

Inorganic Nanoparticles as Drug Delivery Systems and Their Potential Role in the Treatment of Chronic Myelogenous Leukaemia

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Abstract

Chronic myeloid leukemia is a myeloproliferative disease where cells of myeloid lineage display a t(9;22) chromosomal translocation leading to the formation of the BCR/ABL fusion gene and the continuous activation of tyrosine kinases. This malignancy has a peak incidence at 45 to 85 years, accounting for 15% of all leukemias in adults. Controlling the activity of tyrosine kinase became the main strategy in chronic myeloid leukemia treatment, with imatinib being placed at the forefront of current treatment protocols. New approaches in future anticancer therapy are emerging with nanomedicine being gradually implemented. Setting through a thorough survey of published literature, this review discusses the use of inorganic nanoparticles in chronic myeloid leukemia therapy. After an introduction on the basics of chronic myeloid leukemia, a description of the current treatment modalities of chronic myeloid leukemia and drug-resistance mechanisms is presented. This is followed by a general view on the applications of nanostrategies in medicine and then a detailed breakdown of inorganic nanocarriers and their uses in chronic myeloid leukemia treatment.

Keywords

myeloid, leukemia, inorganic, nanoparticles, drug resistance

Abbreviations

Ap-SWNTs, antibody oxidized single-walled carbon nanotubes; BrTet, 5-bromotetrandrine; CCyR, complete cytogenetic response; CDK, cyclin-dependent kinase; CLSM, confocal laser scanning microscopy; CML, chronic myeloid leukemia; CMR, complete molecular response; CNTs, carbon nanotubes; CTAB, cetyltrimethylammonium bromide; DNA, deoxyribonucleic acid; DNR, daunomycin; ER, endoplasmic reticulum; f-SWNTs, ammonium functionalized carbon nanotubes; FACS, fluorescence-activated cell sorting; FDA, Food and Drug Administration; HSA-SWNTs, human serum albumin single-walled carbon nanotubes; MDR, multidrug resistance; MNPs, magnetic nanoparticles; mRNA, messenger RNA; NIR, near infrared radiation; NPs, nanoparticles; o-SWNTs, oxidized single-walled carbon nanotubes; P-gp, permeability glycoprotein; Ph, Philadelphia chromosome; RNA, ribonucleic acid; RNAi, RNA interference; ROS, reactive oxygen species; siRNA, small interfering ribonucleic acid; SWNTs, single-walled carbon nanotubes; TKIs, tyrosine kinase inhibitors; TNF, tumor necrosis factor; UV, ultraviolet.

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Introduction and Clinical Features

Chronic myeloid leukaemia (CML) is a hematopoietic malignancy characterized by neoplastic proliferation of mature myeloid cells, especially granulocytes and their precursors. Chronic myeloid leukaemia results from genetic abnormalities marked by the presence of the Philadelphia chromosome (Ph),^{1,2} which is the product of a reciprocal translocation of Abl gene (chromosome 9, long arm) to BCR (chromosome 22, long arm), that is, t(9;22)(q34;q11.2). This fusion results in a constitutive tyrosine kinase activity that leads to the phosphorylation and activation of various downstream proteins promoting cellular proliferation while inhibiting apoptosis.³

Chronic myeloid leukaemia accounts for 15% of leukemias in adults with an incidence of 1/100 000,⁴ and a median age of diagnosis of 60 to 65 years.⁵ Bone marrow transplantation and chemotherapeutics such as tyrosine kinase inhibitors (TKIs) are the prevalent approaches for CML treatment.⁶ Cure from CML is unattainable via TKI monotherapy as it remains a clinical challenge especially in the advanced cases. The only potential curative approach is allogenic bone marrow transplantation. This statement remains true especially with CML cells developing multidrug resistance (MDR) properties, thus decreasing the efficacy of certain TKIs such as imatinib.⁶ The potential development of cancer and drug resistance are the main disadvantages of chemotherapy.⁷ As per the literature, the cytogenetic response achieved with TKI monotherapy is seen in only 12% of the cases, whereas the hematological response achieved with TKI monotherapy is seen in 50% of the cases. Moreover, the median range of survival ranges between 6 months and 11.8 months.^{8,9} New research indicates that means to overcome such disadvantages and lack of complete cure from CML lies in the combination of new innovative agents with current treatments. Nanotechnology has shown significant potential as a new approach in the treatment of CML.¹⁰

