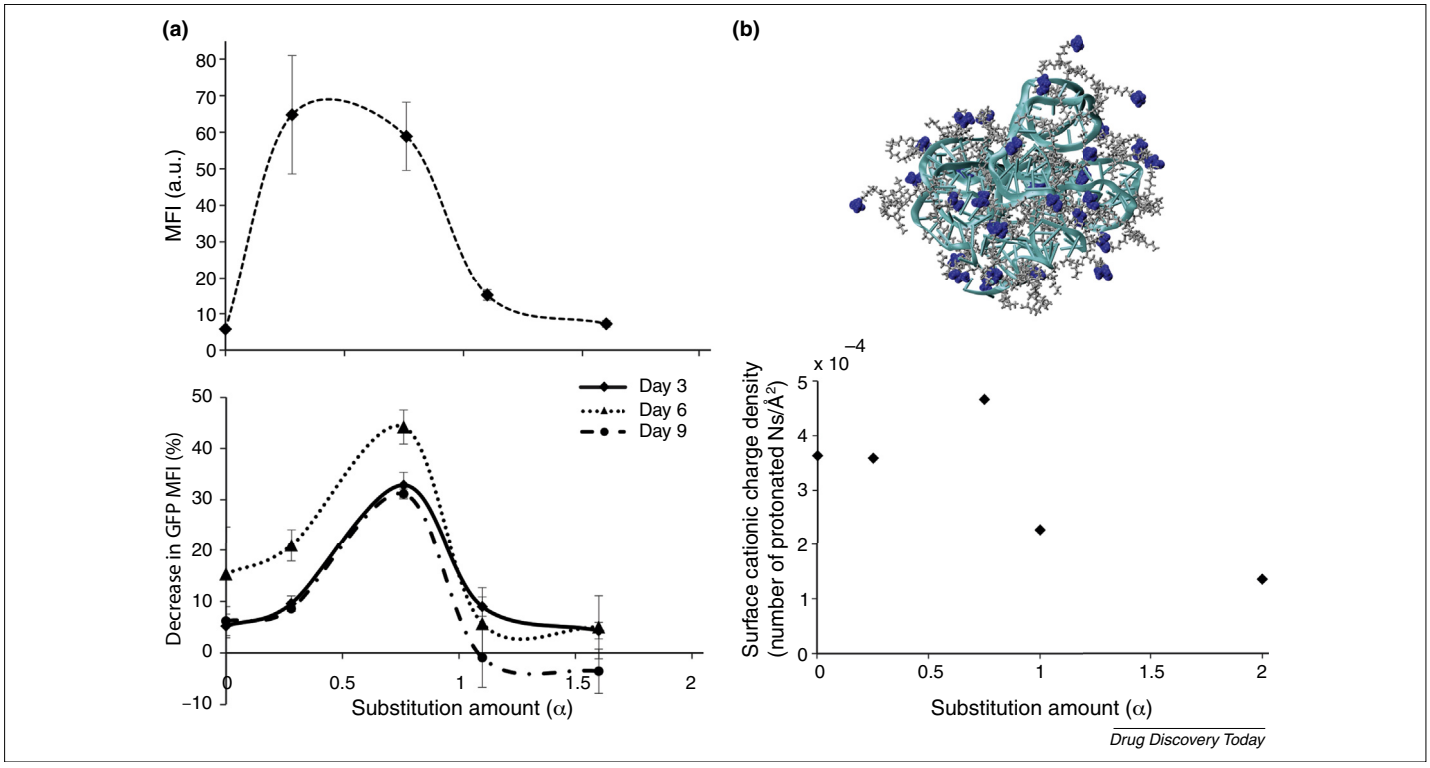

FIGURE 4

(a) Uptake (top) and silencing (bottom) efficiency in CML K562 cells by using siRNA delivered with propionic acid (PrA)-modified polyethylenimine (PEI) complexes. **(b)** Atomistic details (top) and surface charge density (bottom) of sRNA–polymer complexes from molecular dynamics (MD) simulations. Adapted, with permission, from [50].

analysis (and validated with qPCR), whereas the expected consequence of silencing (i.e. apoptosis induction) was secured in culture [51]. Exploring other modulated intermediates will be worthwhile to find complementary targets for a more comprehensive molecular approach to leukemia therapy and to predict the therapeutic response in clinical testing better. In one CML patient [9], where Bcr-Abl siRNA was administered, some evidence of unresponsiveness was noted, which was attributed to clonal evolution of tumors or reaction to the delivery system, an issue that will require close attention when RNAi technology is translated to clinical management of leukemic patients.

