
Review

Recent Developments in Nanomedicine for Pediatric Cancer

Shicheng Yang¹, Mia Wallach², Apurva Krishna³, Raushan Kurmasheva^{4,5*†} and Srinivas Sridhar^{1,3,6*†}

¹ Department of Chemical Engineering, Northeastern University, Boston, MA, United States,

² School of Business, Northeastern University, Boston, MA, United States,

³ Department of Bioengineering, Northeastern University, Boston, MA, United States,

⁴ Department of Molecular Medicine, The University of Texas Health at San Antonio, San Antonio, TX, United States

⁵ Greehey Children's Cancer Research Institute, San Antonio, TX, United States,

⁶ Division of Radiation Oncology, Harvard Medical School, Boston, MA, United States

*† Co-senior and co-corresponding authors: Kurmasheva@uthscsa.edu; s.sridhar@northeastern.edu.

Abstract: Cancer is the second biggest cause of death in children. With the development of chemotherapy, there is a substantial increase in overall survival rate over recent 30 years. However, the overall mortality rate in children with cancer still remains 25%, and many survivors experience a decline in overall quality of life and long-term adverse effects caused by treatments. Although cancer cells share common characteristics, pediatric cancers are different from adult cancers in their prevalence, mutation load, and drug response. Therefore, there is an urgent unmet need to develop therapeutic approaches specifically designed for children with cancer. Nanotechnology can potentially overcome the deficiencies of conventional methods of administering chemotherapy and ultimately improve clinical outcomes. The nanoparticle-based drug delivery systems can decrease the toxicity of therapy, provide a sustained or controlled drug release, improve pharmacokinetic properties of loading contents, and achieve a targeted drug delivery with achievable modifications. Further, therapeutic approaches based on combining nanoformulated drugs with novel immunotherapeutic agents are emerging. In this review, we discuss the recently developed nanotechnology-based strategies for treating blood and solid pediatric cancers.

Keywords: Pediatric cancer, nanoparticles, drug delivery system, liposome, leukemia, lymphoma, osteosarcoma, Ewing sarcoma, glioma, blood-brain barrier.

1. Introduction to pediatric cancer

Pediatric cancer is the second biggest cause of death for children under the age of 14 [1]. Since the mid-1970s, pediatric cancer has been a salient research topic, and new research on the subject is frequently emerging. There has been a substantial decline in the mortality rates for various cancers over the last 30 years among children under the age of 19 [2, 3]. However, despite recent progress, the 5-year death rate in children with cancer still remains as high as 26%, and many survivors experience long-term adverse effects that worsen their quality of life [1, 4].

1.1 Traditional and modern chemotherapy

Cytotoxic chemotherapy utilizing DNA alkylating agents and antimetabolites, has been the most widely used cancer treatment in the past 50 years. Current chemotherapies produce a host of unintended effects that can pose lifelong implications for survivors, such as severe sequelae and decreased quality of life. can cause fatigue, nausea, diarrhea, mouth sores, hair loss, and anemia. These apoptosis-inducing therapies cannot differentiate rapidly dividing normal cells from cancerous cells causing systemic toxicity. Furthermore, chemotherapy is typically administered daily through oral or intravenous injection,

which constrains patients to daily medical appointments or inpatient care and further reduces quality of life.

A deeper understanding of cancer biology in the last decades has accelerated the design of molecules that target and inhibit proteins (such as BRAF) and pathways (like angiogenesis, blocking DNA repair or inducing DNA damage) that are crucial for tumor growth or contribute to cancer cell proliferation [5]. In the last 30 years, the U.S. Food and Drug Administration (FDA) has approved over 30 small molecules for cancer treatment. Most of these compounds are inhibitors with molecular weights of less than 500 Daltons that aim to slow or stop the cell cycle and lead to the eventual death of cancer cells. Inhibitors induce apoptosis by blocking key receptors or enzymes, interfering with downstream intracellular signaling molecules, introducing genetic damage, or preventing DNA repair [6]. These chemotherapies can often shrink or delay growth of solid tumors, which allows patients to live longer with better quality of life. Additionally, chemotherapy can reduce the possibility of cancer recurrence after tumor reduction surgery.

