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RESEARCH ARTICLE

# An Overview of Nanoparticles for Treatment of Retinoblastoma: Disease Characteristics and Experimental Approaches

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ABTRACT

Retinoblastoma is the most common type of eye cancer in infants and children. Probability of saving vision and survival depends upon two main factors: progression of the disease from unilateral to bilateral and severity of the disease. In order to effectively treat retinoblastoma and retain vision, it is crucial to focus treatment options on reducing toxicity and nonspecific targeting while enhancing drug delivery, cellular uptake, and accumulation of chemotherapeutic agents to their specific target sites. Rapid elimination from blood circulation is the greatest obstacle that conventional chemotherapeutic agents face on journey to their target sites. Target specific nanoparticles have proven to be a useful tool in efforts to overcome challenges typically encountered by targeting strategies. Development of nanoparticles loaded with chemotherapeutic agents can allow for more selective tumor targeting, extended drug circulation times, and reduced drug-associated toxicity. Nanoparticles can significantly improve the treatment efficacy in retinoblastoma. The purpose of this review is to discuss the important characteristics and differences of nano delivery systems used against cellular and in vivo models of retinoblastoma, particularly as they relate to the popular Y79 retinoblastoma cell line.

**Keywords:** retinoblastoma, nanoparticles, drug delivery, nanotechnology, cancer therapy, liposomes, pediatric tumor

**Background**

Retinoblastoma is the most common intraocular malignancy in childhood.[1] The incidence of retinoblastoma is approximately 1 in 20,000 live births and there are about 200 to 300 new cases of retinoblastoma in the United States each year.[1] A biallelic mutation of the RB1 genes and the inheritance of one mutant RB1 allele strongly increases the likelihood of developing retinoblastoma.[2] It is recognized that the inactivation of the tumor suppressor gene RB1 in retinoblastoma broadly contributes to the development of the tumor and it is described by the two-hit model of tumor suppressor gene inactivation.[2] There are two RB1 genes in each cell and the formation of retinoblastoma requires both RB1 genes to be mutated or not working properly. Non-heritable (sporadic) form is unilateral and more common. An estimated 2 out of 3 children with retinoblastoma have the sporadic form and they do not have the RB1 gene mutation in all the cells of their body. Instead, the RB1 mutation happens early in life and first occurs only in one cell in one eye.[3] The remaining 1 out of 3 children with retinoblastoma have a germline mutation in one RB1 gene in all the cells of their body. This is known as heritable form of retinoblastoma and is classified as bilateral (affecting both eyes) unless if it is detected early enough, then it is possible for it to be unilateral.[3,4] The average age of appearance of first signs is 24 months for unilateral retinoblastoma cases, and 7 months for bilateral cases.[5] The children that have heritable retinoblastoma are at a risk of passing on the mutated RB1 gene. The most common presenting sign in both types of retinoblastoma is leukocoria (60%), it is when the tumor is visible through the pupil when light entering the eye is reflected outward.[1,2] If retinoblastoma is detected early, immediate treatment can help remove the tumor and save the eye. Otherwise, delaying the treatment can lead to metastasis to other parts of the body including the optic nerve and brain.[1]

There are multiple treatment options currently available for intraocular or extraocular disease.[3,6,7] For small tumors near the front of the eye, cryotherapy is a noninvasive treatment option where a probe is used to freeze and eradicate the tumor cells. Another option for small tumors is thermotherapy in which lasers are utilized to generate heat and kill cancer cell growth. External beam radiation therapy also exists as a treatment option to eradicate the tumor cells, but it is no longer recommended for first line therapy since radiation imposes a high risk of secondary cancers. There are also various chemotherapeutic agents such as doxorubicin, carboplatin, and vincristine that can be used alone or in combination for treatment of systemic or regional tumors. Last line of treatment available is surgery, also referred to as enucleation. The whole eye and part of the optic nerve is removed, and an orbital implant is put in after the surgery. Choice of primary treatment is based on the likelihood of patient survival and the probability of salvaging the eye and its vision, weighed against short term and long-term complications of treatment.[2] The status of the other eye is also considered before initiating a treatment option. The 5-year survival rate for children with retinoblastoma is 96%.[8,9] However, that rate may depend on several factors, including whether the cancer has spread from the eye to other parts of the body.[4] There are no known lifestyle-related or environmental causes of retinoblastoma, so it's important to develop treatment options that can eliminate the tumor as quickly as possible before the spread of disease.[1,2,3]

**Issues with Current Therapies**

The main goal for the treatment of retinoblastoma is a complete elimination of the tumor with minimal to no collateral injury to other tissues, preserving the child's vision whenever possible. Treatment options vary depending on different stages of the disease. Recent developments of treatment have made early detection of retinoblastoma possible, and most often two treatment types are used in conjunction for more optimal effect. Early detection typically means the cancer is confined within the eye, however later detection with wider spread of retinoblastoma leads to a more dismal prognosis and would require a more comprehensive treatment plan to eradicate both the primary tumor and the spread of disease. For these reasons, the main goal of retinoblastoma therapy is to eradicate tumor burden and to reduce the chance of metastasis and disease recurrence.