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References

1 National Cancer Institute Surveillance Research Program (2013) *Fast Stats: An Interactive Tool for Access to SEER Cancer Statistics*. Available at: http://seer.cancer.gov/faststats

2 Estey, E.H. (2013) Acute myeloid leukemia: 2013 update on risk-stratification and management. *Am. J. Hematol.* 88, 318–327

3 Stefanachi, A. *et al.* (2012) New strategies in the chemotherapy of leukemia: eradicating cancer stem cells in chronic myeloid leukemia. *Curr. Cancer Drug Targets* 12, 571–596

4 Assouline, S. and Lipton, J.H. (2011) Monitoring response and resistance to treatment in chronic myeloid leukemia. *Curr. Oncol.* 18, e71–e83

5 O'Brien, S.G. *et al.* (2003) Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N. Engl. J. Med.* 348, 994–1004

6 Iorns, E. *et al.* (2007) Utilizing RNA interference to enhance cancer drug discovery. *Nat. Rev. Drug Discov.* 6, 556–568

7 Rossbach, M. (2010) Small non-coding RNAs as novel therapeutics. *Curr. Mol. Med.* 10, 361–368

8 Montazeri Aliabadi, H. *et al.* (2015) Effect of siRNA pre-exposure on subsequent response to siRNA therapy. *Pharm. Res.* 32, 3813–3826

9 Koldehoff, M. (2015) Targeting bcr-abl transcripts with siRNAs in an imatinib-resistant chronic myeloid leukemia patient: challenges and future directions. *Methods Mol. Biol.* 1218, 277–292

10 He, W. *et al.* (2014) Discovery of siRNA lipid nanoparticles to transfect suspension leukemia cells and provide *in vivo* delivery capability. *Mol. Ther.* 22, 359–370

11 Landry, B. *et al.* (2015) Progress in RNAi-mediated molecular therapy of acute and chronic myeloid leukemia. *Mol. Ther. Nucleic Acids* 4, e240

12 Chen, H. *et al.* (2015) HIF-α promotes chronic myelogenous leukemia cell proliferation by upregulating p21 expression. *Cell Biochem. Biophys.* 72, 179–183

13 Koldehoff, M. *et al.* (2013) Additive antileukemia effects by GFI1B- and BCR-ABL-specific siRNA in advanced phase chronic myeloid leukemic cells. *Cancer Gene Ther.* 20, 421–427

14 Gruhn, B. *et al.* (2013) The expression of histone deacetylase 4 is associated with prednisone poor-response in childhood acute lymphoblastic leukemia. *Leuk. Res.* 37, 1200–1207

15 Yang, L. *et al.* (2013) Hsp27: a novel therapeutic target for pediatric M4/M5 acute myeloid leukemia. *Oncol. Rep.* 29, 1459–1466

16 Seyed-Gogani, N. *et al.* (2014) Nucleostemin depletion induces post-g1 arrest apoptosis in chronic myelogenous leukemia k562 cells. *Adv. Pharm. Bull.* 4, 55–60

17 Weiler, S. *et al.* (2015) ID helix–loop–helix proteins as determinants of cell survival in B-cell chronic lymphocytic leukemia cells *in vitro*. *Mol. Cancer* 14, 30

18 Uckun, F.M. *et al.* (2015) CD22ΔE12 as a molecular target for RNAi therapy. *Br. J. Haematol.* 169, 401–414

19 Uckun, F.M. *et al.* (2014) A rationally designed nanoparticle for RNA interference therapy in B-lineage lymphoid malignancies. *EBioMedicine* 1, 141–155

20 Satake, N. *et al.* (2014) Targeted therapy with MXD3 siRNA, anti-CD22 antibody and nanoparticles for precursor B-cell acute lymphoblastic leukaemia. *Br. J. Haematol.* 167, 487–499

21 Cerny-Reiterer, S. *et al.* (2014) Identification of heat shock protein 32 (Hsp32) as a novel target in acute lymphoblastic leukemia. *Oncotarget* 5, 1198–1211

22 Ratajczak, M.Z. *et al.* (2012) A novel perspective on stem cell homing and mobilization: review on bioactive lipids as potent chemoattractants and cationic peptides as underappreciated modulators of responsiveness to SDF-1 gradients. *Leukemia* 26, 63–72

23 Wang, Y. *et al.* (2014) CXCL12/CXCR4 axis confers adriamycin resistance to human chronic myelogenous leukemia and oroxylin A improves the sensitivity of K562/ADM cells. *Biochem. Pharmacol.* 90, 212–225

24 Landry, B. *et al.* (2016) Targeting CXCR4/SDF-1 axis by lipopolymer complexes of siRNA in acute myeloid leukemia. *J. Control. Release* 224, 8–21

25 Gül-Uludag, H. *et al.* (2014) Polymeric nanoparticle-mediated silencing of CD44 receptor in CD34+ acute myeloid leukemia cells. *Leuk. Res.* 38, 1299–1308

26 Subramaniam, V. *et al.* (2007) Suppression of human colon cancer tumors in nude mice by siRNA CD44 gene therapy. *Exp. Mol. Pathol.* 83, 332–340

27 Li, C-Z. *et al.* (2008) Inhibition of CD44 expression by small interfering RNA to suppress the growth and metastasis of ovarian cancer cells *in vitro* and *in vivo*. *Folia Biol.* 54, 180–186

28 Shah, V. *et al.* (2013) Targeted nanomedicine for suppression of CD44 and simultaneous cell death induction in ovarian cancer: an optimal delivery of siRNA and anticancer drug. *Clin. Cancer Res.* 19, 6193–6204