Nanoparticles (NPs) are considered as 2- or 3-dimensional particles measuring anywhere between 1 and 100 nm.^{11,12} Their usefulness has seen their popularity surge in recent times to be utilized in a wide variety of fields. They have begun to be applied in the management of malignancies^{13,14} and have been found to be useful in medical imaging. Recent discoveries show the use of NPs as antimicrobial agents and more importantly as targeted drug delivery systems.^{11,15} Nanoparticles exhibit revolutionary advantages in the medical field. However, more studies should be conducted to assess for potential toxicity on the human body and adverse effects on the environment. For example, some studies showed that certain NPs such as ZnO NPs may lead to oxidative stress and DNA damage.^{16,17}

This review aims to compile and discuss current advances in nanomedicine in which NPs are used as a drug-delivery system in the treatment of CML. Both the chemical properties and the clinical applications of NPs are detailed in this study.

Basics of Chronic Myeloid Leukemia

Molecular Changes

Chronic myeloid leukaemia is a myeloproliferative malignancy affecting hematopoietic stem cells and is caused by a hyperactive tyrosine kinase activating antiapoptotic and cellular proliferation proteins (such as ERKs and PKB/pAkt) via phosphorylation. Some of the antiapoptotic effects are due to inhibition of the p38 and JNK pathways as well as mutations in p53 and Apaf-1 and an induced deficit in FASR.¹⁸⁻²⁰ The cellular proliferation proteins activate pathways involving RAS, MEK, Erk, Stat5, and PI3K/AKT.²¹ The constitutive tyrosine kinase activity is most commonly due to a 9;22 chromosome reciprocal transformation leading to the fusion of BCR and ABL (BCR/ABL fusion gene); the product of which is the p210bcr-abl fusion protein.¹⁹

Clinical Features

As much as 50% of patients with CML are asymptomatic and are diagnosed incidentally after routine check-ups.²² Even in symptomatic cases, symptoms are not always specific. Around 50% to 75% of patients complain of left upper quadrant pain owing to splenomegaly. Patients may also experience early satiety, display symptoms of anemia, and night sweats. Other symptoms may include bleeding (due to platelet dysfunction) and thrombocytosis.^{22,23} A minority of patients (5%) may present with hyperviscosity symptoms due to the high degree of leukocytosis.¹

Chronic myeloid leukaemia progression can be broken down into 3 different stages depending on the following factors: chronic (the most common at presentation), accelerated, and blast.²⁴

Conventional Methods in CML Treatment: Advantages and Drawbacks

As hematological malignancies are not treatable by radiotherapy or surgery, chemotherapy is the main approach in the management of leukemias. In patients with CML, treatment depends on many factors including phase of the disease and availability of stem cell donor.

Tyrosine kinase inhibitors such as Imatinib (Gleevec), Dasatinib (Sprycel), and Nilotinib (Tasigna) are considered the standard of CML treatment. Drug dosage increase can be considered throughout treatment; however, higher doses impose greater toxicity. Generally, the treatment starts with Imatinib and it can be replaced by other TKIs. Ponatinib, for example, is used in cases of T315I mutation development in the leukemic cells.^{25,26} If these drugs fail or ponatinib appears not to be tolerated, a trial of chemotherapy or interferon can be initiated. Omacetaxine has been used with varying degrees of success in T315I mutation cases nonresponsive to standard therapy. Finally, stem cell transplantation is especially helpful for patients who have been successful in finding a suitable donor and remains the only treatment to cure CML.^{27,28}

Within 1 year of chronic-phase CML treatment with imatinib, up to 70% of patients showed complete cytogenetic response (CCyR) and even higher response rate with the newer TKIs. Moreover, complete molecular response (CMR) was observed after a year in many patients. Current recommendations are to continue TKI therapy indefinitely due to the ability of the BCR-ABL translocation and CML cells to recur, as seen in half of the treated patients. Unfortunately, the prognosis of CML is worse in both the accelerated phase and the blast phase of the disease with fewer successful pharmaceutical treatments and transplants.^{27,28}

As previously stated, Imatinib (on account of its superior anti-leukemic effects) is currently mainstay therapy used in CML treatment.²⁹ However, Imatinib resistance has been described and has been associated with BCR/ABL mutations in most cases.^{30,31} Resistance has also been associated with BCR/ABL amplification, high drug efflux from leukemic cells, BCR/ABL mRNA overexpression, T315I mutation, or other cytogenetic abnormalities.^{31,32} Furthermore, leukemic stem cells in CML are imatinib-resistant by the way of quiescence and/or higher expression of drug-efflux-related proteins. Therefore, a major problem of TKIs is the development of drug tolerance which is observed in roughly 30% of patients.³² Although TKIs are the most successful pharmaceutical treatment for CML, complete cure from CML cannot be achieved without allogeneic transplantation.⁶ Drug resistance and cancer relapse are major disadvantages being faced with current treatments.⁷ Therefore, employing new agents in combination with existing treatments is of critical importance to improve cancer cells' response to drugs, to prevent later relapse, and to successfully cure CML without the need for transplantation. The need to overcome current drug drawbacks has pushed researchers to develop new treatment strategies with nanotechnology emerging as a possible new approach in the management of CML.¹⁰