The common treatment therapies include chemotherapy, radiation therapy, cryotherapy, thermotherapy, and surgery as a last line treatment.[3,6] Some side effects of current therapies include vision deterioration and many retinopathies.[1] Chemotherapy is used for eyes with optic nerve invasion or massive choroidal invasion.[1] These chemotherapy treatments include two regimens from vincristine, cyclophosphamide, and doxorubicin or vincristine, carboplatin, and

etoposide.[1] While systemic chemotherapy is effective in treating retinoblastoma, there are many unwanted side effects affecting other body systems that can prove highly toxic for pediatric patients. Recent progress has occurred in terms of intravenous and intra-arterial chemotherapy, however, in cases of intravenous seeding, where tumor cells start to spread outside the eye, intravenous chemotherapy is ineffective because of the avascular nature of the vitreous fluid.[2] In radiation therapy, depending on the dose, radiation could inhibit normal growth of the eyes and the bone structure around the eyes. Radiation therapy could also lead to increased risk of tumor recurrence.[3] In some cases, conservative and eye-salvaging method is recommended for unilateral retinoblastoma cases if it is an early-stage disease. This approach is recommended especially in young children with heritable retinoblastoma that later develop a tumor in the contralateral eye.[1] Consolidation approach includes cryotherapy for small anterior tumors and thermotherapy or laser photocoagulation for posterior tumors.[1] Some of the main reasons for treatment failures are vitreous seeds of tumor, subretinal seeds of tumor, and intraretinal tumor.[1] Enucleation is the last line therapy to remove the tumor, and it also has the largest impact on the child's quality of life due to the permanent vision loss.[3,4] With early detection efforts and better treatments, enucleation could hopefully be reduced, and patients would be able to survive retinoblastoma with intact vision.

The main reason for these failures in treatment is due to an inability of drug to reach tumor in clinically relevant concentrations. One of the most important reason for the treatment failure is tumor resistance mainly due to well differentiated, non-cycling tumor cells that are resistant to treatments that are dependent on cell division. Well-differentiated retinoblastoma include characteristics such as smaller nuclei, abundant amounts of cytoplasm, and cells that do not actively enter the cell cycle. Because these cells are not actively dividing, targeting these with chemotherapy and radiation presents a challenge, as these agents typically target vital steps in the cell cycle. Differentiated cells are resistant to irradiation and show less tumor regression when compared to their mitotically active undifferentiated counterparts.[10] Common chemotherapeutic drug agents used to treat retinoblastoma such as carboplatin, vincristine, and etoposide are radiomimetic agents that work by targeting DNA synthesis and cell division, which is impaired when cells are not actively entering the cell cycle.[10] In vivo studies utilizing different retinoblastoma cell lines found that undifferentiated tumors were more sensitive to carboplatin, doxorubicin, thiotepa, and ifosfamide when compared to differentiated tumors.[11] The tumor suppressor gene, RB1 plays an essential role in preventing cells from progressing through the cell cycle until they are ready to divide; therefore mutations of RB1 are a risk factor to developing retinoblastoma.[12]

**Effective Treatment Approach**

To overcome tumor barriers in treatment of retinoblastoma cells, various formulations of nanoparticles have been used to selectively deliver the chemotherapeutic agent to the tumor mass. These nanoparticles enhance delivery and efficacy by selectively targeting drugs to cellular models of retinoblastoma such as Y79. Although other retinoblastoma cell lines exist, the Y79 retinoblastoma cells develop metastatic growth patterns in vivo murine models and are often employed for experimental drug testing in vitro .[13,14,15] Specific features of the tumor are exploited to strengthen the nanoparticle's targeting efficacy leading to an improvement in the chemotherapeutic agent's action on Y79 cells.[16]

In order to accelerate the process of drug discovery, emerging methods such as orthotopic patient-derived xenografts to discover novel therapeutic combinations are currently being explored.[17] This method provides better preclinical models resulting in improved outcomes. One effective combination resulting from this method is topotecan with carboplatin efficiently killing retinoblastoma cells with its synergistic mechanism of action.[17] Due to high metabolic demand and the hypoxic environment that retinoblastoma grows in, oxidative stress leads to DNA breaks.[17] The two agents exploit and enhance that mechanism resulting in more retinoblastoma cell death. Alternative pharmaceutical dosage forms such as nanoparticles offer a way around this. Inorganic-based nano-delivery systems include nanoparticles derived from gold, iron oxide, and mesoporous silica.[18] Organic-based nano-delivery systems include micelles, liposomes, and solid lipid nanoparticles.[18]

