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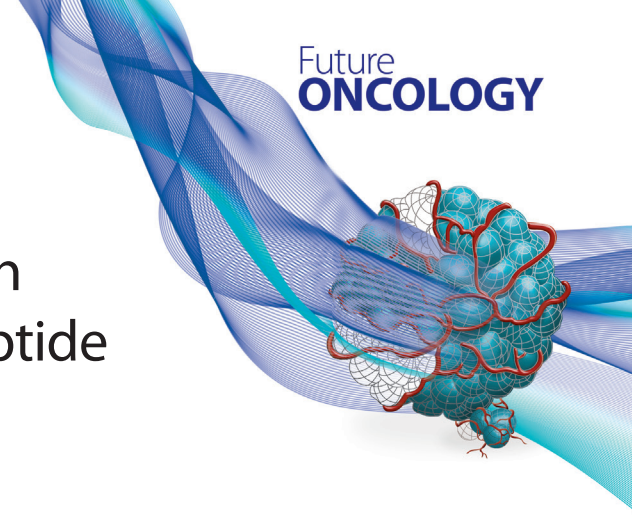

COMMENTARY

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Targeting leukemic stem cells with multifunctional bioactive polypeptide nanoparticles



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KEYWORDS
• biotherapy • cancer • CD19 • health • leukemia • nanotechnology • personalized medicine

RNA interference (RNAi) using synthetic small interfering RNA (siRNA) is being explored as a potential therapeutic strategy in oncology for silencing the expression of specific mRNA species that have been linked to chemotherapy resistance of cancer cells [1–9]. Systemically administered unformulated siRNA lack RNAi activity *in vivo* due to rapid enzymatic degradation in blood and very poor entry into target cells. As nanoparticles (NPs) can protect siRNA from degradation, facilitate their cellular uptake by endocytosis and enable an effective RNAi by allowing the endosomal escape of the endocytosed siRNA into the cytoplasm, they are generally considered the appropriate delivery platforms for siRNA as a new class of therapeutic agents against otherwise undruggable molecular targets. Several nanoscale formulation platforms have been developed for systemic delivery of siRNA [1–9]. However, a rapid development of nanoscale RNAi therapeutics has been hampered by the limited knowledge about the identity of the critical driver lesions in specific types of cancer, safety concerns about certain formulations and a very poor siRNA delivery efficiency into the target cancer cells. There is an urgent and unmet need to develop novel materials and delivery systems capable of safely and efficiently delivering siRNA to molecular targets in the most common cancer types.

B-precursor acute lymphoblastic leukemia (ALL) is the most common cancer type and the most common cause of cancer-related deaths in children [10,11]. Leukemic clones in aggressive forms of pediatric B-precursor ALL are characterized by a genetic defect involving CD22, a negative regulator of signal transduction pathways controlling proliferation and survival [12–16]. Specifically, B-precursor ALL cells express an abnormal CD22 due to deletion of exon 12 (CD22ΔE12) [12,13]. Forced expression of the mutant CD22ΔE12 protein in transgenic (Tg) mice in early B-cell ontogeny causes fatal $CD19^+CD24^+CD45R/B220^+CD127/$

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"Polypeptide-based siRNA nanoparticles with CD19-binding functionality could represent an important addition to the emerging new personalized treatment options for B-lineage lymphoid malignancies."

IL7-R$^+$sIgM$^-$ B-precursor ALL [14,16]. This transgenic mouse model recapitulates the gene expression profile of CD22ΔE12$^+$ human B-precursor ALL, establishing a causal relationship between CD22ΔE12 and B-precursor ALL and indicating that CD22ΔE12 alone as a driver lesion is sufficient for malignant transformation and clonal expansion of B-cell precursors [14,16]. More recent studies revealed a very high incidence of CD22ΔE12 in both pediatric and adult aggressive B-lineage lymphoid malignancies [15]. Our studies using quantitative real-time reverse transcription PCR demonstrated that 89% of newly diagnosed pediatric high-risk B-precursor ALL cases and 100% of infant ALL cases were CD22ΔE12$^+$. This very high incidence of CD22DE12 in high-risk B-precursor ALL was also confirmed using multiprobe CD22 gene-expression profiling [15]. Our most recent preliminary studies have established CD22ΔE12 as a molecular target for therapeutic RNAi [16]. Transfection with CD22ΔE12 siRNA, but not scrambled siRNA, caused selective (albeit partial) depletion of CD22ΔE12 mRNA as well as CD22ΔE12 protein in aggressive B-precursor ALL xenograft cells. This CD22ΔE12-knockdown was associated with a marked inhibition of their clonogenicity *in vitro* [14,16].