Multidrug Resistance

One of the major reasons of the failure of chemotherapy and cancer relapse in hematological malignancies is MDR in which cancerous cells develop resistance against the cytotoxic effects of chemotherapeutic drugs via various complex mechanisms of action. Despite drug resistance being initiated by a certain type of drugs, drug resistance is not drug specific.³³ The MDR is attained through mechanisms of downregulation of the production of apoptosis-related proteins such as Bax, caspase-3, and Bcl-2,³⁴ drug excretion, recovery from drug-induced DNA damage, and changes in the activity of enzymes functioning in drug metabolism.³⁵ Nevertheless, the most important mechanism of MDR is drug excretion via permeability glycoprotein (P-gp),³⁶ which is a transmembrane efflux pump, encoded by *Mdr1* gene functioning to decrease intracellular drug concentrations by actively transporting the drug outside the cells.^{37,38} The overexpression of *Mdr1* gene leads to excessive production of P-gp, increasing cell resistance to chemotherapeutic drugs; thus, increasing the chance of cancer recurrence and worsening the prognosis.³⁹ The overexpression

of P-gp was initially found in tumor stem cells and residual cells remaining after chemotherapy administration.⁴⁰⁻⁴² Understanding the mechanism of P-gp induced MDR has pushed researchers to develop P-gp inhibitors providing a strategy that can improve the treatment of myelodysplastic syndrome with P-gp mediated MDR.¹ In addition, alteration in the expression of Bcl2 is also associated with MDR and tumor development.³⁴ Many chemotherapeutic drugs target intrinsic mitochondrial apoptosis pathway, also known as cytochrome c/Apaf-1/caspase-9 pathway. Excessive activation of Bcl2 in cancerous cells causes arrest in the mitochondrial pathway by inhibiting Bax, which eventually prevents activation of Caspase 3 essential for the final steps of apoptosis.^{43,44} It has been proven that, in hematologic malignancies, there is an increase in Bcl2 and Bax expression to up to 21%.⁴⁵

As previously mentioned, in certain CML patients undergoing treatment with TKIs, the cancer cells develop resistance against TKIs via T315I mutation. Moreover, Ponatinib, the only effective TKI drug against this mutation, is the treatment of choice in such patients despite its many side effects.

With an ever-increasing rate of resistance to treatment, new therapeutic targeting methods must be developed. Nanomedicine emerges as a promising therapeutic approach of targeted drug delivery to overcome drug resistance.

Nanomedicine in CML Treatment

Targeted drug delivery provides an efficient approach for the specific delivery of the chemotherapeutic drug(s) to cancer cells sparing normal cells from their cytotoxic effect.^{46,47} While designing a drug delivery system, several measurements must be taken into consideration to ensure success. The drug delivery system must ensure an efficient delivery of the drug to the targeted cells while preserving the drug's molecular bioactivity. Moreover, the kinetics of the drug loading and drug release should be controlled to achieve a desirable and adequate loading and release of the drug.^{48,49} Nanoparticles present several advantages when used as drug delivery systems. For example, drug delivery via NPs improves the water solubility of the drug in cancer cells. Moreover, drug delivery by NPs enhances the intracellular uptake of the drug and aids in preserving its metabolic stability. The circulation time of the drug is also enhanced. The NPs allow targeted drug delivery (by both passive and active strategies), thus sparing the cytotoxic effect of the drug to the normal cells.^{50,51} In short, when loaded into nanocarriers, chemotherapeutic drugs can escape degradation while displaying lower toxicity, superior efficacy, and solubility.^{52,53}

