Organic Nanoparticles as Drug Delivery Systems and Their Potential Role in the Treatment of Chronic Myeloid Leukemia

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Abstract
Chronic myeloid leukemia is a myeloproliferative neoplasm that occurs more prominently in the older population, with a peak incidence at ages 45 to 85 years and a median age at diagnosis of 65 years. This disease comprises roughly 15% of all leukemias in adults. It is a clonal stem cell disorder of myeloid cells characterized by the presence of t(9;22) chromosomal translocation, also known as the Philadelphia chromosome, or its byproducts BCR-ABL fusion protein/messenger RNA, leading to the expression of a protein with enhanced tyrosine kinase activity. This fusion protein has become the main therapeutic target in chronic myeloid leukemia therapy, with imatinib displaying superior antileukemic effects, placing it at the forefront of current treatment protocols and displaying great efficacy. Alternatively, nanomedicine and employing nanoparticles as drug delivery systems may represent new approaches in future anticancer therapy. This review focuses primarily on the use of organic nanoparticles aimed at chronic myeloid leukemia therapy in both in vitro and in vivo settings, by going through a thorough survey of published literature. After a brief introduction on the pathogenesis of chronic myeloid leukemia, a description of conventional, first- and second-line, treatment modalities of chronic myeloid leukemia is presented. Finally, some of the general applications of nanostrategies in medicine are presented, with a detailed focus on organic nanocarriers and their constituents used in chronic myeloid leukemia treatment from the literature.

Keywords
nanomedicine, CML, drug delivery systems, organic nanoparticles, multidrug resistance

Abbreviations
AAV, adeno-associated virus; Bcl-2, B-cell lymphoma-2; BCPV, biodegradable charged polyester-based vector; BIM, Bcl-2-interacting mediator; CML, chronic myeloid leukemia; CNS, central nervous system; HAS, human serum albumin; IFN, interferon; IL, interleukin; LDL, low-density lipoprotein; MCL-1, myeloid cell leukemia 1; MDR, multidrug resistance; miRNA, microRNA; mRNA, messenger RNA; MW, molecular weight; NPs, nanoparticles; PCL, poly-caprolactone; PEG, polyethylene glycol; PEI, polyethylenimine; Peg-IFN-α2b, pegylated IFN-α2b; PLGA, poly lactic-co-glycolic acid; Ps, spermine-introduced pullulan; RNAi, RNA interference; siRNA, small-interfering RNA; TKI, tyrosine kinase inhibitor; Trf, transferrin; TrfR, transferrin receptor; QSC, quiescent stem cells; sLDL, synthetic LDL

Received: August 01, 2019; Revised: August 26, 2019; Accepted: September 04, 2019.

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Introduction and Clinical Features

Chronic myeloid leukemia (CML) is a neoplasm that affects mature myeloid cells, especially granulocytes and their precursors. It results from the reciprocal translocation of the ABL gene on chromosome 9 to the BCR gene on chromosome 22, that is, t(9;22) forming the Philadelphia chromosome (BCR-ABL gene fusion). Consequently, tyrosine kinase activity is expressed constitutively, leading to the phosphorylation and activation of various downstream proteins that promote cellular proliferation and simultaneous inhibition of apoptosis.

Chronic myelogenous leukemia represents roughly 15% of leukemias in the adult population, with an incidence of 1 to 2 new cases per 100,000 individuals and a median age of diagnosis of 60 to 65 years.

The most common clinical presentation of CML is an incidental finding in an asymptomatic patient, representing 50% of patients with CML. Additionally, even in symptomatic patients, symptoms are largely nonspecific.

They can range from dyspnea on exertion or fatigue due to anemia, to left upper quadrant pain and early satiety from splenomegaly. Rarely, 5% of patients may present with symptoms of headache, retinopathy, and vertigo among others, owing to the hyperviscosity syndrome from the large-scale leucocytosis.

Regarding treatment, the most widespread therapeutic approach for CML is the use of imatinib, a tyrosine kinase inhibitor (TKI). Nonetheless, TKIs do not offer a cure for CML. Rather, a bone marrow transplantation is required for cure. Additionally, current treatment protocols are hampered by drug resistance and cancer relapse.

For this reason, the combination of newer agents with existing ones offers a new prospect to address cancer cells’ resistance to drugs, cancer recurrence, and importantly a cure without the need for transplantation. Of these strategies, nanotechnology is emerging as a possible new approach in the management of CML.

Multidrug resistance (MDR) emerged as a major reason of chemotherapy failure and relapse in hematological neoplasms. Specifically, cancerous cells develop resistance against the cytotoxic effects of various drugs, through a complex mechanism involving different pathways. To note, resistance developed to one drug is not specific to that drug only and may involve multiresistance.

One pathway involved is the down-regulation of the production of apoptosis-related proteins, such as Bax and B-cell lymphoma-2 (Bel-2). Other mechanisms affect recovery from drug-induced DNA damage, drug excretion, and alterations in the activity of enzymes functioning in drug metabolism.

Drug excretion via P-gp, the permeability glycoprotein, remains the most important mechanism of resistance. In essence, it is an adenosine triphosphate–dependent transmembrane efflux pump encoded by the Mdr1 gene, which functions to decrease intracellular drug concentrations through active transportation of drugs out of the cells. This overexpression of Mdr1 increases resistance to drugs and consequently increases the chance of cancer recurrence, a sign of worse prognosis.

A promising new approach to combat resistance is through the use of nanoparticles (NPs) as drug delivery systems. This review aims to collect and discuss current advancements in organic nanomedicine, in which organic NPs are made to function as drug delivery systems for the treatment of CML, with a focus on both the chemical properties of NPs and their clinical applications.

Nanomedicine in CML Treatment

Designing a drug delivery system is a complex process requiring several steps to be taken to ensure success. The delivery system must be developed in a way that balances efficiency in delivery and preservation of drug bioactivity.

Moreover, adequate loading and release of the drug must be ensured through the control of the kinetics of both drug loading and release. Herein lies the advantage in using NPs as delivery systems, as they enhance the intracellular uptake of the drug, improve water solubility of the drug in cancer cells, and enhance circulation time of the drug. Importantly, NPs allow sparing of normal cells from the cytotoxic effects of drugs through their targeted delivery system. As such, chemotherapeutic drugs loaded into NPs can resist degradation, display lower toxicity, and show enhanced solubility and efficacy.

However, NP use is not without its own complexities. Both intrinsic properties, such as surface charge, particle size and shape, zeta potential, and surface area, as well as extrinsic properties, such as the activity of the reticuloendothelial system, and renal clearance affect toxicity, uptake, and half-life of NPs. The reticuloendothelial system, mostly the macrophages, specifically mediates the half-life of NPs. A method to circumvent this system is to coat NPs with a hydrophilic layer, using polymers such as polyethylene glycol (PEG) through a process called PEGylation. Endocytosis is the last step through which NPs are transported into cells when they reach their target.