A liposomal nanoformulation (LNF) of CD22ΔE12 siRNA duplex effectively delivered CD22ΔE12 siRNA into CD22ΔE12$^+$ human leukemia cells and caused apoptotic cell death within 48 h. CD22ΔE12- leukemia cells were not affected by the treatments demonstrating that cytotoxic effects of the CD22ΔE12 siRNA-loaded nanoscale liposomes are dependent on the presence of the CD22ΔE12 molecular target in the treated cell population [14]. *In vitro* and *in vivo* clonogenic leukemic cells were particularly sensitive to CD22ΔE12 depletion [14]. Furthermore, combinations of CD22ΔE12 siRNA LNF with dexamethasone, pegaspargase, adriamycin and vincristine were significantly more effective than the chemotherapy drugs alone. Thus, CD22ΔE12 depletion significantly impairs the ability of leukemic clones to resist standard chemotherapy drugs. CD22ΔE12 siRNA LNF significantly impaired the ability of leukemia-initiating *in vivo* clonogenic human leukemia cells (i.e., putative leukemic stem cells) to engraft and cause fatal leukemia in non-obese diabetic/severe combined immunodeficiency mouse xenograft models of B-precursor ALL [14]. In pharmacokinetics (PK) studies, effective anti-leukemic concentrations of CD22ΔE12 siRNA LNF were achieved in mice at nontoxic dose levels. The estimated mean residence time was 8 h and the plasma half-life was 5.5 h. CD22DE12 siRNA LNF exhibited significant therapeutic activity in non-obese diabetic/severe combined immunodeficiency mouse models of relapsed B-precursor ALL and improved the survival outcome in a dose-dependent manner and without systemic toxicity [14]. These results provided proof of concept that CD22ΔE12 mRNA could be an appropriate target for effective RNAi therapy against B-precursor ALL. The potent *in vitro* and *in vivo* anti-leukemic activity of the LNF of CD22ΔE12 siRNA against human leukemia cells from relapsed BPL patients indicates that chemotherapy-resistant leukemic clones could be destroyed using nanoformulations of CD22ΔE12-specific siRNA as a new class of RNAi therapeutics.

Cheng *et al.* have recently developed a platform for the facile generation of cationic helical polypeptides [17]. Similar to cell-penetrating peptides found in nature, these rationally designed cationic helical polypeptides display excellent membrane penetration properties. They exhibit unprecedented thermodynamic and physicochemical stability [17] and unique biological properties with exceptional endosomal escape and siRNA delivery efficiency [18,19]. The high molecular weight, cationic, α-helical polypeptide, termed poly(γ-(4-((2-(piperidin-1-yl)ethyl) aminomethyl)benzyl-L-glutamate) (PVBLG-8) was identified as the top-performing peptide for nucleic acid delivery [17,18]. We demonstrated that the stabilized helical structure of PVBLG-8 markedly contributed to its membrane penetrating capacity via the pore formation mechanism; its relatively high molecular weight and cationic charge density facilitated the efficient condensation of nucleosides [17,18]. Our first proof-of-principle showed that this helical polypeptide is uniquely suited for the preparation of siRNA NPs by using the TNF-α gene as a target for RNAi [19]. Importantly, these helical polypeptide hybrid NP carrying TNF-α-specific siRNA displayed membrane-disruptive and endosomolytic properties, did not cause hemolysis at ≤50 μg/ml polypeptide concentrations and exhibited a promising safety profile in mice with no signs of organ-specific toxicity in mice at 50 μg/kg (3.5 nmol/kg) or 500 μg/kg (35 nmol/kg) dose levels [19]. We have also complexed CD22ΔE12 siRNA with a 200-mer polymer of PVBLG-8 to prepare a nanoscale formulation of CD22ΔE12 siRNA. By