However, using NPs requires a deep understanding of various parameters that determine their function inside a living entity such as a cell or the entire human body. The toxicity, uptake, and half-life of NPs depends on both intrinsic factors such as surface charge, particle size, and shape like zeta potential, and surface area and extrinsic factors such as the activity of the reticuloendothelial system and renal clearance.^{54,55} For example, the shape and size of NPs were shown to affect their

cytotoxicity as well as optical, catalytic, and electromagnetic characteristics.¹ The NPs with less than three facets such as spherical NPs are less reactive than truncated triangular nanoplates.⁵⁶ On the other hand, the half-life of NPs is highly dependent on their interaction with the reticuloendothelial system, especially macrophages.⁵⁷ The ability of the reticuloendothelial system to clear NPs depends on the absorption of opsonins by NPs, activating macrophages that are the main leukocytes responsible for NP elimination. Reducing NP clearance by macrophages could be achieved by coating NPs with a hydrophilic layer, using polymers such as polyethylene glycol through a process called PEGylation.^{58,59} Other advantages of PEGylated NPs are the low toxicity demonstrated in both *in vivo* and *in vitro* studies^{58,59} and the ability to decrease renal drug clearance.^{60,61} Finally, when NPs reach the targeted tissues, endocytosis is the main mechanism by which these hydrophilic NPs are transported into cells. This active transport mechanism consists of engulfing molecules in incised cytoplasmic membrane-derived vesicles, thus absorbing these molecules into the interior of cells.⁶²

Classification of Inorganic NPs

According to RSC Advances by Aula *et al* (2015),⁶³ NPs can be divided into organic and inorganic. In this review, inorganic NPs will be discussed and categorized as follows:

- Carbon nanotubes (CNTs)
- Noble metal NPs
- Silver-based NPs
- Gold-based NPs
- Magnetic NPs (Fe₃O₄ NPs)
- ZnO NPs
- Copper oxide NPs (CuO NPs)

In contrast to the inorganic NPs, lipid nanocapsules and polymer NPs are widely studied, and have outstanding advantages in biocompatibility, but possess major drawbacks such as instability and a low-loading capacity. So far, only 6 types of inorganic NPs including ZnO,⁶⁴ copper, gold,⁶⁵ silver and Fe₃O₄ NPs,⁶² and CNTs have been studied as possible drug delivery systems for CML.

Inorganic NPs for CML Treatment

Carbon Nanotubes (CNTs)

Carbon nanotubes are hollow tubes formed by rolling carbon polymer sheets that can cross cellular membrane without generally inflicting cellular injury.^{66,67} Although CNTs are generally considered nontoxic and biocompatible,^{66,68} using CNTs without surface modification could be cytotoxic to cells and it has been shown that residual heavy metals in CNTs induce cellular cytotoxicity.^{12,69} The CNT toxicity remains the most concern for their use in the clinical setting. However, studies appearing in the literature related to the toxicology of CNTs presented confusing results. Some studies claimed that CNTs

are responsible for both acute and chronic toxicity while some studies showed insignificant toxicity, should reaction condition be optimal.⁷⁰ Functionalized CNTs with no residual heavy metals, especially single-walled carbon nanotubes (SWNTs), are considered safe at the cellular level with remarkable biocompatibility.^{71,72} The biocompatibility of functionalized SWNTs, their ability to be used as vectors, and the ease of CNT endocytosis make them useful as delivery vehicles for various biomolecules including RNA,^{73,74} proteins,^{67,75} DNA,^{75,76} and siRNA. Additionally, RNA and DNA could be adsorbed as double or single strands while binding noncovalently to SWNT surfaces.⁷⁷ An important characteristic of CNTs is that drugs such as doxorubicin could be carried by CNTs through physical adsorption without being covalently bound, thus avoiding chemical interactions between CNTs and the drug.⁷⁸ SNX-2112 is a promising chemotherapeutic agent with potential use in various types of cancer since it is a Hsp90 inhibitor. However, SNX-2112 is both hydrophobic and lipophobic, which limits its use in clinical settings. Zheng *et al* (2016) added chitosan (CHI) noncovalently to SWNTs to increase their biocompatibility. The CHI-SWNTs were then used as delivery system for SNX-2112 delivery to the K562 cells. The results showed significant inhibition of the K562 cells and the abundant expression of apoptosis-related proteins.⁷⁹ Since CNTs could absorb near-infrared radiations (NIR) and laser effectively, exposing CNTs based nanocarriers to NIR at the level of the targeted cells improves drug release.^{80,81} The large aspect ratio of CNTs compared to other drug delivery systems, allows CNTs to have more carrying capacity and more efficient transfer across phospholipid cellular membranes. This was demonstrated by comparing the transfer of siRNA using CNTs to that using liposomes.^{82,83} Moreover, the condensation of nucleic acids and their delivery across the cellular membrane and into mammalian cells was achieved and showed to be effective using CNTs bound to ammonium as the functional group.^{84,85}