Classification of NPs

According to RSC Advances by Aula et al., NPs can be divided into organic and inorganic. Inorganic NPs for CML treatment were detailed in a previous work. In this review, organic NPs are discussed and categorized as presented in Table 1 and below:

- Lipid-based NPs (liposomes)
- Polymer-based NPs and micelles
  - Poly lactic-co-glycolic acid (PLGA) NPs
  - Lipophilic polyethylenimines (PEIs) polymers
  - Lipophilic polyethylene glycol (PEG) NPs
- Micelles
  - Multipurpose caniste-diisopropylamino)ethyl methacrylate deblock copolymers
  - PEG
- Synthetic low-density lipoprotein (LDL)
- Dendrimers
| Nanocarrier Type | Ligand | Target | Coating | Drug | Application | In vitro (Cell Lines) | In vivo | Ref |
|-----------------|--------|--------|---------|------|-------------|----------------------|---------|-----|
| Liposome        | --     | --     | --      | Cytarabine | Treatment of isolated CNS relapse in imatinib-treated patients with CML | -- | 2 cases of isolated CNS CML relapse | 30 |
| Liposome        | Trf    | TrfR   | PEG     | Imatinib + siRNA, Paclitaxel + curcumin | Induction of apoptosis | K562, LAMA-84, K562, KCL2, KU812 | -- | 31 |
| PLGA            | --     | --     | --      | Paclitaxel + curcumin | Induction of apoptosis | -- | -- | 32 |
| sLDL            | Apo-B  | LDLR   | Lipid components coupled with a synthetic amphiphatic peptide | Targeting of quiescent stem cells | Primitive CD34**+**38**α**-subpopulation of CML cells, K562 | -- | -- | 33 |
| PEG             | --     | --     | --      | IFN-α2b + imatinib | Treatment of CML in newly diagnosed patients | -- | Newly diagnosed patients with CML | 34 |
| PEI             | --     | --     | PEG     | siRNA, miRNA | Induction of apoptosis | K562 | -- | 35 |
| BCPVs           | --     | --     | --      | siRNA | Induction of apoptosis | K562 | -- | 36 |
| AAV core encapsulated in an acid-degradable PK polymeric shell | -- | -- | Eosin-5-isothiocyanate | Viral core encoding BIM, siRNA | Induction of apoptosis | K562 | BCR-ABL+ FL5.12/p190 mice models | 37 |
| HAS             | Trf    | TrfR   | --      | Sorafenib | Induction of apoptosis | K562, cell samples from imatinib-resistant patients with CML | -- | -- | 38 |
| Realgar (α-As<sub>4</sub>S<sub>4</sub>) | --     | --     | --      | Realgar | Induction of apoptosis | K562 | -- | 39 |
| Pullulan        | --     | --     | Spermine | miRNA | Induction of apoptosis | K562, CML CD34**+** cells from clinical isolates | -- | -- | 40 |
| Citrus lemon–derived nanovesicles | -- | -- | -- | Induction of apoptosis | -- | -- | -- | 41 |
| PCL             | --     | --     | --      | Imatinib | Induction of apoptosis | BCR-ABL+ KU812 | -- | -- | 42 |
| PECs            | --     | --     | --      | Doxorubicin | Induction of apoptosis | BCR-ABL+ KU812 | -- | -- | 42 |

Abbreviations: AAV, adeno-associated virus; BCPV, biodegradable charged polyester-based vectors; CML, chronic myeloid leukemia; CNS, central nervous system; HSA, human serum albumin; LDLR, LDL receptor; miRNA, microRNA; PCL, poly-caprolactone; PECs, polyelectrolyte complexes; PEI, polyethylenimine; PK, polyketal; PLGA, poly lactic-co-glycolic acid; siRNA, small-interfering RNA; sLDL, synthetic low-density lipoprotein; Trf, transferrin; TrfR, transferrin receptor.
- Biodegradable polyesters
  - Biodegradable charged polyester-based vector (BCPVs)
  - Poly-caprolactone (PCL)
- Albumin-based nanovehicles
- Realgar NP
- Polysaccharide polymer
  - Spermine-introduced pullulan (Ps)
- Citrus lemon–derived nanovesicles

**Organic NPs for CML Treatment**

**Liposomes**

Aichberger et al investigated the usage of liposomal cytarabine for the treatment of myeloid central nervous system (CNS) relapse in CML. When compared to free drugs, the advantages of liposomes in drug vehiculation can be attributed to a reduction in drug toxicity, the ability to couple them with antibodies and other signaling molecules for further targeted delivery, as well improved altered bioavailability and pharmacokinetics.

Although considered an extreme rarity in the past, data show that a CNS relapse can appear in imatinib-treated patients with CML. Although imatinib has been shown to be extremely effective in CML treatment, its chemical properties don’t allow for adequate crossing of the blood–brain barrier. This “anatomic” resistance may lead to a possible CNS relapse. This was demonstrated and studied on 2 patients who had a CNS relapse after being treated with imatinib for 4 years.

Both patients received intrathecal liposomal cytarabine. In the first patient, additional CNS radiation was performed, while a systemic relapse in the other patient was being concurrently treated with dasatinib. In both cases, symptoms resolved, and lumbar puncture revealed no leukemic cells in the CSF. Furthermore, 3 months of posttherapy monitoring showed no evidence of systemic or CNS relapse in either patient and both were shown to be in complete cytogenetic and molecular response. Therefore, liposomal cytarabine with/without radiation is effective as local therapy in patients with CNS relapse.

Furthermore, there is no concrete data regarding the efficacy of the newer BCR-Abl kinase inhibitors with regard to CNS relapse, implying that they cannot be regarded as standard therapy alone. These data suggest that liposomal cytarabine may be an effective therapeutic approach in such patients.

In addition to treating CNS relapses, liposomes have been used to coencapsulate anti-BCR-ABL small-interfering RNA (siRNA) and imatinib in CML treatment. Overexpression of the transferrin receptor (TrfR) on tumor cell surface and constitutive endocytosis has made it a prime target for liposomal delivery into cancer cells. Transferrin (Trf) is the ligand for this TrfR.

Small-interfering RNAs are small double-stranded RNA molecules composed of 20 to 25 base pairs that target messenger RNA (mRNA), leading to its cleavage. Nonetheless, the therapeutic use of siRNA is burdened by impediments such as degradation by enzymes, a low uptake by cells, as well as a rapid clearance by the kidneys, leading to the observed poor pharmacokinetic properties in an in vivo environment. The vehiculation of siRNA in liposomes confers protection from nuclease degradation, leading to a sustainable plasma concentration while also allowing for targeting of tumor cells by these nucleic acids.

Mendonca et al demonstrated an in vitro triple targeting strategy within a single system using TrfR-targeted liposomes to coencapsulate imatinib with anti-BCR-Abl siRNA. Cellular targeting was accomplished via Trf, molecular targeting of BCR-Abl mRNA was accomplished via BCR-Abl siRNA, and protein targeting was accomplished with imatinib. Not only is such a combination strategy a promising approach to the heterogeneity of tumor cells and their varying response to individual drugs, it is also an equally promising therapeutic approach to overcoming the resistance phenomena.

Rather surprisingly, imatinib encapsulation yields were improved by siRNA. This possibly owes to interactions between imatinib and siRNA, positively and negatively charged, respectively, which could promote further entrapment of imatinib in the liposomes.

Antileukemic activity against the K562 cell line was recorded for varying concentrations, with higher cellular toxicity being achieved with formulations carrying a higher siRNA to imatinib ratios. Furthermore, the highest siRNA to imatinib ratio was achieved at the lowest imatinib to lipid ratio. As such, it was possible to achieve therapeutic concentrations of each of imatinib and siRNA in a single liposome.

**Poly Lactic-Co-Glycolic Acid**

Polylactic acid as well as polylactic acid and their copolymer PLGA have been thoroughly explored due to their biocompatibility and biodegradability. They manifest physical strength and biological compatibility while also possessing great flexibility. Moreover, their degradation can be modulated with control over the molecular weight (MW) of the NPs to fit the desired dose for sustained drug release. Loading the therapeutic drugs into PLGA provides many advantages, such as protecting the drug from enzymatic degradation and providing sustained release. Recent reports demonstrate that PLGA NPs complexed to anticancer drugs can be used for efficient drug delivery and for reduced toxicity against normal cells.