Several nano-delivery systems are currently on the market, including liposomes (lipid bilayers with an enclosed aqueous core compartment in the center of the vesicle). Doxil is one such liposomal delivery system. The lipid-based

liposome incorporates doxorubicin.[19] The product is used to treat several different types of cancers, including breast and ovarian.[19] Another example is AmBisome (which incorporates amphotericin B within the delivery vehicle). The formulation product has been used to treat fungal infections.[19] Doxil and Ambisome are FDA approved liposomal preparations and offer significant advantages over their respective free drug counterparts. Such advantages include (a) specific targeting to tissues, (b) reduced side effects, (c) and, extended drug circulation times.[19,20] Solid-lipid nanoparticles have pharmacokinetic and physiological effects similar to liposomes.[21] Cellular membrane lipid-extracted nanoliposomes (CLENs) are also an option, using cellular-derived lipids that enhance target tissue specificity.[22] The lipid extract used to create CLENs are derived from the intended target cell, and thus should resemble the target cell membrane.[22] This resemblance provides improved recognizance of the nano-delivery system by the target cell population resulting in enhanced uptake when compared to conventional liposomes.[22]

Solid lipid nanoparticles (SLN) can incorporate both hydrophilic and lipophilic drugs while delivering the drug in a controlled manner.[21] A study has shown that SLN improved sensitivity to the Y79 retinoblastoma cell line. Doxorubicin-loaded SLN had a 2.4-fold increase in intracellular accumulation leading to a stronger inhibition of cell growth, specifically a 64% decrease in IC50 when compared to both free doxorubicin and doxorubicin liposomes.[23] This enhanced cytotoxic effect of doxorubicin when compared to the other forms of doxorubicin was associated with enhanced uptake into cells via SLN.[23] Additionally, the epithelial cell lines were found to be more sensitive to doxorubicin loaded SLN when compared to control cells.[23]

Cationic SLN improved cytotoxicity when compared to controls. A study evaluated the cytotoxicity profiles of cationic SLN on various cancer cell lines, including Y79, using cetyltrimethylammonium bromide (CTAB) or dimethyldioctadecylammonium bromide (DDAB) as surfactants in the preparation of drug free SLN.[24] Results showed that CTAB-SLN had a 99% IC50 decrease compared to DDAB-SLN suggesting CTAB significantly increased the cytotoxicity profile of the nanoparticles on Y79 retinoblastoma.[24]

Chitosan nanoparticles (CNP) have a positive surface charge and thus a relatively high affinity for target cell membranes, providing a controlled release of encapsulated contents.[25] Retinoblastoma tumor masses overexpress folate receptors by 100-300 times compared to in normal tissues.[26] Conjugation of folic acid to nanoparticles is a potential method that has been used to selectively target Y79 retinoblastoma cells. Folic acid on CNP has resulted in a 16% increase in intracellular uptake compared to CNP without folic acid conjugation.[27] The conjugation of folic acid to doxorubicin-loaded CNP increased tumor cell death by 43% when compared to free doxorubicin.[27]

Topotecan (TPH), a water-soluble derivative of camptothecin, has a reasonably safe toxicity and stability profile. TPH is an effective therapeutic option for treatment of retinoblastoma.[28] TPH is hydrolyzed to a biologically inactive compound under physiological pH, and has a reduced half-life in the vitreous humor, reducing its efficacy as a pharmacotherapeutic treatment.[29] Chitosan can be modified to form N-trimethyl chitosan (TMC), improving chitosan solubility in neutral pH environments.[28] Another modification is the covalent attachment of thiol groups to chitosan.[28] Thiolated chitosan (TCs) have increased control of drug release and permeability without changing biodegradability.[28] Delrish et al compared both TPH-TMC-NPs and TPH-TCs-NPs with free TPH to demonstrate the advantage of using TPH-TCs-NPs over the previously mentioned delivery methods. TPH-TCs-NPs demonstrated significant cellular uptake compared to TPH-TMC-NPs, and free TPH.[28] The results of the cytotoxicity studies favored TPH-TCs-NPs, over TPH-TMC-NPs and free TPH controls.[28]