Li *et al* (2010) used P-glycoprotein antibody functionalized CNTs in an attempt to overcome MDR CML.⁸⁶ This study investigated the specificity and cytotoxicity of P-gp antibody oxidized single-walled carbon nanotubes (Ap-SWNTs) loaded with Dox to MDR K562R CML cells. First, the experiment showed 458 times higher expression of P-gp in K562R compared to K562 sensitive (K562S) cells. The overexpression of P-gp on leukemic cellular membranes was considered to infer the specificity of the antibody Ap-SWNTs to MDR cells. This was showed by the increased binding affinity of Ap-SWNTs to K562R where the affinity of Ap-SWNTs to K562R was 23-folds higher than with K562S. Additionally, by physical adsorption, Dox was loaded on the Ap-SWNTs. This makes it possible to release the drug at the level of targeted cells using near infrared radiation (NIR) thus increasing drug specificity and drug release capacity while preserving molecular integrity by minimizing reaction between doxorubicin and CNTs. The high surface to volume ratio of SWNTs and the peripheral oxidation of the CNTs increase Dox loading capacity thus better cytotoxic effect even after antibody coupling. The increase

in DOX delivery via Ap-SWNTs into K562R cells compared to free Dox, Dox/human serum albumin-SWNTs, was confirmed using confocal laser scanning microscopy (CLSM). Regarding cytotoxicity, Dox/Ap-SWNTs composite demonstrates the superior cytotoxicity compared to Dox with o-SWNTs (oxidized SWNTs), Ap-SWNTs, HSA-SWNTs, free Dox and Dox/anti-Pgp, with free Dox and Dox/anti-Pgp showing minimal cytotoxicity, where the K562R cell showed increase survival and proliferation with increased incubation time. This increased cytotoxicity using Dox/Ap-SWNTs could be due to the targeted delivery of Dox and due to difficulty of efflux of Ap-SWNTs outside the MDR cells caused by the relatively large size of nanotubes compared to DOX and the steric hindrance of the P-gp produced by the A-SWNTs reducing DOX efflux. Wu *et al* (2014) showed similar results regarding the efficacy and versatility of CNTs for the delivery of chemotherapeutic agents to malignant cells. They first optimized the length of the CNTs by ultrasonication and oxidative acid treatment. Verapamil and Dox were then co-loaded into the CNTs for their delivery into MDR CML cells K562/A02 in which verapamil was the reversal agent for real-time inhibition of P-gp. Their results showed that there was enhanced uptake of DOX, apoptosis induction, and increased sensitivity to the drug.⁸⁷

Yet another approach by Wang *et al* (2008) used targeted RNAi of cyclin A2 mediated by functionalized SWNTs to arrest proliferation and induce apoptosis in CML K562 Cells.⁸⁸ Here, cells were targeted using ammonium functionalized CNTs (f-SWNTs) vectors carrying siRNA *in vitro*. The siRNA was used to modulate the genes expression of cancer-related genes such as cyclin A2 and CDK. The siRNA with specificity to cyclin A2 was used to suppress the overexpression of cyclin A2 thus arresting cellular G1–S phase and G2–M transitions by decreasing the levels of formation of cyclin–CDK complex leading to increased intracellular E2F activity and eventually arrest in proliferation and apoptosis. The experiment showed reduced expression of cyclin a2 RNA and protein, with no detectable toxicity induced by the f-SWNTs. The siRNA showed specificity to CML cells that were attributed to an increased sensitivity of rapidly proliferating cells to siRNA compared to normal dividing cells that could be caused by differences in cellular membrane.

Silver Nanoparticles

The applications of silver NPs (AgNPs) are broad and diverse. The antimicrobial properties of AgNPs make them suitable for usage as antiseptics in various manufacturing and consumer products in addition to their usage in the biomedical field.^{89,90} Although the cytotoxicity of AgNPs might limit their use,^{91,92} AgNPs show potential uses in the treatment and management of various diseases including ocular neovascular diseases, MDR tumors, and immunological and inflammatory diseases.^{93,94} In the treatment of cancers, AgNPs show cytotoxic effects against a number of leukemia cell lines including Jurkat cells and THP-1.^{95,96} How AgNPs affects cell cycle and

apoptotic pathways varies with AgNPs concentration and the targeted cell types.^{15,95} Possible mechanisms of AgNP cellular uptake include endocytosis, micropinocytosis, or phagocytosis.⁹⁷