1.2 Critical differences between pediatric and adult cancers

Cancer is an extremely heterogeneous disease with complex and tissue-specific impairments from genetic, epigenetic, and environmental factors. Understanding the critical differences between pediatric and adult cancers is integral for investigating underlying pathophysiologic and molecular mechanisms for developing new diagnostic/therapeutic approaches [7].

First, types of cancers are different between children and adults. Childhood cancers are not triggered by lifestyle as in adults, and very few are inherited from parents. Although the risk of developing most types of cancer significantly increases with age, there are several exceptions. For example, while brain and bone cancers are rare in adults, they have much higher rates in children [8, 9]. Similarly, leukemia is also more prevalent in children; it accounts for 28% of all pediatric cancers, but only 3% to 4% of cancers among all age groups [9, 10].

Second, even though cancers in children tend to respond better to therapy, children and adults differ in genitourinary pH and transit, intestinal motility and conjugation, and transport of bile salts. All of these factors affect metabolism of chemotherapeutic drugs. Pediatric tissues and organs are immature, and because children are rapidly developing, they have higher metabolic rates. Treatment dosages that are safe to administer to adults are often severely toxic to a child's developing organs, which absorb, distribute, and eliminate substances more rapidly than the same type of organ in an adult.

Finally, genetic stability in children and adults is also different, which affects mutation rates in cancer [11]. In a study of pan-cancer genome and transcriptome analyses of all reported driver genes in pediatric cancer, only 45% matched those found in adult cancers [12, 13]. Children have 'quieter' genomes, and many pediatric cancers, such as Ewing sarcoma, alveolar rhabdomyosarcoma, synovial sarcoma, and acute lymphocytic leukemia (ALL), are driven by fusion oncogenes resulting from chromosomal translocations. Compared to children, adult genomes have a much higher mutational rate, which provides unique molecular targets for treatment strategies. The lack of mutation targets makes development of targeting treatments for pediatric cancer more challenging. Thus, types of mutations in pediatric cancer can differ from those in adults, leading to a different integration of chemotherapies with drug targets, and in turn making the strategy of targeting specific mutations less effective for children.

For the above reasons, chemotherapy doses in children cannot be simplified by direct adjustment to the body weight/surface, as they often are in adults. Therefore, there is an urgent unmet need to develop therapeutic methods specifically designed for children with cancer, which may provide clinicians with more powerful weapons to treat them.

1.3 Challenges in pediatric cancer treatment

While clinical trials in children are more complicated due to scientific, ethical, and technical factors that have stunted research progress over the years, the lack of studies about drug disposition in pediatric patients poses a key difficulty for clinical applications. Extrapolation of adult dosing is often used to determine the dosage for pediatric patients. However, pharmacokinetic and pharmacodynamic properties of roughly 70% of drugs prescribed to pediatric patients have not been appropriately studied in children, necessitating the development of new approaches for determining the dosage for pediatric patients [14].

Over the past several decades, astounding progress has been made in development of chemotherapy and the emergence of novel treatments for the adults, such as immunotherapy and combination therapy. Nevertheless, conventional anti-cancer treatments can have late side effects or cause severe long-term health problems that are not well studied once former pediatric cancer patients mature into adults [7]. Additionally, in some instances, pediatric cancers have shown less successful responses than adult cancers to the same dosage and treatment. Consequently, there is a pressing need to develop targeted therapies or drug carriers that can deliver therapeutic agents with higher efficiency to lower the dosage needed and minimize side effects.

One of the significant recent developments aimed to advance pediatric research is the Research to Accelerate Cures and Equity for Children Act (RACE for Children Act) and the Public-Private Partnership, which is aimed to necessitate having sufficient pediatric models with the appropriate genetic alterations. The RACE Act requires the FDA to develop a list of molecular targets of known and new drugs, and pediatric agents substantially relevant to cancer growth and progression will require pediatric trials. This expectation applies both to drugs or biologics developed by pharmaceutical companies and by academic institutions.

2. Nanomedicine

2.1 Introduction to nanomedicine

Nanotechnology is an emerging interdisciplinary strategy in cancer diagnosis and treatment. Nanotechnology has rapidly developed over the past ten years and has been intensively applied in fields of engineering, medicine, biology, and chemistry. It has been considered as a novel strategy not only in therapeutics but also in the diagnosis of cancer research [15, 16].