Y79 cell line model has been used to evaluate various chemical methods and applications for retinoblastoma. For example, the combination of Poly(lactic-co-glycolic acid) (PLGA) and PEG has been explored for targeting.[20] Targeting ligands (such as folate) have been linked to PEG chains which has permitted selective cellular binding of overexpressed folate receptors.[20,30] Doxorubicin loaded in PLGA-PEG-folate micelles using various solvents (dimethyl sulfoxide, acetone, and dimethyl formamide) has been studied in Y79 retinoblastoma cells to examine the effects of the solvents on entrapment efficiency, particle size, and polydispersity.[31] Dimethylformamide was found to be the most suitable solvent for the preparation of micelles, showing the highest intracellular uptake, and killing effects against Y79 cells.[31] Doxorubicin loaded within PLGA-PEG-folate micelles resulted in four times the intracellular uptake compared to free

doxorubicin.[31] Carboplatin-loaded PLGA and SA-PLGA were studied to investigate their antiproliferative effect on retinoblastoma cells.[32] Following 7 days after treatment in vitro, the authors observed significant intracellular uptake when compared to the control group. The study revealed an improved growth inhibitory effect when compared to native carboplatin.[32]

Nanoparticles offer interesting possible solutions for the treatment for retinoblastoma. For example, the anticancer drug nutlin-3a, activates the tumor suppressor gene p53.[33] However, nutlin-3a is the substrate of multidrug resistance protein MRP-1 and thus its application is somewhat limited.[34] Curcumin can reverse multidrug resistance; however, it has poor bioavailability and plasma instability.[33] As a possible work around, nutlin-3a and curcumin were loaded into PLGA-nanoparticles covered in folate, and the results confirmed that administering both agents together in PLGA-nanoparticles enhanced the therapeutic efficacy of nutlin-3a.[33]

Mesoporous silica nanoparticles (MSN) contain pores which allow for efficient loading and a sustained release of therapeutic agents to the target site.[35,36] The silica component provides additional stability compared to other nanoparticles such as liposomes.[35] Carbohydrate (galactose or mannose) conjugated MSN loaded with camptothecin and a photosensitizer were evaluated for treating Y79 retinoblastoma.[37] Both galactose and mannose allowed effective internalization of MSN leading to an enhanced cell death of Y79 retinoblastoma.[37] The results showed that Y79 has preferential affinity for galactose and mannose residues in addition to potential for synergistic use of camptothecin and photosensitizer when encapsulated within the same nano-delivery system.[37]

Carboplatin has been studied via intravitreal injections loaded into polymethylmethacrylate nanoparticles (NPC). When compared to free carboplatin, animal studies showed that NPC increased the intravitreal concentration of carboplatin 3 – 4 times more than free carboplatin due to higher trans-scleral permeability, indicating high efficacy of NPC as an intravitreal dosage form for retinoblastoma.[38] A study was also done on humans using carboplatin loaded NPC to evaluate intraocular distribution of the chemotherapeutic agent on six patients with advanced retinoblastoma scheduled to undergo enucleation.[39] The nano-delivery system was administered via subtenon injections and the highest concentration of carboplatin in retina was seen at 24 hours.[39] Additionally, carboplatin-loaded apotransferrin and lactoferrin nanoparticles have been studied compared to free carboplatin, and show greater intracellular uptake, sustained retention, and antiproliferative activity via receptor mediated endocytosis delivery to malignant retinoblastoma cells.[40]

Lactoferrin nanoparticles (Lf-Nps) have been evaluate for cytotoxicity of etoposide, (topoisomerase inhibitor) and carboplatin in vitro, against the growth of retinoblastoma-Y79 cells.[41] Lactoferrin is an iron transporting glycoprotein (transferrin family) shown to improve target recognition in several studies.[42] The drug localization observed with lactoferrin nanoparticles is associated with expression of lactoferrin receptors in target cancer cells.[42] The rapidly dividing cancer cells demonstrate significant expression of lactoferrin receptors due to increased iron demand for metabolic needs.[42] Narayana et al, confirmed significant intracellular drug uptake, and cytotoxicity profile, for carboplatin (CPT)- and etoposide (ETP)-loaded lactoferrin nanoparticles (Lf-Nps) in retinoblastoma Y79 cells.[41] Drug agents loaded in lactoferrin nanoparticles (Lf-Nps) doubled overall cellular uptake compared to carboplatin (CPT) or etoposide (ETP) alone.[41] Additionally, the cytotoxic effect increased up to 50% with carboplatin (CPT)- and etoposide (ETP)-loaded lactoferrin nanoparticles(Lf-Nps) compared to controls.[41]