Guo *et al* (2008) investigated the cellular uptake and cytotoxic effects of AgNPs on CML.⁶⁴ The uptake of AgNPs was shown to increase in a dose-dependent manner, which was demonstrated using dark field microscopy and further confirmed by AAS [Please provide expansion for AAS.] analysis. When coated with PVP [Please provide expansion for PVP.], inhibitors of endocytosis, their uptake was prevented demonstrating the role of receptor-mediated endocytosis in AgNPs uptake.⁹⁸ This study and another more recent study conducted by Guo *et al* demonstrated that exposure to AgNPs causes the generation of reactive oxygen species (ROS) causing oxidative stress-induced apoptosis and cellular arrest at the S-phase of the cell cycle. The initial addition of AgNPs displayed a dose-dependent increase in ROS, and the number of cells at the S phase of cell cycle. The addition of vitamin C (an antioxidant) to K562 cells subjected to AgNPs reversed cellular cytotoxicity, reduced the apoptosis induced by AgNPs, and increased expression of Cyclin A and CDK2 genes expression, which were initially inhibited by AgNPs.^{64,99}

Cytotoxic effects of AgNPs were also evaluated and shown to induce cell death in a dose-dependent manner as flow cytometry analysis suggested significant cell shrinkage at the respective high AgNPs concentrations by the decrease of forward scatter's mean intensity of the treated k562 cells compared to untreated cells.

Interestingly, the effect of AgNPs on CML cells isolated from 4 patients was evaluated. AgNPs showed overall suppression of the viability of the isolated CML cells, but the samples showed variable sensitivity to AgNPs treatment in contrast to the normal cells isolated (human bone marrow mononuclear cells and human cord blood mononuclear cells). Compared to normal cells, CML cells isolated from 3 of the patients showed sensitivity to AgNPs at concentrations lower than that of normal cells (from 1.25 to 5 $\mu\text{g/ml}$), which indicates that some patients are more sensitive to AgNPs treatments than others, and that AgNPs exhibit increased cytotoxicity against CML cells compared to normal cells. The data obtained suggested that AgNPs could provide a novel opportunity for the treatment of CML.⁹⁸

Gold Nanoparticles

Gold NPs (AuNPs; approved by the FDA) are used in various biomedical applications due to their good biocompatibility, small size, low toxicity, easy surface modification, and controlled drug release.^{100,101} Historically, gold-based chemicals were used for various therapeutic purposes^{102,103} and now gold NPs are being explored for application in cancer treatment^{104,105} where they have demonstrated effectiveness in reducing tumor necrosis factor (TNF)-mediated toxicity in antitumor treatment.¹⁰⁶ They have also showed potential applications in photothermal cancer therapy.^{107,108} The cytotoxicity

of AuNPs is attributed to surface-modified ligands and particle size. That is, larger AuNPs have lower cytotoxicity, and AuNPs conjugated with, for example, PEG, cysteine, citrate, biotin, and glucose, are less toxic to cells than those linked to cationic ligands, such as cetyltrimethylammonium bromide (CTAB).^{12,109} Nanoparticles such as AuNPs are rapidly eliminated by the reticuloendothelial system, particularly macrophages, thus limiting their use in cancer therapy. This limitation could be overcome when AuNPs are PEGylated, which limits the recognition and clearance of NPs by macrophages, prolonging their circulating half-life.^{58,59} Therefore, due to its important functions, PEG has been used to stabilize the AuNP and to facilitate its use in biomedical applications. Huang *et al* (2014) showed that PEG-AuNPs kill K562 cells in a dose- and time-dependent manner.¹¹⁰ The K562 cells treated with PEG-AuNPs were observed under a light and Transmission Electron Microscope (TEM). The cells showed a decrease in size after initiation of PEG-AuNPs treatment, with a number of cells showing apoptotic changes. TEM showed that PEG-AuNPs preferentially accumulated in cytoplasmic vacuoles, disrupted the mitochondria, and had an increased intensity around the nuclear membrane without penetrating the nucleus. Additionally, after 48 and 72 h of treatment, PEG-AuNPs resulted in a decrease in the number of cells in G0/G1 and G2/M phases and an increase in the number of cells in the S phase. Induction of apoptosis was also suggested by the time-dependent increase of the percentage of sub-G1 population after PEG-AuNPs treatment. K562 cells were evaluated by flow cytometry to analyze the number of permeabilized cells stained with the potential-sensitive dye, DiOC6. The mitochondrial transmembrane potential was significantly reduced after 24 to 72 h of treatment. This indicates that PEG-AuNPs may induce mitochondrial dysfunction leading to cellular apoptosis. Moreover, in an attempt to understand the mechanism of AuNP-induced cytotoxicity, Tsai *et al* (2011) used various omic approaches to identify the phosphorylated proteins and differentially expressed proteins/genes in K562 cells treated with AuNPs.¹¹¹ Systems biology analysis was used to systematically analyze the omic data to identify the cellular mechanisms influenced by AuNPs. Unfolded protein-associated endoplasmic reticulum (ER) stress response was the predominant event as revealed by Systems biology analysis of the proteomic data. In parallel with transcriptomic analysis using mRNA expression, microarrays showed ER stress response in the AuNP-treated cells. The AuNPs were shown to be efficient cellular ER stress inducers using ER stress protein markers' expression assay. Upon ER stress, a cascade of cellular responses was observed chronologically involving ROS increase, mitochondrial cytochrome C release, and mitochondrial damage. Therefore, this study concluded that induction of unmanageable ER stress is the main mechanism by which AuNPs induces cell death. Gossai *et al* (2016) showed *in vitro* and *in vivo* significant study results regarding the efficacy of AuNPs in the delivery and selective release of dasatinib to the malignant cells where the chemotherapeutic agent was customized to be released when cancer cell-specific mRNA was