2.2 Nanoparticles as a delivery method

Nanoparticles, defined as particles ranging from 1 nm to 1000 nm, have many unique properties compared to larger particles, and have been widely studied for their potential as anti-cancer drug delivery platforms (**Figure 1, top**). Nanoparticles can deliver drugs selectively to tumors, and modification of nanoparticle surfaces allows loaded drug molecules to avoid immune system recognition and elimination by the body. The most often used strategy is fine-tuning the nanodelivery system, in which specially designed nanoparticles are loaded with small drug molecules such as chemotherapeutic agents or inhibitors and targeted directly to the tumor site to block metabolism or knock down protein expression. Nanoparticles can be fabricated with various materials to further increase their encapsulation capacity and modify the surface properties to functionalize the target. Thus, nanoparticles could improve the solubility of hydrophobic drugs and prolong drug circulation time in the bloodstream, which would allow lower effective therapeutic doses and therefore fewer side effects.

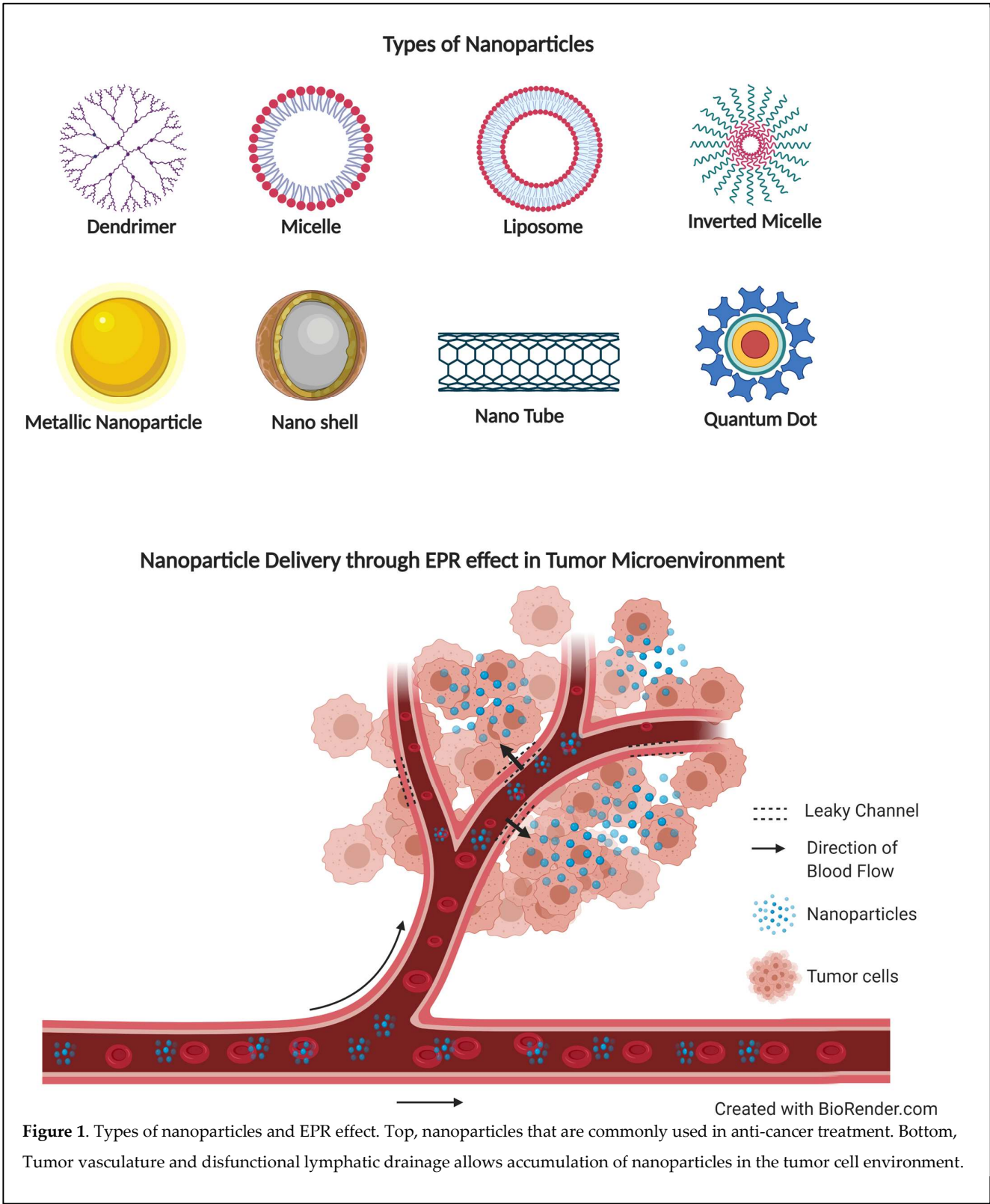
Although the anti-tumor efficacy of traditional chemotherapy drugs, such as anthracyclines or DNA alkylating agents, is well-proven, the risk of toxicity in pediatric patients creates clinical limitations. Many promising drugs, such as the CDK12 inhibitor dinaciclib, have limited clinical application because of their short half-life and high toxicity [17]. In addition, some orally administered PARP inhibitors, such as talazoparib, must