Epithelial cell adhesion molecule (EpCAM) is a cell surface molecule which is overexpressed in retinoblastoma resulting in cell proliferation.[43] Selectively inhibiting EpCAM on retinoblastoma is shown to deregulate genes controlling growth; thereby, reducing cell survival.[43]Conjugation of EpCAM antibody to a polyethyleneimine capped gold nanoparticles resulted in a 29% increase in intracellular accumulation compared to unconjugated gold nanoparticles.[44] The loading of siRNA specific to EpCAM knockdown in these nanoparticles for retinoblastoma cells was twice as effective as free siRNA.[44] Another study showed that EpCAM conjugated to mesoporous silica nanoparticles loaded with carboplatin (EpCMSN) resulted in a controlled release kinetics, as well as enhanced internalization when compared to nanoparticles not conjugated to EpCAM antibodies.[45] EpCMSN also showed superior anticancer effects and enhanced apoptosis of cancerous retinoblastoma cells, as well as a significantly lower IC50.[45]

Combinatorial nanoparticles are another technology used to target cancer cells by boosting synergistic anti-tumor activities while also reducing off-target effects.[46] Glycol chitosan-coated ceria nanoparticles (GCCNPs) were used to prepare combinatorial nanoparticles containing doxorubicin, AMD11070 (an inhibitor of CXCR4), and cerium oxide nanoparticles (nanoceria).[46] Nanoceria is an antioxidant at physiological pH, but acts as an oxidase in acidic environments both in vitro and in vivo, increasing cytotoxicity.[46,47] The resulting combinatorial nanoparticles (AMD-GCCNPs-DOX) were able to induce reactive oxygen species and release doxorubicin intracellularly, and were tested on Y79. Results found that at a pH of 6.5, the AMD-GCCNPs-DOX nanoparticles easily accumulated within Y79 cells and released doxorubicin in response to the tumors environment.[46]

Survivin, an inhibitor of apoptosis protein (IAP), is one of the most specific cancer targets, as there is high expression in malignant cancerous cells compared to healthy cells, which have almost no expression of the IAP.[48] Retinoblastoma overexpresses survivin, making it a promising target for drug therapy.[48] Switchable lipid nanoparticles (LNP) loaded with siRNA (siLNP) targeted against survivin were evaluated for their capability of enhancing the therapeutic effect of several chemotherapy agents in Y79 retinoblastoma.[49] The switchable lipid nanoparticles used were able to undergo a conformational switch when exposed to retinoblastoma's acidic pH, promoting membrane destabilization and cytosolic release of the siRNA into the target cell.[49] Following treatment with siLNP, retinoblastoma cells were incubated with either carboplatin, melphalan, topotecan, or teniposide.[49] Results found that silencing of survivin via siLNP enhanced the cytotoxicity of carboplatin and melphalan to Y79 retinoblastoma.[49]

Switchable LNP have also been used to deliver two agents simultaneously, specifically melphalan and miR-181a, to treat seeded retinoblastoma. Approximately 171 nm switchable LNP were loaded with both melphalan and miR-181a that had an encapsulation efficiency of 93%.[50] The switchable LNP released their contents in retinoblastoma's acidic cellular environment and all Y79 cells were transfected within 24 hours.[50] Preclinical studies using bilateral Y79 retinoblastoma murine models assessed the efficiency of chemotherapeutic agents in LNP by comparing the number of live cells remaining in treated eye versus untreated eye.[50] Following 48 hours after treatment, miR-181a-loaded LNP reduced viable tumor cells by 37% compared to free melphalan, whereas dual loading of melphalan and miR-181a in LNP reduced viable tumor cells by 72%.[50] The switchable LNP significantly improved the therapeutic effects of both melphalan and miR-181a and showed enhanced apoptotic effects compared to free melphalan.[50] Switchable LNP ultimately allowed for lower administration of melphalan while increasing efficacy and minimizing melphalan's cytotoxic drug side effects.[50]

**Concluding Remarks**

Established treatments for retinoblastoma focus on getting rid of the cancer, saving the patient's life, and saving the eye if possible. However, challenges related to the side effects resulting from conventional treatments still exist. For example, the inhibition of normal growth of the eyes and the inhibition of growth of normal bone structures surrounding the eyes increase the risk of tumor recurrence (from radiation and some non-specific drug therapies). Enucleation is a last line therapy that focuses on rescuing both vision and the eye using locally directed therapies with or without systemic chemotherapy. This treatment approach has a significant impact on child's quality of life because delayed detection of retinoblastoma can result in higher enucleation and to vision loss. To overcome these challenges, various formulations of nanoparticles selectively target tumor cells, enhance drug accumulation, and reduce toxicity effects. Table 1 summarizes nanotherapeutics evaluated for the treatment of retinoblastoma. In comparison to free drug delivery, specialized nano delivery systems enhance intracellular accumulation of agents and improve cytotoxicity of Y79 retinoblastoma cells. Nanoparticle drug delivery systems also permit lower administered doses of chemotherapeutic agents to achieve similar overall drug action against retinoblastoma, thus suggesting a potential to improve tolerability and decrease side effects in clinical applications. Finally, no one particular cell line addresses all desired experimental needs. However, this report supports the continued use of the suspension Y79 cell line for vitro and in vivo experiments.