Acharya et al explored an approach using PLGA NPs to simultaneously deliver 2 drugs to the Bcr-Abl fusion protein. In this study, paclitaxel, doxorubicin, etoposide, rapamycin, curcumin, and nutilin were encapsulated alone, and then paclitaxel was combined with each of the other drugs and encapsulation efficiency was calculated. When encapsulated alone, each of the drugs showed high encapsulation that slightly dropped when combined with paclitaxel. The lowered encapsulation efficiency may be due to differences in the partition coefficient between the drugs and their competition to occupy the NPs. Nevertheless, drug loading was still capable of reaching a therapeutically optimal concentration in vitro that requires further in vivo experimentation to prove its efficacy.
The highest therapeutic efficacy in vitro experimentation was observed with a paclitaxel/curcumin combination. When compared to their native equivalents, the encapsulated dual formulations used in this study showed better antileukemic effects on K562. When comparing the IC50 values of encapsulated dual formulations to their singly encapsulated counterparts, singly encapsulated formulations were effective only after 5 days versus 2 days when combined, showing that such drug combinations and their encapsulation in PLGA NPs could potentially reduce the treatment duration. Thus, NPs could potentially be used to codeliver drugs to the same target site and increase efficacy while decreasing treatment duration.

**Synthetic LDL**

Low-density lipoprotein is a lipoprotein in plasma responsible for the transport of cholesterol. This spherical particle has a diameter of approximately 22 nm. It’s use as a drug carrier in chemotherapy dates back to the 1980s when Gal et al61 established an increase in the uptake of LDL in cancer cells of gynecological origin compared to normal cells. This observation has been shown to apply to many other types of cancer, including CML.62 There is an elevation in LDL receptor activity leading to a higher LDL uptake and particularly in cases of CML with a worse prognosis related to lower concentrations of plasma lipids.63

There is an overexpression of Bcr-Abl in CML stem cells. These cells do respond to imatinib therapy; however, this response is one of reversible block in proliferation and without a significant amount of apoptosis. Therefore, achieving a complete cure is hindered by the subsistence of leukemic quiescent stem cells (QSC) with the ability to initiate relapse. Zhou et al38 have previously shown that intracellular levels of imatinib in CML progenitor cells (CD34+), and in other commonly used leukemic cell lines are remarkably higher than in primary primitive QSC (CD34+ 38 lo−), which were significantly subtherapeutic. Achieving complete cure with imatinib and other TKIs, therefore, is dependent on targeting QSC.

Zhou et al33 also aimed to determine whether drug delivery in synthetic LDL (sLDL) targeting QSC could overcome the subtherapeutic levels of drug concentrations in these cells. First, nondrug-loaded NPs were used to target BCR-Abl-positive cell lines, and an increase in the sLDL uptake by these cells compared to their BCR-Abl-negative counterparts was noted. Furthermore, compared to non-CML CD34+ cells, primitive CD34+ 38 lo− cells were reported to accumulate significantly higher levels of sLDL. Therefore, loading sLDL NPs with drugs could present as a potential mechanism to enhance the level of intracellular drugs in primitive CML cells and aid in their elimination. Further studies, in vivo and with the inclusion of drugs in sLDL particles, must be carried out before a definitive conclusion that sLDL-loaded NPs may be beneficial in the therapeutic management of CML via the eradication of QSC.

**Polyethylene Glycol**

Polyethylene glycol is a water-soluble block used in forming polymer micelles. The advantages that PEG presents are that it makes micelles biocompatible and increases the bioavailability of certain drugs.64 Simonsson et al64 combined imatinib with pegylated interferon (IFN)-z2b (Peg-IFN-z2b) to potentially increase the molecular response rates in patients with CML.

Combining imatinib with IFN-α is an approach sometimes used in the management of CML.65-68 However, when using IFN-α, it seems that it is barely detectable after 24 hours of administration. Subsequently, a modification was necessary and 2 types of IFN-α have been developed: Peg-IFN-α2a and Peg-IFN-α2b, which involve the attachment of PEG.69 Studies have compared the difference between the combination of imatinib with IFN-α2a and Peg-IFN-α2a on one side and IFN-α2b and Peg-IFN-α2b on another side.69 The results indicated the efficacy of the pegylated form of IFN-α along with the combination of each with imatinib.34,69

Newly diagnosed patients with CML were randomly chosen and either received a weekly dosage of 40 μg of Peg-IFN-α2b along with a daily dosage of 400 mg of imatinib or only a dosage of 400 mg of imatinib.34 The results were remarkable in that the combination therapy showed much better results after 12 months, where major molecular response rate was greatly increased in the former than in patients receiving imatinib alone. Therefore, this combined therapy shows a promise for future CML therapy, keeping in mind that Peg-IFN-α2b appeared to have some toxicity levels leading to its discontinuation in 34 patients receiving the combined therapy.34

**Polyethylenimines**

Polyethylenimine is a polycation used as a delivery system for DNA70 and RNA carrying. Polyethylenimine has positively charged amine groups that complex with the negatively charged phosphate groups of nucleic acids.71-78 This modality is, however, limited by its toxicity79; therefore, methods for lowering the toxicity levels have been enhanced and involve the linkage with nonionic/hydrophilic polymers such as the PEG.35 With further modifications, the PEG could be rendered more hydrophobic and hence have a better interaction with the cell membrane making transfection much more feasible along with the endocytic uptake of the nucleic acids.35 Consequently, modifying the hydrophobicity of the low-molecular PEI increases the safety as well as efficacy of drug delivery to cancer cells and induces silencing of the target protein.

RNA interference (RNAi) provides an alternative approach for the treatment of CML with acquired resistance. However, for RNAi to reach the cell and exert its actions, an efficient carrier must be provided. Therefore, Valencia-Serna et al35 explored low-MW lipid-modified PEIs as potential carriers for therapeutic BCR-ABL downregulation. Results showed that palmitic acid-substituted PEI (1.2 kDa) was the most efficient (when compared to other lipid-substituted polymers) in delivering siRNA to the cells and silencing the reporter green
fluorescent protein gene (GFP). In addition, PA-substituted PEI was able to reduce the mRNA levels in K562 cells when the BCR-ABL protein was targeted and was also able to induce apoptosis (early and late stages). Thus, low-MW PA-substituted PEI may be used as an efficient delivery system of siRNA for CML treatment.

Another strategy to transfect CML cells is the usage of PEG–PEI complex as nonviral carriers of microRNA (miRNA). In CML, a beneficiary tumor suppressor miRNA is the miR-150 that functions as an inducer for the phosphoinositide-3-kinase, protein kinase B (AKT) pathway activation, pioneering telomerase activation, and immortalization of cancer cells. One study showed that the usage of PEG–PEI-based NPs encapsulating the miRNA maintains a proper transfection of CML cells. The high efficiency in miRNA transfection and the low cytotoxic effects make PEG–PEI a promising nonviral delivery system in CML treatment. The data indicate that PEG–PEI may be a promising nonviral carrier for the treatment of CML, with many advantages such as relatively high mRNA transfection efficiency and low cytotoxicity.