pass through the portal vein, where part of the molecules is degraded before entering the main bloodstream, resulting in lower bioavailability (known as the first pass effect). However, if drugs are encapsulated in specially designed nanocarriers, smaller dosages are needed to generate the same therapeutic effect, which lowers toxicity and accomplishes a more stable blood concentration [18].

Tumors can induce growth of blood vessels to supply their cells with necessary nutrients and oxygen supply, resulting in highly disorganized and abnormal vascular networks containing poorly aligned, defective endothelial cells with wide fenestrations. These tissues lack a smooth muscle layer and adequate lymphatic drainage and have innervation with a broader lumen and impaired functional receptors. Such features improve permeability and increase accumulation for some nanoparticles, such as liposomes. This phenomenon is known as the enhanced permeability and retention (EPR) effect (**Figure 1, bottom**) and is considered an advantage for nanocarriers [19]. With more drug molecules retained in the target tissue, a lower systemically administered dose can be given without compromising therapeutic efficacy. Nanoparticle formulations have far superior pharmacokinetics compared with the current chemotherapy delivery methods. Furthermore, specific antibodies can be conjugated onto the surfaces of nanoparticles; in this way, nanoparticles can recognize and selectively accumulate in designated cancer cells and deliver the loaded therapeutic molecules with increased accuracy.

Cancer cells have different metabolic activity and active signaling pathways than normal cells, which leads to a significant divergence in surface receptor expression. Monotherapies can achieve higher drug levels in tumors by conjugating specially designed antibodies on the surface of nanocarriers targeting tumor cells. This enhances therapeutic efficacy and reduces side effects compared to the same dosage administered conventionally.

A variety of types of nanoparticles developed for drug delivery or as diagnostic agents are described below.



2.2.1 Metallic nanoparticles

Metallic nanoparticles range from 1-100 nm in size and are frequently used as drug carriers and bioimaging agents. They are useful carriers based on their physicochemical

properties, high stability, high reactivity, and photothermic and plasmonic properties [20]. Gold nanoparticles have a large surface-to-volume ratio, and their surface chemistries allow customization to optimize charge, hydrophilicity, and functionality [21].

2.2.2 Dendrimers, micelles, and liposomes

Polymeric micelles, dendrimers, and liposomes are widely used nanocarriers of hydrophobic drugs used to enhance aqueous solubility and prolong the half-life of chemotherapeutic agents in circulation. These nanoparticles are usually 10-100 nm in size and consist of a hydrophilic polyethylene glycol (PEG) outer shell and a hydrophobic core [22, 23]. They are self-assembling in water at a particular concentration and often used for passive targeting as drug carriers. This strategy takes advantage of the EPR effect to deliver increased payloads specifically to tumor sites.