present in the cell. As a result, the cytotoxic effect of both the NPs and the chemotherapeutic agent is significantly reduced while the drug delivery remains highly specific and efficient.¹¹²

Magnetic Nanoparticles

Low toxicity, minimal impact on metabolism and magnetic properties makes magnetic NPs (MNP) one of the most commonly used materials in various biomedical applications, especially cancer treatment.^{113,114} The MNPs help improve the efficiency of cancer treatment via targeted delivery of anticancer drugs and reversal of drug resistance.^{115,116} To avoid MNPs aggregation *in vivo*, they can be coated with hydrophobic or hydrophilic polymers. Additionally, MNPs should retain their magnetic properties while being biocompatible, having high drug-loading capacity, aqueous dispersibility, and the desired drug release profile.¹¹⁷

Chen *et al* (2008) evaluated the MDR reversal activity of MNP Fe₃O₄ (nano-Fe₃O₄) and 5-bromotetrandrine (BrTet) on MDR cell line K562/A02 solitarily or in combination; and investigated the mechanism of this reversal.¹¹⁸ BrTet is a more potent derivative of tetrandrine (a calcium channel antagonist), which possesses anti-MDR characteristics attributed to its ability to reinforce drug-induced apoptosis by increasing intracellular drug concentration mainly through downregulation of *mdr1* mRNA or P-gp.¹¹⁹ The proliferation of MDR K562/A02 cells that were cultured with daunomycin (DNR) alone or in combination with nano-Fe₃O₄, BrTet, or both for 48 h was evaluated by MTT assay. The DNR accumulation and P-gp of MDR K562/A02 were analyzed by fluorospectrophotometry. A comparison of IC₅₀ values showed that combination therapy had a powerful and statistically significant effect on the cell lines as the IC₅₀ value was significantly reduced when culturing K562/A02 with combined therapy. Flow cytometry assay showed that nano-Fe₃O₄ and BrTet increased the DNR accumulation in K562/A02 cells, especially in the group of synergy of these two agents. The mean fluorescence intensity of intracellular DNR significantly increased when incubated with combination reversal reagents compared to either nano-Fe₃O₄ or BrTet alone. Fluorospectrophotometry assay showed that P-gp was downregulated when pre-treating with nano-Fe₃O₄, BrTet alone, and in combination with K562/A02 cells. A study by Cheng *et al* (2009) using the same NPs to overcome CML cell P-gp-induced MDR also showed a synergistic effect of combination therapy.¹¹⁹

Another approach developed by Singh *et al* (2011) used long circulating lectin-conjugated paclitaxel-loaded MNPs for the treatment of CML.¹²⁰ Paclitaxel is approved by the FDA for various anti-cancer treatments.¹²¹ It acts by binding microtubules leading to sustained mitotic arrest¹²² and activates stress signalling pathways like JNK and p38MAPK leading to apoptosis.¹²³ This study investigated the efficacy of paclitaxel-loaded MNPs, functionalized with a lectin targeting moiety. The use of lectin moiety helps in delivering appropriate drug

Table 1. Summary of the Main Nanoparticles Discussed in the Text.