leveraging the unique biofunctions of PVBLG-8, we have developed a unique NP of CD22ΔE12 siRNA as a novel anti-leukemic nanomedicine candidate with a high impact potential for the most common form of pediatric cancer [16]. This unique NP formulation effectively delivered Cy3-labeled CD22ΔE12 siRNA into the cytosol of ALL-1 cells, caused marked CD22ΔE12 mRNA depletion and inhibited their clonogenic growth. We are planning to develop PVBLG-8-based, advanced multifunctional bioactive nanomaterials with optimized properties for siRNA delivery in attempts to further improve the potency and broaden the therapeutic window of their nanocomplexes with therapeutic siRNA. We hypothesize that the resulting siRNA NPs prepared using reconfigured PVBLG-8 building blocks, especially PEG-PVBLG-8 star copolymers with a spherical architecture and high density of PPBLG-8 [20], will exhibit unprecedented *in vivo* RNAi potency owing to improved serum stability, PK properties, biodistribution and cellular uptake. The development of polypeptide-based multifunctional NPs with therapeutic siRNA targeting a driver lesion such as the CD22ΔE12-specific siRNA will be a significant step forward to overcome chemotherapy resistance in BPL and other CD22ΔE12$^+$ B-lineage lymphoid malignancies.

NPs can also be functionalized with a tumor-targeting moiety directed against a surface receptor on cancer cells in order to achieve optimal tumor targeting and site-specific drug delivery for reduction of their toxicity and potentiation of their anti-cancer efficacy [21]. The pharmacological effectiveness of siRNA-based therapeutics depends on their cellular uptake, intracellular trafficking, endosomal release and productive delivery to their target subcellular compartments [21]. The favorable leukemic cell versus normal tissue expression profile of CD19 and its abundant expression on relapse BPL clones make it an attractive molecular target for biotherapy in relapsed ALL. Several hundred thousand CD19 molecules on the surface of each B-lineage leukemia/lymphoma cell are rapidly internalized upon ligation with anti-CD19 mAb or immunoconjugates [22]. We are particularly interested in directing polypeptide-based NPs to leukemia cells with monospecific recombinant human CD19-ligand (CD19L) protein [23] or the bispecific recombinant human CD19L-TRAIL fusion protein [24]. This could be accomplished by conjugating the CD19-directed monospecific 54 kDa CD19L protein as well as the bispecific 75 kDa CD19L-sTRAIL fusion protein onto the side chain of the PVBLG-8 to prepare NPs capable of active targeting to B-precursor ALL cells. We postulate that the CD19L and CD19L-sTRAIL targeting of the siRNA NPs will improve the selective uptake of their siRNA cargo by CD19$^+$ BPL cells and thereby enhance their antileukemic potency. In the case of CD19L-sTRAIL, the targeting protein itself has femtomolar anti-leukemic activity, which is expected to contribute to the development of a targeted NP with unprecedented potency at nontoxic dose levels.

Future perspective

Polypeptide-based siRNA NPs with CD19-binding functionality could represent an important addition to the emerging new personalized treatment options for B-lineage lymphoid malignancies. We hypothesize that the decoration of the siRNA NPs with targeting moieties such as CD19L or CD19L-sTRAIL will markedly improve their PK, biodistribution, tolerability and therapeutic window by directing the NP to CD19$^+$ leukemia cells. This would reduce the potentially toxic interactions of the NP with non-hematopoietic normal tissues and enhance their cell type-specific cytotoxicity thereby reducing dose levels required for therapeutic efficacy.

Disclaimer

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Financial & competing interests disclosure

The project described was supported in part by the Keck School of Medicine Regenerative Medicine Initiative Award (FM Uckun) and by DHHS grants P30CA014089 (FM Uckun), U01-CA-151837 (FM Uckun), R01CA-154471 (FM Uckun) from the National Cancer Institute. J Cheng acknowledges the support from NIH (Director's New Innovator Award 1DP2OD007246-01). This work was also supported in part by Nautica Triathalon and its producer Michael Epstein (FM Uckun), Couples Against Leukemia Foundation (FM Uckun) and Saban Research Institute Merit Awards (FM Uckun). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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• **This is the first report of a unique targeting moiety for functionalizing nanoparticles so they are directed to CD19-positive leukemic B-cell precursors.**