**Phosphorus Dendrimers**

Dendrimers are small NPs that have radial symmetry that make an efficient carrier for the delivery of a given drug. Several types of dendrimers exist, and each has certain biological characteristics. These biological properties include electrostatic interactions, chemical stability, polyvalency, self-assembling, low cytotoxicity, and solubility. These properties allow for the wide usage of the dendrimers in the medical field. Phosphorus dendrimers are amphiphilic having a hydrophilic surface and a hydrophobic backbone, which facilitates their entry through the cell membrane. The formation of dendriplexes with siRNA is favored due to the interaction between the negatively charged siRNA and the positively charged dendrimer surface. Moreover, the success of the formation of dendriplexes depends on external factors such as pH and temperature.

With regard to dendrimers potential usage in CML treatment, it was demonstrated that phosphorus dendrimers can be used to carry siRNAs specific for BCR gene, but no experiments were done to test their effect on CML cells. Therefore, further investigations should be done.

**Biodegradable Charged Polyester-Based Vectors**

Yang et al. showed that BCPVs are efficient nonviral transfection NPs for gene knockdown of the BCR-ABL hybrid oncogene in K562 cell line. Their results showed that cancer cell proliferation can be decreased by enhancing cell apoptosis by downregulation of the oncoprotein (BCR-ABL) via RNAi. The significance of such results is that BCPVs may potentially be used as potential carriers of siRNA for CML treatment. The positive charge on the surface of the BCPV NPs allows for the penetration of the negatively charged cell membrane of the cells via electrostatic interactions. Furthermore, since the BCPV–siRNA complex is encapsulated, the obstacle of siRNA degradation is eliminated. When compared to Lipofectamine2000 (common transfection reagent), BCPV had a larger suppressive effect on K562 cells proliferation. This is due to the higher transfection efficiency of BCPV, subsequently leading to the enhancement of apoptosis. The mRNA expression knockdown-induced apoptosis is positively correlated with transfection efficiency. In summary, what makes BCPVs potentially successful carriers for siRNAs delivery is that they have low toxicity, high transfection efficiency, and manageable charge density. It is important to note that these studies were performed on adherent cells whereas leukemic cells are of nonadherent nature necessitating further research.

**Viral/Nonviral Chimeric NPs**

One interesting approach by Hong et al. used viral/nonviral chimeric NPs to synergistically suppress leukemia proliferation via simultaneous gene transduction and silencing. In this study, a dual-modal gene therapy was developed. A new therapeutic approach to apoptosis-mediated gene therapy was led through analyzing the Bcr-Abl pathways, where it appeared to be directly regulated by the Bcl-2 family proteins, such as Bcl-2-interacting mediator (BIM) of cell death. B-cell lymphoma-2 interacting mediator is responsible for the proapoptotic BIM expression and silencing the MCL-1 through a dual-modal gene manipulation. This study made use of NPs consisting of an adeno-associated virus (AAV) core encapsulated in an acid-degradable polymeric shell where proapoptotic BIM transduction was induced by the AAV core and silencing of prosurvival MCL-1 was achieved by siRNA encapsulated in the shell. The AAV was shielded in a nonviral chimeric NP that protected the virus from the immune system as well as encapsulating the siRNA responsible for the MCL-1 silencing. This, in turn, suppressed the proliferation of the Bcr-Abl-positive cells as was shown to occur in this study.

**Albumin-Bound Paclitaxel to Treat Refractory CML**

One of the challenges of using TKIs are point mutations in the kinase domain imparting drug resistance and contributing to future relapses. Studies showed undesirable results due to point mutations in the tyrosine kinase–binding domain, the most critical being the tyrosine-isoleucine mutation at 315 position (T315I). One strategy is to target the other survival kinases; however, this might inflict damage to healthy cells where the pathway might also be present. Therefore, Retnakumari et al. developed a dual targeting method, where 2 pathways would be targeted at the same time in order to target the receptors of the refractory cancer cells along with their intracellular network of kinases. The chemo drugs chosen needed to be transferred in a nontoxic manner and be readily bioavailable; therefore, a polymeric encapsulation for the
drugs was chosen and that polymer was albumin-encapsulating paclitaxel (Abraxane), which is a key formulation of the human serum albumin (HSA). Since albumin is part of the blood serum, it is a suitable integration of the delivery system due it is nonimmunogenic, nontoxic, and biocompatible nature. This HSA-based NP was loaded with STAT5 inhibitor (sorafenib) designed to inhibit the phosphorylation of an activated secondary survival kinase (STAT5) and the surface was conjugated with Tf ligands to target TfR on the CML cells. The results were promising when performed in vivo, where the dual targeting method led to the apoptosis of the leukemia cells without any side effects, inflammatory responses, or toxicity to the blood while showing maximal toxicity to the most resistant clinical samples. This study demonstrates the potential of nanomedicines in tackling critical challenges such as drug resistance by engineering endogenous proteins abundant in our own body.

**Realgar NPs**

One of the methods to treat leukemia is by modifying the pathways involved in the cells’ life cycles, especially those concerned with cell apoptosis and autophagy. One of the well-known pathways is the PI3K/Akt/mTOR (class I phosphoinositide 3-kinase/protein kinase B mammalian target of rapamycin). Such pathway appears to be enhanced when treated with As$_2$S$_2$ such as realgar; however, realgar has been demonstrated to be insoluble in water and most organic reagents. These characteristics play a negative role in its utilization in biological applications and medicine due to its limitations and poor bioavailability. Therefore, Shi et al prepared realgar NPs with a minimum size of 100 nm and proved to have higher bioavailability compared to coarse realgar while degrading the oncoprotein involved (Bcr-Abl). Moreover, one of the known membrane proteins involved in the PI3K/Akt/mTOR pathway, Caveolin-I (Cav-1), appeared to be enhanced when treated with the realgar NPs.

**Pullulan/MiRNA NPs**

Ma et al aimed to modulate the growth and imatinib sensitivity of CML stem/progenitor cells using pullulan/miR-181a NPs in vitro. A miR-181a is a miRNA that has been shown to inhibit the proliferation of a CML cell line, K562 cells. Moreover, miR-181a binds to 3-UTR of RALA (an oncogene) and subsequently inhibit its expression. Spermine-introduced pullulan were used as vectors to deliver miRNA to CML cells. Delivery efficiency and mean fluorescence intensity showed successful transfection of CML CD34$^+$ cells by Ps miRNA in vitro. In CML, the expression of miR-181a is extremely low while the expression of RALA is very high compared to control cells. RALA is the direct target of miR-181a. The delivery of miR-181a induced a decrease in CML cells proliferation, while it had no effect on normal CD34$^+$ cells. When TKIs were used in combination with miR-181a, a higher specific inhibition of CML cells growth was achieved, even in cells manifesting resistance. Consequently, Ps was presented as an efficient nonviral delivery system of miRNA to CML cells. The delivery of miR-181a not only inhibited the growth the CML cells (CD34$^+$), but it also increased their sensitivity to imatinib mesylate. Overall, Ps provides an improvement in the treatment of CML.