Nanocarrier Type	Ligand	Target	Coating	Drug/Active Compound	Suggested Mechanism of Action	<i>In vitro</i> (Cell Lines)	Reference
Carbon nanotubes	–	–	P-Glycoprotein antibody functionalized	Doxorubin	Inhibition of transcription	MDR K562R CML	86
Carbon nanotubes	siRNA	Cyclin A2	Ammonium functionalized		Proliferation arrest and apoptosis induction	CML K562	88
Carbon nanotubules	–	Hsp90 inhibition	Chitosan	SNX-2112	Apoptosis induction	CML K562	79
Carbon nanotubules	–	P-gp	–	Verapamil and Doxorubicin	Inhibition of P-gp, increase sensitivity to drug, and induction of apoptosis	CML 562/A02	87
Silver nanopeptides (AgNPs)	–	–	–	AgNPs	Induction of cytotoxicity in CML cells via ROS generation	CML K562	63, 99
Gold nanoparticles (AuNPs)	–	–	Polyethylene glycol	AuNPs	Mitochondrial damage, induction of unmanageable ER stress	K562	110
AuNPs	–	Cancer-specific mRNA		Dasatinib	Release of the TKI when activated by cancer cell specific mRNA		112
Magnetic nanoparticle: 5-bromotetrandrine	5-Bromotetrandrine	Calcium channel		5-Bromotetrandrine	Downregulation of <i>mdr1</i> mRNA or P-gp	K562/A02	118
Magnetic nanoparticles: Fe ₃ O ₄	–			Fe ₃ O ₄	Synergistic effect with 5-bromotetrandrine	K562	118
Magnetic nanoparticles			Lectin	Paclitaxel	Induction of apoptosis	K562	120
ZnO nanoparticles	–	–	–	Daunorubicin	Synergistic effect with daunorubicin	K562/A02	63
CuO nanoparticles	–	–	–	CuO	Increase in P53 and in Bax/Bcl-2 ratio	K562	126

Abbreviations: CML, Chronic myeloid leukemia; ER, endoplasmic reticulum; mRNA, messenger RNA; ROS, reactive oxygen species; TKI, tyrosine kinase inhibitors.

concentration specifically to the target tissues through receptor-mediated endocytosis.

In vivo studies showed better bioavailability of drug bound to MNPs, which was concluded to be due to the longer circulation half-life of pac-MNPs and lec-pac-MNPs compared to native 6-coumarin. In k562 cells, mitogenic assay showed that, after 48 h of drug administration, lec-pac-MNPs were up to 10 times more cytotoxic than pac-MNPs and 67 times more cytotoxic than intact pac. Fluorescence-activated cell sorting (FACS) analysis showed that cells treated with lec-pac-MNPs had a higher proportion of cells in G2-M compared to other

formulations. Using confocal microscopy FACS analysis, it was shown that apoptosis was best induced the most with Lec-pac-MNPs followed by pac-MNPs and intact pac.

ZnO Nanoparticles

Guo *et al* (2008) investigated the synergistic cytotoxic effects of different sized ZnO NPs and daunorubicin against k562/A02 cells under UV irradiation.⁶⁴ ZnO NPs are able to absorb a large fraction of the UV zone and generate various ROS such as hydrogen peroxide, hydroxyl radical, and superoxide.^{124,125}

Therefore, ZnO NPs were combined with daunorubicin for the treatment and the inhibition of MDR in leukemia K562/A02 cells that possess P-gp overexpression. This combined therapy was effective and efficiently enhanced the suppression of the leukemic cell lines when subjected to UV light. The main hindrance to this technique is the difficulty of its application in the treatment of hematological malignancies.

CuO Nanoparticles

Shafagh *et al* (2015), using MTT assay, showed that CuO NPs selectively kill cancer cells in a dose-dependent (manifested as ROS generation) manner without affecting normal cells.¹²⁶ Acridine orange and propidium iodide double staining was used to confirm CuO NPs induced apoptosis. CuO NPs exposure was associated with increase in tumor suppressor gene P53 and in Bax/Bcl-2 ratio indicating possible mitochondria-mediated cell death pathway.

Conclusion

Chronic myeloid leukaemia is a neoplastic proliferation of mature myeloid cells resulting from a translocation between Abl gene and BCR gene, leading to abnormal growth of these cells due to constitutively active TK. Tyrosine kinase inhibitors remain at the forefront of CML treatment. However, they do not lead to complete cure due to lingering quiescent stem cells. The development of resistance to imatinib is not uncommon, and functional “anatomic” resistance has also been reported. The use of NPs, as summarized in Table 1, for the vehiculation of anti-CML drugs may present new approaches in future therapy. The results displayed are promising, with certain NPs overcoming MDR, and successful targeting of quiescent stem cells that are common culprits in relapse. Most of these delivery systems demonstrated safety by specifically targeting CML cells and sparing normal cells. However, the majority of NPs were used in experimental settings (*in vitro*) necessitating further studies and clinical trials in this field. This review dealt with inorganic NPs used in CML therapy, a follow-up review article discussing organic NPs would be a useful addition to the literature regarding NP usage in CML therapy.

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