**Table 1.** Summary of currently evaluated nanoparticle delivery systems for retinoblastoma

| | Study | Delivery system | In vitro/ vivo | Intracellular accumulation | Cytotoxicity |
|---|---|---|---|---|---|
| **1** | Serpe et al. 2006 | Doxorubicin loaded solid lipid nanoparticles (**DOX-SLN**) | In vitro Y79 | • DOX-SLN had 2.4-fold increase in intracellular accumulation compared to free drug | Cytotoxicity, $IC_{50}$ (ng/mL), at 48 hrs<br>• Free DOX (>300)<br>• Liposomes (>300)<br>• DOX-loaded SLN (108.3 ± 18.3)<br>DOX-SLN had 64% IC50 decrease compared to free DOX |
| **2** | Parveen et al. 2010 | Doxorubicin-loaded chitosan nanoparticles conjugated to folic acid (**DOX-CNPs-FA**) | In vitro Y79 | • Free DOX (5.01%)<br>• DOX-CNPs (13.24%)<br>• DOX-CNPs-FA (30%) | Cytotoxicity<br>• Free DOX (16.43%)<br>• DOX-CNPs (38.04%)<br>• DOX-CNPs-FA (60.2%) |
| **3** | Boddu et al. 2010 | DOX-loaded poly(d,l-lactide-co-glycolide)-poly(ethylene glycol)-folate (PLGA-PEG-FOL) micelles (**DOXM**) | In vitro Y79 | • DOXM had ~4 times higher uptake compared to free DOX | Cytotoxicity<br>• DOXM resulted in a more decrease in tumor cell viability compared to free DOX |
| **4** | Das et al. 2012 | Nutlin-3a and curcumin encapsulated in PLGA nanoparticle surface functionalized with folate (**Fol-Nut-Cur-NP**) | In vitro Y79 | • Fol-Cur-NPs had ~3.3 times greater uptake than unconjugated NPs and 9 times greater than native curcumin | Cytotoxicity, $IC_{50}$ (µg/ml)<br>• Curcumin (10.70)<br>• Nutlin-3a (2.86)<br>• Nutlin-3a + curcumin (2.5)<br>• Fol-Nut-Cur-NPs (0.07) |
| **5** | Gary-Bobo et al. 2012 | Camptothecin loaded in mesoporous silica possessing a photosensitizer and surface bound carbohydrates (galactose or mannose) (**MSN-PS-man-CPT**) (**MSN-PS-gal-CPT**) | In vitro Y79 | • Galactose and mannose allowed for efficient internalization of MSN compared to MSN without carbohydrates on surface | Cytotoxicity, after 3 days<br>• MSN-man-CPT (35%)<br>• MSN-gal-CPT (34%)<br>Cytotoxicity with PS<br>• MSN-PS-man (28%)<br>• MSN-PS-man-CPT (58%)<br>• MSN-PS-gal (40%)<br>• MSN-PS-gal-CPT (68%) |
| **6** | Mitra et al. 2013 | EpCAM antibody (EpAb) conjugated to polyethyleneimine (PEI) capped gold nanoparticles (AuNPs) loaded with EpCAM-specific siRNA molecules (**AuNP-PEI-EpAb-siRNA**) | In vitro Y79 | • EpCAM conjugated AuNP-PEI-siRNA (59%)<br>• EpCAM unconjugated AuNP-PEI-siRNA (29.2%) | Cytotoxicity<br>• EpCAM conjugated AuNP-PEI-siRNA as effective as twice the amount of naked siRNA |
| **7** | Ahmed et al. 2014 | Carboplatin loaded apotranferrin and lactoferrin nanoparticles (**Apo-nano-carbo**) (**Lacto-nano-carbo**) | In vitro Y79 | • Both NPs demonstrated higher cellular uptake and maintained intracellular drug concentration for a longer period compared to free carboplatin | Cytotoxicity, $IC_{50}$ (µg/ml)<br>• Free carboplatin (13.5)<br>• Apo-nano-carbo (4.31)<br>• Lacto-nano-carbo (4.16) |