**Citrus Lemon–Derived Nanovesicles**

Under specific circumstances, when some natural compounds are used in association with chemotherapeutic drugs, lower dose of drugs can be used without compromising efficiency. Nanoparticles extracted from lemon by Raimondo et al induced apoptotic effects on the cancer cells via the TRAIL mechanism. These NPs express anticancerous properties since they inhibit cell proliferation. One of the main advantages of TRAIL lies in its specificity to induce apoptosis only in transformed cells and not in normal cells. Subsequently, the therapeutic approach can overcome 2 main obstacles: drug resistance and toxicity on normal tissues. Based on morphology and size, the NPs extracted from the citrus lemon juice are exosome like. According to data, citrus lemon nanovesicles reduced cell proliferation in vivo on CML (LAMA84) xenograft models in both time- and dose-dependent manner. The nanovesicles were also active in vitro against the following cell lines: A549, SW480, and LAMA84. The nanovesicles were able to induce apoptosis via the TRAIL pathway in cancer cells without affecting the normal cells. Moreover, the nanovesicles inhibited angiogenesis by inhibiting the secretion of angiogenic cytokines (vascular endothelial growth factor A, interleukin [IL]-6, and IL-8).

**Poly-Caprolactone**

In order to maintain well-controlled drug delivery, NPs usage became important and studied more thoroughly; therefore, Palama et al investigated the usage of coupled delivery of imatinib and doxorubicin carried using biodegradable pH sensitive core–shell PCL NPs and enzyme-sensitive polyelectrolyte complexes for a sustained downregulation of BCR-ABL in CML. The main purpose of the combination is to increase the therapeutic index of the drug by making it more target specific while simultaneously making it less toxic at increased concentrations. The experimental results suggest that the nanof ormulations maintained the biological activity of drugs for longer periods and led to a continuous release of active drug. Moreover, at low concentrations, the combined drug-loaded nanof ormulation was relatively more efficient against CML cells than the single drug formulation. Western blot analysis was used to assess the effective inhibition of the BCR-ABL tyrosine kinase activity after 3 days of incubation with a low concentration (10 nM) of free combination drugs and same concentration of drug-loaded NPs. Cell viability of CML was permanently blocked, using the drug-loaded NPs cocktail, but BCR-ABL inhibition was only partially inhibited using the free combination, demonstrating this nanoformulation’s ability to improve drug kinetics and efficacy.
Conclusion

Chronic myeloid leukemia is a neoplastic proliferation of mature myeloid cells, especially granulocytes and their precursors. It is the result of the BCR-ABL fusion gene, leading to a constitutively active TK resulting in uncontrolled growth of myeloid cells. Imatinib and other TKIs are the most widespread treatment modalities available for CML treatment. However, as good as the response rates to these drugs are, they are not without their downfalls. For instance, imatinib fails to achieve cure status due to persistent QSC. Furthermore, resistance plays a major part in the decreased efficacy of imatinib and its sister drugs, with a functional “anatomic” resistance also being reported.

Therefore, nanomedicine and the use of NPs as drug delivery systems may provide an alternative and a superior treatment modality to CML. This review highlighted organic examples of such treatments in vitro and in vivo settings, with inorganic NPs highlighted in a previous study. Preliminary results are encouraging, with NPs displaying the ability to overcome both “anatomic” resistance and MDR, and successfully targeting the QSC. Additionally, these NPs demonstrated safety through specific targeting of CML cells with sparing of normal cells. However, the majority of these studies were conducted in a controlled laboratory environment, while only a few studies included clinical trials; therefore, further studies and clinical trials in this field are required.

Acknowledgments

The authors thank Ahmad El Mahmoud, Khodor Terro, Bachar El Baba, and Nadia El Harake for helping with revision of the text.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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References

1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405.
2. Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. Nat Rev Cancer. 2005;5(3):172-183.
3. Hazlehurst LA, Bewry NN, Nair RR, Pinilla-Ibarz J. Signaling networks associated with BCR–ABL–dependent transformation. Cancer Control. 2009;16(2):100-107.
4. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2016 update on diagnosis, therapy, and monitoring. Am J Hematol. 2016;91(2):252-265.
5. Hochhaus A, Saussele S, Rosti G, et al. Chronic myeloid leukaemia: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017;28(suppl 4):iv41-iv51.
6. Chereda B, Melo JV. Natural course and biology of CML. Ann Hematol. 2015;94(suppl 2):107.
7. Korubo KI, Omunakwe HE. Chronic myeloid leukemia: clinical and laboratory features at presentation to a referral hospital in Southern Nigeria. Blood. 2013;122(21):5174.
8. Cotta CV, Bueso-Ramos CE. New insights into the pathobiology and treatment of chronic myelogenous leukemia. Ann Diagn Pathol. 2007;11(1):68-78.
9. Bhatia R, Holtz M, Niu N, et al. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. Blood. 2003;101(12):4701-4707.
10. Subbiah R, Veerapandian M, Yun KS. Nanoparticles: functionalization and multifunctional applications in biomedical sciences. Curr Med Chem. 2010;17(36):4559-4577.
11. Gottesman MM. Mechanisms of cancer drug resistance. Ann Rev Med. 2002;53(1):615-627.
12. Park MT, Kang JA, Choi JA, et al. Phytosphingosine induces apoptotic cell death via caspase 8 activation and Bax translocation in human cancer cells. Clin Cancer Res. 2003;9(2):878-885.
13. Ünlü M, Kiraz Y, Kaci FN, et al. Multidrug resistance in chronic myeloid leukemia. Turkish J Biol. 2014;38(6):806-816.
14. Li X, Li JP, Yuan HY, et al. Recent advances in P-glycoprotein-mediated multidrug resistance reversal mechanisms. Methods Find Exp Clin Pharmacol. 2007;29(9):607-618.
15. Ross DD. Modulation of drug resistance transporters as a strategy for treating myelodysplastic syndrome. Best Pract Res Clin Haematol. 2004;17(4):641-651.
16. Aller SG, Yu J, Ward A, et al. Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. Science. 2009;323(5922):1718-1722.
17. Mahon FX, Belloc F, Lagarde V, et al. MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. Blood. 2003;101(6):2368-2373.
18. Falchi L, Kantarjian HM, Wang X, et al. Significance of deeper molecular responses in patients with chronic myeloid leukemia in early chronic phase treated with tyrosine kinase inhibitors. Am J Hematol. 2013;88(12):1024-1029.
19. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. Adv Drug Deliv Rev. 2013;65(1):36-48.
20. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. Science. 2004;303(5665):1818-1822.
21. Vauthier C, Dubernet C, Chauvierre C, Brigger I, Couvreur P. Drug delivery to resistant tumors: the potential of poly (alkyl cyanoacrylate) nanoparticles. J Control Release. 2003;93(2):151-160.
22. Xu P, Van Kirk EA, Zhan Y, Murdoch WJ, Radosz M, Shen Y. Targeted charge-reversal nanoparticles for nuclear drug delivery. Angew Chem Int Ed Engl. 2007;46(26):4999-5002.
23. Gindy ME, Prad’homme RK. Multifunctional nanoparticles for imaging, delivery and targeting in cancer therapy. Expert Opin Drug Deliv. 2009;6(8):865-878.
24. Haley B, Frenkel E. Nanoparticles for drug delivery in cancer treatment. *Urol Oncol.* 2008;26(1):57-64.

25. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol.* 2007;2(12):751-760.

26. Karakoti AS, Hench LL, Seal S. The potential toxicity of nanomaterials—the role of surfaces. *JOM.* 2006;58(7):77-82.

27. Owens DE, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *Int J Pharm.* 2006;307(1):93-102.

28. Niidome T, Yamagata M, Okamoto Y, et al. PEG-modified gold nanorods with a stealth character for in vivo applications. *J Control Release.* 2006;114(3):343-347.

29. Aula S, Lakkireddy S, Jamil K, et al. Biophysical, biopharmaceutical and toxicological significance of biomedical nanoparticles. *RSC Adv.* 2015;5(59):47830-47859.