2.2.3 Iron oxide nanoparticles

Superparamagnetic iron oxide nanoparticles have been thoroughly studied as inorganic nanocarrier systems for drug delivery [24-26]. Iron oxide nanoparticles are uniquely advantageous as they are non-toxic, biodegradable, biocompatible, and efficiently cleared from the body through the iron metabolism pathway. Their magnetic behavior also allows them to serve as both contrast agents in MRI imaging for diagnostic purposes and be guided to targeted therapeutic sites by external magnetic fields [27]. As iron oxide nanoparticles are hydrophobic and negatively charged, they are recognized by the phagocytic system and are therefore cleared from the body. Uncoated iron oxide nanoparticles generate dose-dependent cytotoxicity in microbial and murine models [28].

2.2.4 Nanotubes

Carbon nanotubes can be functionalized with bioactive peptides, proteins, nucleic acids, and drugs. When employed in this fashion, they are not immunogenic and display low toxicity in drug delivery. Carbon nanotubes have a high propensity for crossing cellular membranes, resulting in high levels of cellular uptake [29].

2.2.5 Quantum dots

Quantum dots range from 2-10 nm and are nanometric semiconductors with distinctive optical properties, including high quantum yield, size-tunable light emission, and good chemical and photo-stability. They are used for fluorescent imaging with increased transmission of visible light through biological tissue for diagnostic purposes, drug delivery, and optical agents in sensor systems of biomarkers. Some quantum dots that contain heavy metals such as cadmium and mercury exhibit high levels of toxicity; this can be reduced by functionalizing the surface of quantum dots with biocompatible molecules [30].

Thus, nanoformulations can potentially overcome the deficiencies of conventional methods of administering chemotherapy and improve clinical results. Accordingly, the key advantages of the nanoformulations are:

1. More precise dosing in preclinical studies compared to free drugs.
2. Higher dose with less toxicity.
3. Improved pharmacokinetic properties of drugs.
4. More selective, antibody-targeted drug delivery for cancers with specific surface protein expression.

3. Blood cancers

3.1 Leukemia

Leukemias account for about 28% of all pediatric malignancies, and are the most common cancers in children [9]. The disease is characterized by abnormal white blood cells that originated from tissues that produce blood cells, such as bone marrow.

Lipid-based nanoparticles have been tested to treat leukemia. One study reported the use of lipid-based solid nanoparticles to deliver mitoxantrone and a P-glycoprotein (P-gp) inhibitor β -element to overcome multidrug resistance [31]. P-gp overexpression is believed to block the mechanism of multidrug resistance. Thus, co-delivery of the P-gp and mitoxantrone inhibitor can synergistically affect in inhibition and maximize treatment effects. This nanocarrier size is 120 nm with a negative surface charge, was effectively loaded with the drug combination, and maintained colloid stability after administration. Mice treated with the nanoparticle showed higher drug accumulation and slower tumor growth than free drug administration [31].

Immunotherapy is another strategy for cancer treatment, in which immune cells are activated or enhanced in their ability to detect and kill cancer cells. One group explored the use of ionizable lipid nanoparticles to deliver mRNA for chimeric antigen receptor (CAR) T cell therapy [32]. CAR is an FDA-approved treatment for acute lymphoblastic leukemia (ALL), in which T cells are collected from the patient and are engineered by introducing DNA or RNA to produce CARs on their surfaces. CAR T cells can recognize and attack cells with the targeted antigen on their surfaces. When lipid nanoparticles comprised of several ionizable lipids were compared to the traditional RNA delivery method of electroporation in delivering mRNA to Jurkat cells, they exhibited lower cytotoxicity while achieving a high transduction ratio, and both CAR T cell engineering methods elicited potent cancer-killing activity [32].

Magnetic hyperthermia is also applied in anti-tumor therapy by targeting magnetic nanoparticles (MNPs) to the tumor site. In this paper, a kind of leukemia targeting MNPs was developed by immobilizing the epithelial cellular adhesion molecule (EpCAM) antibody on the surface of MNPs (EpCAM-MNPs). EpCAM-MNPs can target and remove leukemia cells circulating in the bloodstream and were reported to decrease viability of human monocytic leukemia (THP1) cells by over 40% [33].