| | | | | | |
|---|---|---|---|---|---|
| **8** | Qu et al. 2017 | Carboplatin loaded in EpCAM-conjugated mesoporous silica nanoparticles (**EpCMSN**) | In vitro Y79 | • EpCMSN showed enhanced internalization compared to CMSN (carboplatin in MSN) | Cytotoxicity, $IC_{50}$ (µg/ml)<br>• Free carboplatin (3.26)<br>• EpCMSN (1.38) |
| **9** | Gao et al. 2018 | Doxorubicin and AMD11070 tumor cell targetable (CXC chemokine receptor 4 antagonist) loaded in glycol chitosan-coated ceria nanoparticles (GCCNPs) (**AMD-GCCNPs-DOX**) | In vivo/ In vitro Y79 | • At pH 6.5, the AMD-GCCNPs-DOX easily accumulated within Y79 cells and released DOX in response to the tumor microenvironment | Cytotoxic at pH 6.5, $IC_{50}$ (nM)<br>• DOX (283.4)<br>• AMD-GCCNPs-DOX (192.3) |
| **10** | Tabatabaei et al. 2019 | miR-181a and melphalan loaded in switchable lipid nanoparticles (**mi181a-mephalan-LNP**) | In vivo/ In vitro Y79 | • Significant transfection levels observed within 24 hours | Cytotoxicity, in vivo<br>• miR-181a-LNP reduced cell viability by 37% as compared to free melphalan<br>• miR-181a-melphalan-LNP reduced cell viability by 72% |
| **11** | Zhuang et al. 2020 | Carboplatin loaded in surface modified nanoparticles- **PLGA and SA-PLGA (PLGA-NPs and SA-PLGA-NPs)** | In vitro Y79 | • Significant intracellular uptake of SA-PLGA or PLGA compared with that of control CBP-FITC | Cytotoxicity<br>• SA-PLGA and PLGA loaded with carboplatin decreased cell viability compared to free carboplatin.<br>• Significant cell growth inhibitory effect of SA-PLGA loaded with carboplatin, compared to PLGA loaded with carboplatin |
| **12** | Delrish et al. 2021 | Topotecan (TPH) loaded in N-trimethyl chitosan nanoparticles and thiolated chitosan nanoparticles (**TPH-TMC-NPs and TPH-TCs-NPs**) | In vivo/ In vitro Y79 | • TPH-TCs-NPs demonstrated greater cellular uptake when compared to TPH-TMC-NPs and free TPH. | Cytotoxicity after 24h, $IC_{50}$<br>• TPH-TCs-NPs, 53 nM<br>• TPH-TMC-NPs, 85 nM<br>• Free TPH, 138 nM |
| **13** | Narayana et al. 2021 | Carboplatin- and etoposide-loaded lactoferrin protein nanoparticles (**Lf-CPT and Lf-ETP NPs**) | In vitro Y79 | • Lf-CPT and Lf-ETP Nps had ~ 2 times greater cellular uptake compared to free drug | Cytotoxicity<br>• Lf-CPT and Lf-ETP Nps increased cytotoxicity ~50% compared to free drug |

**Future Direction**

We should invest in new and innovative nanotherapies for the future treatment of disease. These should include an ability to exploit differential expression, regulation, and control when possible. For example, healthy retinal cells contain approximately 10% molar content of cholesterol, and the synthesis of cholesterol happens within the inner layers of the retina.[51] HMG CoA reductase is the major rate-limiting enzyme in the retina that regulates cholesterol levels.[52] Retinoblastoma cells are thought to have higher cholesterol content than healthy retinal cells. Therefore, cholesterol content may represent a determinant of targeting retinoblastoma.[51] Lipid-based nanoparticles are known to be less toxic for in vivo applications compared to inorganic nanoparticles. For this reason, nanoparticles such as CLENs (cell membrane lipid-extracted nano-liposomes) could lend its highly tailored-specific

nature to improve selective targeting of malignant retinoblastoma cells in vitro and in vivo.[21,22,53] In the case of retinoblastoma, the differential expression of cholesterol (between normal and disease) would naturally match the elevated cholesterol content. Moreover, the additional inclusion of cholesterol content could well improve stability or possibly facilitate drug uptake mechanisms.[54]

Target specific nanoparticles have demonstrated effectiveness as a drug carrier system in some experimental models. Examples include an ability to overcome treatment barriers, and limit unwanted side effects of medications. This is a great starting point, but efforts devoted to novel design & development, with translations from the bench to the bedside, are critically important too. Lastly is the consideration of cost-effectiveness. The cost for the development and commercialization of nanomedicine (consisting mostly of medical devices and drug delivery systems) has an estimated market value of over 300 billion US dollars by 2025. The reports go on to suggest that the market value of nanodrug products will continue to rise with novel advances in technology, and from products expected to come off patents relatively soon.[55] Therefore, based on the success with nanotherapeutics in experimental models and in the clinic, it is fair to predict that emerging nanoproducts will continue to add market value. Additionally, the issues surrounding affordability for patient use should remain part of the discussion.