30. Aichberger KJ, Herndlhofer S, Agis H, et al. Liposomal cytara- 

31. Mendonça LS, Moreira JN, de Lima MCP, Simões S. Co-encapsulation of anti-BCR-ABL siRNA and imatinib mesylate in transferrin receptor-targeted sterically stabilized liposomes for chronic myeloid leukemia treatment. *Biotecnol Bioeng.* 2010;107(5):884-893.

32. Acharya S, Sahoo SK. Sustained targeting of Bcr–Abl+ leukemia cells by synergistic action of dual drug loaded nanoparticles and its implication for leukemia therapy. *Biomaterials.* 2011;32(24):5643-5662.

33. Zhou P, Hatzieremia S, Elliott MA, et al. Uptake of synthetic low density lipoprotein by leukemic stem cells—a potential stem cell targeted drug delivery strategy. *J Control Release.* 2010;148(3):380-387.

34. Simonsson B, Gedde-Dahl T, Markevärn B, et al. Combination of pegylated IFN-a2b with imatinib increases molecular response rates in patients with low-or intermediate-risk chronic myeloid leukemia. *Blood.* 2011;118(12):3228-3235.

35. Valencia-Serna J, Gul-Uludağ H, Mahdipoor P, Jiang X, Uludağ H. Investigating siRNA delivery to chronic myeloid leukemia K562 cells with lipophilic polymers for therapeutic BCR-ABL down-regulation. *J Control Release.* 2013;172(2):495-503.

36. Yang C, Panwar N, Wang Y, et al. Biodegradable charged polyester-based vectors (BCPVs) as an efficient non-viral transfection nanogenen for gene knockdown of the BCR–ABL hybrid oncogene in a human chronic myeloid leukemia cell line. *Nanoscale.* 2016;8(17):9405-9416.

37. Hong CA, Cho SK, Edson JA, et al. Viral/nonviral chimeric nanoparticles to synergistically suppress leukemia proliferation via simultaneous gene transduction and silencing. *ACS Nano.* 2016;10(9):8705-8714.

38. Retnakumari AP, Hanumanthu PL, Malarvizhi GL, et al. Ration- 

39. Shi D, Liu Y, Xi R, et al. Caveolin-1 contributes to realgar nanoparticle therapy in human chronic myelogenous leukemia K562 cells. *Int J Nanomed.* 2016;11:5823-5835.

40. Ma W, Liu J, Xie J, et al. Modulating the growth and imatinib sensitivity of chronic myeloid leukemia stem/progenitor cells with pullulan/microRNA nanoparticles in vitro. *J Biomed Nanotechnol.* 2015;11(11):1961-1974.

41. Raimondo S, Naselli F, Fontana S, et al. Citrus limon-derived nanovesicles inhibit cancer cell proliferation and suppress CML xenograft growth by inducing TRAIL-mediated cell death. *Oncotarget.* 2015;6(23):19514-19527.

42. Palamã IE, Cortese B, D’Amone S, Arcadio V, Gigli G. Coupled delivery of imatinib mesylate and doxorubicin with nanoscaled polymeric vectors for a sustained downregulation of BCR-ABL in chronic myeloid leukemia. *Biomater Sci.* 2015;3(2):361-372.

43. Ryting ME, Wierda WG. Central nervous system relapse in two patients with chronic myelogenous leukemia in myeloid blastic phase on imatinib mesylate therapy. *Leuk Lymphoma.* 2004;45(8):1623-1626.

44. Rajappa S, Uppin SG, Raghunadharao D, Rao IS, Surath A. Isolated central nervous system blast crisis in chronic myeloid leukemia. *Hematol Oncol.* 2004;22(4):179-181.

45. Mendonça LS, Firmino F, Moreira JN, Pedroso de Lima MC, Simões S. Transferrin receptor-targeted liposomes encapsulating anti-BCR-ABL siRNA or asODN for chronic myeloid leukemia treatment. *Bioconjug Chem.* 2009;21(1):157-168.

46. Daniels TR, Delgado T, Helguera G, Penichet ML. The transferrin receptor part II: targeted delivery of therapeutic agents into cancer cells. *Clin Immunol.* 2006;121(2):159-176.

47. Li H, Qian ZM. Transferrin/transferrin receptor-mediated drug delivery. *Med Res Rev.* 2002;22(3):225-250.

48. Chiu SJ, Liu S, Perrotti D, Marcucci G, Lee RJ. Efficient delivery of a Bcl-2-specific antisense oligodeoxyribonucleotide (G3139) via transferrin receptor-targeted liposomes. *J Control Release.* 2006;112(2):199-207.

49. Visser CC, Stevanović S, Voorwinden LH, et al. Targeting lipo- 

50. Dykshoorn DM, Novina CD, Sharp PA. Kiling the messenger: short RNAs that silence gene expression. *Nat Rev Mol Cell Biol.* 2003;4(6):457.

51. Dykshoorn DM, Palliser D, Lieberman J. The silent treatment: siRNAs as small molecule drugs. *Gene Ther.* 2006;13(6):541.

52. Huang C, Li M, Chen C, Yao Q. Small interfering RNA therapy in cancer: mechanism, potential targets, and clinical applications. *Expert Opin Ther Targets.* 2008;12(5):637-645.

53. Kumar LD, Clarke AR. Gene manipulation through the use of small interfering RNA (siRNA): from in vitro to in vivo applications. *Adv Drug Deliv Rev.* 2007;59(2):87-100.

54. Kawakami S, Hashida M. Targeted delivery systems of small interfering RNA by systemic administration. *Drug Metab Pharmacokinet.* 2007;22(3):142-151.

55. Zhang C, Tang N, Liu X, Liang W, Xu W, Torchilin VP. siRNA- 

56. Rytting ME, Wierda WG. Central nervous system relapse in two patients with chronic myelogenous leukemia in myeloid blastic phase on imatinib mesylate therapy. *Leuk Lymphoma.* 2004;45(8):1623-1626.

57. Rajappa S, Uppin SG, Raghunadharao D, Rao IS, Surath A. Isolated central nervous system blast crisis in chronic myeloid leukemia. *Hematol Oncol.* 2004;22(4):179-181.

58. Mendonça LS, Firmino F, Moreira JN, Pedroso de Lima MC, Simões S. Transferrin receptor-targeted liposomes encapsulating anti-BCR-ABL siRNA or asODN for chronic myeloid leukemia treatment. *Bioconjug Chem.* 2009;21(1):157-168.

59. Daniels TR, Delgado T, Helguera G, Penichet ML. The transferrin receptor part II: targeted delivery of therapeutic agents into cancer cells. *Clin Immunol.* 2006;121(2):159-176.

60. Li H, Qian ZM. Transferrin/transferrin receptor-mediated drug delivery. *Med Res Rev.* 2002;22(3):225-250.

61. Chiu SJ, Liu S, Perrotti D, Marcucci G, Lee RJ. Efficient delivery of a Bcl-2-specific antisense oligodeoxyribonucleotide (G3139) via transferrin receptor-targeted liposomes. *J Control Release.* 2006;112(2):199-207.

62. Visser CC, Stevanović S, Voorwinden LH, et al. Targeting liposomes with protein drugs to the blood–brain barrier in vitro. *Eur J Pharm Sci.* 2005;25(2):299-305.

63. Dykshoorn DM, Novina CD, Sharp PA. Killing the messenger: short RNAs that silence gene expression. *Nat Rev Mol Cell Biol.* 2003;4(6):457.