29 Theis, M. *et al.* (2015) Targeting human long noncoding transcripts by endoribonuclease-prepared siRNAs. *J. Biomol. Screen.* 20, 1018–1026

30 Kaymaz, B.T. *et al.* (2015) Revealing genome-wide mRNA and microRNA expression patterns in leukemic cells highlighted hsa-miR-2278 as a tumor suppressor for regain of chemotherapeutic imatinib response due to targeting STAT5A. *Tumour Biol.* 36, 7915–7927

31 Jiang, K. *et al.* (2015) Targeted delivery of CXCR4-siRNA by scFv for HER2(+) breast cancer therapy. *Biomaterials* 59, 77–87

32 Wang, L. *et al.* (2014) Nanocomposite-siRNA approach for down-regulation of VEGF and its receptor in myeloid leukemia cells. *Int. J. Biol. Macromol.* 63, 49–55

33 Omedes Pujol, M. *et al.* (2013) Determination of key structure–activity relationships in siRNA delivery with a mixed micelle system. *J. Control. Release* 172, 939–945

34 Oliveira, A.C.N. *et al.* (2015) Stealth monoolein-based nanocarriers for delivery of siRNA to cancer cells. *Acta Biomater.* 25, 216–229

35 Kornblau, S.M. *et al.* (2013) Proteomic profiling identifies distinct protein patterns in acute myelogenous leukemia CD34+CD38− stem-like cells. *PLOS ONE* 8, e78453

36 Yang, Z. *et al.* (2014) A microfluidic method to synthesize transferrin-lipid nanoparticles loaded with siRNA LOR-1284 for therapy of acute myeloid leukemia. *Nanoscale* 6, 9742–9751

37 van Asbeck, A.H. *et al.* (2013) Molecular parameters of siRNA – cell penetrating peptide nanocomplexes for efficient cellular delivery. *ACS Nano* 7, 3797–3807

38 Tezgel, A.Ö. *et al.* (2013) Novel protein transduction domain mimics as nonviral delivery vectors for siRNA targeting NOTCH1 in primary human T cells. *Mol. Ther.* 21, 201–209

39 Landry, B. *et al.* (2012) Effective non-viral delivery of siRNA to acute myeloid leukemia cells with lipid-substituted polyethylenimines. *PLoS ONE* 7, e44197

40 Valencia-Serna, J. , Gul-Uludağ, H. , Mahdipoor, P. , Jiang, X. and Uludağ, H. (2013) Investigating siRNA delivery to chronic myeloid leukemia K562 cells with lipophilic polymers for therapeutic BCR-ABL down-regulation. *J Control Release* 172 (2), 495–503http://dx.doi.org/10.1016/j.jconrel.2013.05.014

41 Hossain, D.M.S. *et al.* (2014) Leukemia cell-targeted STAT3 silencing and TLR9 triggering generate systemic antitumor immunity. *Blood* 123, 15–25

42 Kaymaz, B.T. *et al.* (2013) Repression of STAT3, STAT5A, and STAT5B expressions in chronic myelogenous leukemia cell line K-562 with unmodified or chemically modified siRNAs and induction of apoptosis. *Ann. Hematol.* 92, 151–162

43 Zhang, H. *et al.* (2014) NF-kappa B mediated up-regulation of CCCTC-binding factor in pediatric acute lymphoblastic leukemia. *Mol. Cancer* 13, 5

44 Wang, J. *et al.* (2014) CIP2A is overexpressed and involved in the pathogenesis of chronic myelocytic leukemia by interacting with breakpoint cluster region-Abelson leukemia virus. *Med. Oncol.* 31, 112
45 Nishioka, C. *et al.* (2013) $CD34^{+}/CD38^{-}$ acute myelogenous leukemia cells aberrantly express CD82 which regulates adhesion and survival of leukemia stem cells. *Int. J. Cancer* 132, 2006–2019
46 Tyner, J.W. *et al.* (2009) RNAi screen for rapid therapeutic target identification in leukemia patients. *Proc. Natl. Acad. Sci. U. S. A.* 106, 8695–8700
47 Corbin, A.S. *et al.* (2011) Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. *J. Clin. Invest.* 121, 396–409
48 Daines, D.A. *et al.* (2013) Development of a novel treatment for leukemia directed at tumor-associated mRNA splicing. *Leuk. Res.* 37, 1125–1131
49 Cancer Genome Atlas Research Network (2013) Genomic and epigenomic landscapes of adult *de novo* acute myeloid leukemia. *N. Engl. J. Med.* 368, 2059–2074
50 Meneksedag-Erol, D. *et al.* (2015) A delicate balance when substituting a small hydrophobe onto low molecular weight polyethylenimine to improve its nucleic acid delivery efficiency. *ACS Appl. Mater. Interfaces* 7, 24822–24832
51 Wu, H. *et al.* (2014) Gene expression profile analysis of SUDHL6 cells with siRNA-mediated BCL11A downregulation. *Cell Biol. Int.* 38, 1205–1214