**Conflict of Interest**

No conflicts of interest.

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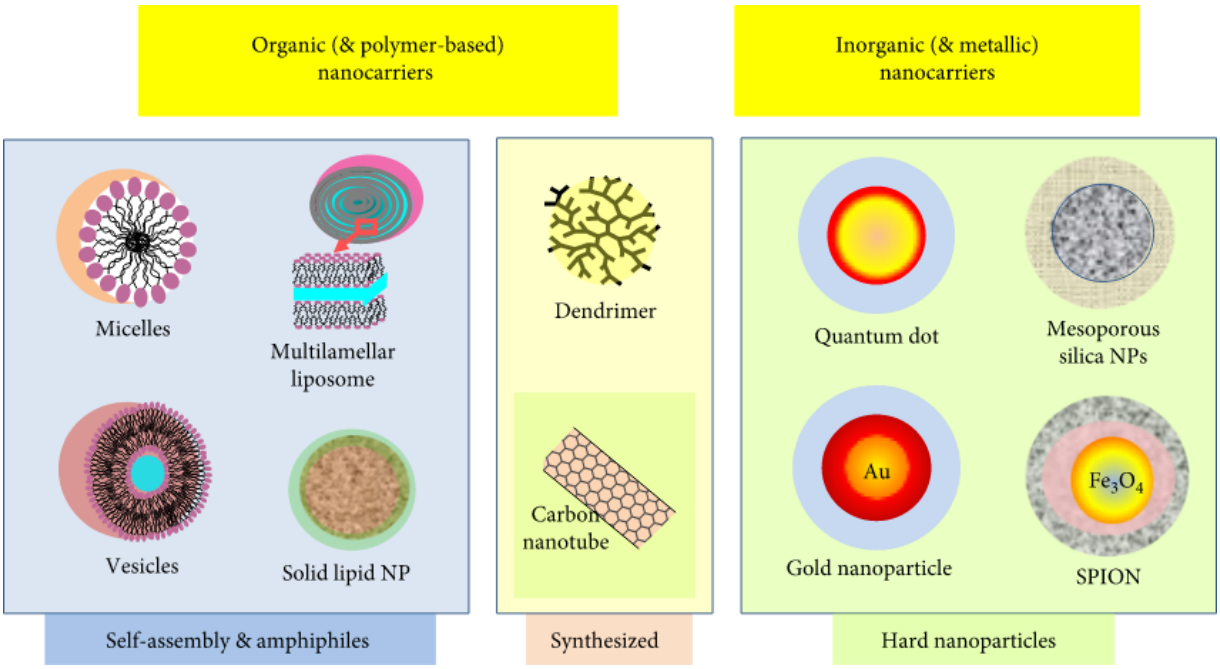


**Figure 1. Examples of nanoparticles.** Schematic showing several organic and inorganic nano-systems used to deliver therapeutic agents.[18]

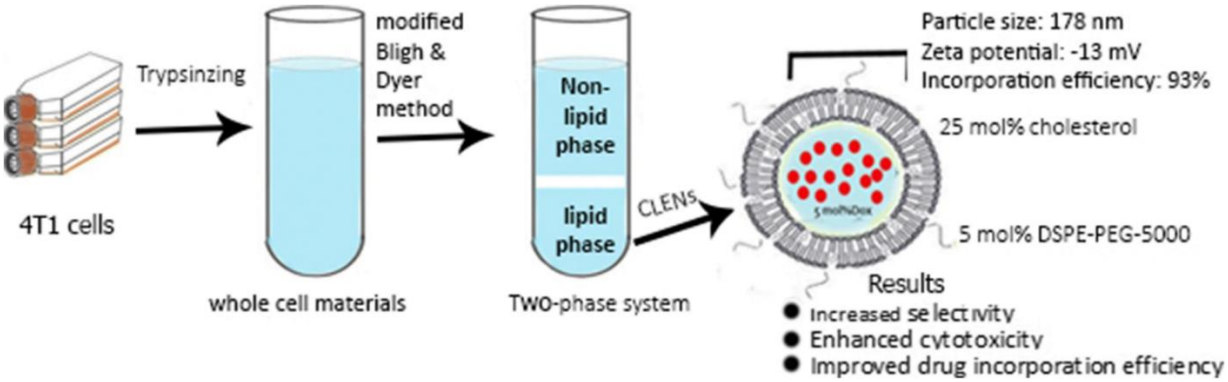


**Figure 2. Formation of CLENs.** Schematic showing the process for preparing CLENs from isolation of lipid extracts to exposure to target cell populations.[22]

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