64. Dykshoorn DM, Palliser D, Lieberman J. The silent treatment: siRNAs as small molecule drugs. *Gene Ther.* 2006;13(6):541.

65. Huang C, Li M, Chen C, Yao Q. Small interfering RNA therapy in cancer: mechanism, potential targets, and clinical applications. *Expert Opin Ther Targets.* 2008;12(5):637-645.

66. Kumar LD, Clarke AR. Gene manipulation through the use of small interfering RNA (siRNA): from in vitro to in vivo applications. *Adv Drug Deliv Rev.* 2007;59(2):87-100.

67. Kawakami S, Hashida M. Targeted delivery systems of small interfering RNA by systemic administration. *Drug Metab Pharmacokinet.* 2007;22(3):142-151.

68. Zhang C, Tang N, Liu X, Liang W, Xu W, Torchilin VP. siRNA-containing liposomes modified with polyarginine effectively silence the targeted gene. *J Control Release.* 2006;112(2):229-239.
56. Bala I, Harirhan S, Kumar MR. PLGA nanoparticles in drug delivery: the state of the art. *Crit Rev Ther Drug Carrier Syst*. 2004;21(5):387-422.

57. Misra R, Acharya S, Sahoo SK. Cancer nanotechnology: application of nanotechnology in cancer therapy. *Drug Discov Today*. 2010;15(19):842-850.

58. Acharya S, Dinawaz F, Sahoo SK. Targeted epidermal growth factor receptor nanoparticle bioconjugates for breast cancer therapy. *Biomaterials*. 2009;30(29):5737-5750.

59. Misra R, Sahoo SK. Intracellular trafficking of nuclear localization signal conjugated nanoparticles for cancer therapy. *Eur J Pharm Sci*. 2010;39(1):152-163.

60. Sahoo SK, Labhasetwar V. Enhanced antiproliferative activity of transferrin-conjugated paclitaxel-loaded nanoparticles is mediated via sustained intracellular drug retention. *Mol Pharm*. 2005;2(5):373-383.

61. Gal D, Ohashi M, MacDonald PC, Buchsbaum HJ, Simpson ER. Low-density lipoprotein as a potential vehicle for chemotherapeutic agents and radiolabeled drugs in the management of gynecologic neoplasms. *Am J Obstet Gynecol*. 1981;139(8):877-885.

62. Zhou P, Hatziieremia S, Elliott MA, et al. Uptake of synthetic low density lipoprotein by leukemic stem cells—a potential stem cell targeted drug delivery strategy. *J Control Release*. 2010;148(3):380-387.

63. Ghiault VS, Pahwa MB, Ghiault PS. Alteration in lipid profile in patients of chronic myeloid leukemia before and after chemotherapy. *Clin Chim Acta*. 2006;366(1):239-242.

64. Jia F, Liu X, Li L, Mallapragad S, Narasimha B, Wan Q. Multifunctional nanoparticles for targeted delivery of immune activating and cancer therapeutic agents. *J Control Release*. 2013;172(3):1020-1034.

65. Baccarani M, Rosti G, de Vivo A, et al. A randomized study of interferon-α versus interferon-α and low-dose arabinosyl cytosine in chronic myeloid leukemia. *Blood*. 2002;99(5):1527-1535.

66. O’Brien SG, Guilhot F, Larson RA, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med*. 2003;348(2):994-1004.

67. Giles FJ, Shan J, Chen S, et al. A prospective randomized study of alpha-2b interferon plus hydroxyurea or cytarabine for patients with early chronic phase chronic myelogenous leukaemia: the International Oncology Study Group CML1 study. *Leuk Lymph*. 2003;50(3):168-174.

68. Küh R, Burgstaller S, Apfelbeck U, et al. A randomized study comparing interferon (IFNα) plus low-dose cytarabine and interferon plus hydroxyurea (HU) in early chronic-phase chronic myeloid leukemia (CML). *Leuk Res*. 2003;27(5):405-411.

69. Lipton JH, Khoroshko N, Golenkov A, et al. Phase II, randomized, multicenter, comparative study of peginterferon-α-2a (40 kD) (Pegasys®) versus interferon α-2a (Roferon®-A) in patients with treatment-naïve, chronic-phase chronic myelogenous leukemia. *Leuk Lymph*. 2007;48(3):497-505.

70. Nimesh S, Chandra R. Polyethylenimine nanoparticles as an efficient in vitro siRNA delivery system. *Eur J Pharm Biopharm*. 2009;73(1):43-49.

71. Lungwitz U, Breunig M, Blunk T, Göpferich A. Polyethylenimine-based non-viral gene delivery systems. *Eur J Pharm Biopharm*. 2005;60(2):247-266.

72. Urban-Klein B, Werth S, Abuharbeid S, Czubayko F, Aigner A. RNAi-mediated gene-targeting through systemic application of polyethylenimine (PEI)-complexed siRNA in vivo. *Gene Ther*. 2005;12(5):461.

73. Read ML, Singh S, Ahmed Z, et al. A versatile reducible polycation-based system for efficient delivery of a broad range of nucleic acids. *Nucleic Acids Res*. 2005;33(9):e86-e86.

74. Hassani Z, Lemkine GF, Erbacher P, et al. Lipid-mediated siRNA delivery down-regulates exogenous gene expression in the mouse brain at picomolar levels. *J Gene Med*. 2005;7(2):198-207.

75. Thomas M, Lu J, Ge Q, Zhang C, Chen J, Klibanov AM. Full decylation of polyethylenimine dramatically boosts its gene delivery efficiency and specificity to mouse lung. *Proc Natl Acad Sci U S A*. 2005;102(16):5679-5684.

76. Merdan T, Kunath K, Fischer D, Kopecek J, Kissel T. Intracellular processing of poly (ethylene imine)/ribozyme complexes can be observed in living cells by using confocal laser scanning microscopy and inhibitor experiments. *Pharm Res*. 2002;19(2):140-146.

77. Aigner A, Fischer D, Merdan T, Brus C, Kissel T, Czubayko F. Delivery of unmodified bioactive ribozymes by an RNA-stabilizing polyethylenimine (LMW-PEI) efficiently down-regulates gene expression. *Gene Ther*. 2002;9(24):1700.

78. Dheur S, Dias N, van Aerschot A, et al. Polyethylenimine but not cationic lipids improve antisense activity of 3’-capped phosphodiester oligonucleotides. *Antisense Nucleic Acid Drug Dev*. 1999;9(6):515-525.

79. Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. *Nat Rev Drug Discov*. 2009;8(2):129-138.

80. Biray Avci Ç, Özcan I, Balci T, Özer Ö, Gündüz C. Design of polyethylen glycol-polyethyleneimine nanocomplexes as non-viral carriers: miR-150 delivery to chronic myeloid leukemia cells. *Cell Biol Int*. 2013;37(11):1205-1214.

81. Machová Poláková K, Lopotová T, Klamová H, et al. Expression patterns of microRNAs associated with CML phases and their disease related targets. *Mol Cancer*. 2011;10:41.

82. Watanabe A, Tagawa H, Yamashita J, et al. The role of microRNA-150 as a tumor suppressor in malignant lymphoma. *Leukemia*. 2011;25(8):1324-1334.

83. Abbasi E, Aval SF, Akbarzadeh A, et al. Dendrimers: synthesis, applications, and properties. *Nanoscale Res Lett*. 2014;9(1):247.

84. Caminade AM, Majoral JP. Water-soluble phosphorus-containing dendrimers. *Prog Polym Sci*. 2005;30(3-4):491-505.

85. Solassol J, Crozet C, Perrier V, et al. Cationic phosphorus-containing dendrimers reduce prion replication both in cell culture and in mice infected with scrapie. *J Gen Virol*. 2004;85(6):1791-1799.

86. Maksimenko AV, Mandrouguine V, Gottikh MB, Bertrand JR, Majoral JP, Malvy C. Optimisation of dendrimer-mediated gene transfer by anionic oligomers. *J Gene Med*. 2003;5(1):61-71.

87. Ferenc M, Pedzwiat-Werbicka E, Nowak KE, Klajnert B, Majoral JP, Byszewska M. Phosphorus dendrimers as carriers
of siRNA—characterisation of dendriplexes. *Molecules*. 2013;18(4):4451-4466.

88. Gschwind A, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: targets for cancer therapy. *Nat Rev Cancer*. 2004;4(5):361-370.

89. Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. *N Engl J Med*. 2005;353(2):172-187.

90. O’Hare T, Deininger MW, Eide CA, Clackson T, Druker BJ. Targeting the BCR-ABL signaling pathway in therapy-resistant Philadelphia chromosome-positive leukemia. *Clin Cancer Res*. 2011;17(2):212-221.

91. Li Q, Wu Y, Fang S, et al. BCR/ABL oncogene-induced PI3K signaling pathway leads to chronic myeloid leukemia pathogenesis by impairing immuno-modulatory function of hemangioblasts. *Cancer Gene Ther*. 2015;22(5):227-237.

92. Koldehoff M, Zakrzewski JL, Beelen DW, Elmaagacli AH. Additive antileukemia effects by GF11B-and BCR-ABL-specific siRNA in advanced phase chronic myeloid leukemia cells. *Cancer Gene Ther*. 2013;20(7):421-427.

93. Kuribara R, Honda H, Matsui H, et al. Roles of Bim in apoptosis of normal and Bcr-Abl-expressing hematopoietic progenitors. *Mol Cell Biol*. 2004;24(14):6172-6183.

94. Essafi A, de Mattos SF, Hassen YA, et al. Direct transcriptional regulation of Bim by FoxO3a mediates STI571-induced apoptosis in Bcr-Abl-expressing cells. *Oncogene*. 2005;24(14):2317-2329.

95. Koss B, Morrison J, Perciavalle RM, et al. Requirement for antiapoptotic MCL-1 in the survival of BCR-ABL B-lineage acute lymphoblastic leukemia. *Blood*. 2013;122(9):1587-1598.

96. Perciavalle RM, Stewart DP, Koss B, et al. Anti-apoptotic MCL-1 localizes to the mitochondrial matrix and couples mitochondrial fusion to respiration. *Nat Cell Biol*. 2012;14(6):575-583.

97. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science*. 2012;304(5670):554-554.

98. Brose MS, Volpe P, Feldman M, et al. BRAF and RAS mutations in human lung cancer and melanoma. *Cancer Res*. 2002;62(23):6997-7000.

99. An X, Tiwari AK, Sun Y, Ding PR, Ashby CR, Jr, Chen ZS. BCR-ABL tyrosine kinase inhibitors in the treatment of Philadelphia chromosome positive chronic myeloid leukemia: a review. *Leuk Res*. 2010;34(10):1255-1268.

100. Weisberg E, Griffin JD. Mechanism of resistance to the ABL kinase domain mutations, drug resistance, and the road to a cure for chronic myeloid leukemia. *Blood*. 2007;110(7):2242-2249.

101. Gruber FX, Ernst T, Porkka K, et al. Dynamics of the emergence of dasatinib and nilotinib resistance in imatinib-resistant CML patients. *Leukemia*. 2012;26(1):172-177.

102. Kindtler T, Breitenbuecher F, Kasper S, et al. In BCR-ABL-positive cells, STAT-5 tyrosine-phosphorylation integrates signals induced by imatinib mesylate and Ara-C. *Leukemia*. 2003;17(6):999-1009.

103. Kindler T, Breitenbuecher F, Kasper S, et al. In BCR-ABL-positive cells, STAT-5 tyrosine-phosphorylation integrates signals induced by imatinib mesylate and Ara-C. *Leukemia*. 2003;17(6):999-1009.

104. Nyman DW, Campbell KJ, Hersh E, et al. Phase I and pharmacokinetics trial of ABI-007, a novel nanoparticle formulation of paclitaxel in patients with advanced nonhematologic malignancies. *J Clin Oncol*. 2005;23(31):7785-7793.

105. Kratz F. Albumin as a drug carrier: design of produgs, drug conjugates and nanoparticles. *J Control Release*. 2008;123(3):171-183.

106. Zhao GX, Pan H, Ouyang DY, He XH. The critical molecular interconnections in regulating apoptosis and autophagy. *Ann Med*. 2015;47(4):305-315.

107. Wang X, Zhang X, Xu Z, Wang Z, Yue X, Li H. Reversal effect of arsenic sensitivity in human leukemia cell line K562 and K562/ADM using realgar transforming solution. *Biol Pharm Bull*. 2013;36(4):641-648.

108. Tian Y, Wang X, Xi R, et al. Enhanced antitumor activity of realgar mediated by milling it to nanosize. *Int J Nanomed*. 2014;9:745-757.

109. Quest AF, Lobos-González L, Nunez S, et al. The caveolin-1 connection to cell death and survival. *Curr Mol Med*. 2013;13(2):266-281.

110. Liu Y, Shi D, Xi R, et al. Cav-1 knockdown enhances autophagy induced by realgar nanoparticles in a hepatocellular carcinoma SMCC-7721 cell line. *J Comput Theor Nanos*. 2016;13(8):5262-5268.

111. Fei J, Li Y, Zhu X, Luo X. miR-181a post-transcriptionally downregulates oncogenic RalA and contributes to growth inhibition and apoptosis in chronic myelogenous leukemia (CML). *PLoS One*. 2012;7(3):e32834.

112. Wang L, Wang J, Fang L, et al. Anticancer activities of citrus peel polymethoxyflavones related to angiogenesis and others. *Biomed Res Int*. 2014;2014:453972.

113. Manthey JA, Guthrie N, Grohmann K. Biological properties of citrus flavonoids pertaining to cancer and inflammation. *Curr Med Chem*. 2001;8(2):135-153.

114. Benavente-Garcia O, Castillo J. Update on uses and properties of citrus flavonoids pertaining to cancer and inflammation. *Curr Med Chem*. 2001;8(2):135-153.

115. Blagosklonny MV. Overcoming limitations of natural anticancer drugs by combining with artificial agents. *Trends Pharmacol Sci*. 2005;26(2):77-81.

116. Raimondo S, Naselli F, Fontana S, et al. Citrus limon-derived nanovesicles inhibit cancer cell proliferation and suppress CML xenograft growth by inducing TRAIL-mediated cell death. *Oncotarget*. 2015;6(23):19514-19527.

117. Nesterov A, Nikrad M, Johnson T, Kraft AS. Oncogenic Ras sensitizes normal human cells to tumor necrosis factor-α-related apoptosis-inducing ligand-induced apoptosis. *Cancer Res*. 2004;64(11):3922-3927.

118. Regente M, Corti-Monzón G, Maldonado AM, Pinedo M, Jorrín J, de la Canal L. Vesicular fractions of sunflower apoplastic fluids are associated with potential exosome marker proteins. *FEBS Lett*. 2009;583(20):3363-3366.

119. Minotti G, Mennà P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol Rev*. 2004;56(2):185-229.