3.2 Lymphoma

Lymphoma is a type of cancer that originates from the lymphatic system, enlarging the lymph nodes and metastasizing to other tissues via the lymphatic fluid. After leukemias and brain tumors, lymphoma is the third most common form of cancer among children. According to the National Cancer Institute, around 2,200 people under age 20 are diagnosed with lymphoma in the United States every year. Hodgkin lymphoma and non-Hodgkin lymphoma comprise approximately 15% of all childhood malignancies [34]. The anti-CD30 antibody-drug conjugate brentuximab and anti-CD20 antibody rituximab are used regularly in adults with lymphoma. However, no targeted agents have been approved for use in pediatric patients with lymphoma [34].

Anaplastic large cell lymphoma (ALCL), the most common T-cell pediatric lymphoma, has an active pathogenic ALK oncogene and shows a high level of cell surface expression of CD30. Zeng *et al.* reported a precision therapy for ALCL using nanoparticles made with RNA-based CD30-specific aptamers and loaded with ALK oncogene-specific siRNA and doxorubicin. The conjugated aptamers allowed the nanoparticles to specifically target ALCL cells, while the loaded gene therapy agent siRNA and the chemotherapy agent doxorubicin enhanced their cancer-killing ability [35].

The overactivation of the PI3K/mTOR signaling pathway in non-Hodgkin lymphoma made the corresponding inhibitor BEZ235 a very promising treatment. However, it was withdrawn from early-phase clinical trials due to its off-target toxicity and poor solubility. To solve these problems, adding specific antibodies on nanoparticles as a targeted drug delivery was a successful strategy [36]. Kin Man Au *et al.* developed a nanoparticle conjugated with two antibodies, anti-CD20 and anti-Lym1, as tumor-targeting components and loaded them with the PI3K/mTOR inhibitor BEZ235 to treat non-Hodgkin lymphoma [37]. Dual antibody conjugation effectively raised the number of nanoparticles retained on target tumor cells and strengthened the anti-tumor activity of BEZ235. This is one example

of how a nanoparticle-based drug delivery system improves the therapeutic window of small-molecule drugs with substantial on- or off-target toxicity.

Besides the delivery of small-molecule chemotherapy agents, using nanoparticles to deliver nucleic acid has been explored as an immediate treatment for cancer cells. A recent study shows the lipid nanoparticle-based delivery of siRNA as interference therapy to attack mantle cell lymphoma by silencing their mRNA associated with cancer cell proliferation [38]. To overcome the compensatory upregulation of the cell cycle regulator cyclin D2 that results from the silencing of cyclin D1 by delivering siRNA, two more target molecules, Bcl-2 and Mcl-1, which prevent apoptosis, were treated with these nanoparticles to encapsulate the corresponding siRNA. JeKo-1 cells showed a 75% apoptosis rate and slower dividing time after the nano-cocktail treatment, demonstrating effective siRNA delivery by the nanoparticle [38].

4. Bone cancer

4.1 Osteosarcoma

Bone cancers occur mostly in older children and teens and account for about 3% of all pediatric cancers [9]. Osteosarcoma is the most common malignancy of the bone tissue and disproportionately affects pediatric patients. It represents 2% of all pediatric cancers and most commonly affects young adults between the ages of 10 and 30 [39]. Most osteosarcomas in this population are high-grade malignant tumors associated with a poor prognosis [39]. For example, according to data from the American Cancer Society, a distant tumor that has spread beyond nearby tissue is associated with a 27% 5-year survival rate across all ages [40]. Because osteosarcoma is the most aggressive bone cancer, it accounts for 9% of pediatric cancer deaths.

The current standard treatment incorporates presurgical chemotherapy to shrink tumors, surgical resection, radiation therapy to remove what cannot be surgically resected, and post-surgery chemotherapy to lower the chance of relapse [41]. However, because surgery is less effective against advanced osteosarcoma, and multidrug resistance makes treatment challenging, new multimodal therapies are being explored.

As with other cancer treatments, nanocarrier delivery to osteosarcoma tumors is an emerging research field aimed at increasing targeted drug delivery and decreasing the necessary dosage and cellular toxicity. This is achieved either through passive delivery, relying on the EPR effect, or active delivery, taking advantage of the osteosarcoma cancer environment's acidic pH and nanoparticle surface modification.

Liposomes have been the most broadly studied vehicle to target osteosarcoma due to their biocompatibility and surface modification ability. [42] Specifically, liposomes have been loaded with doxorubicin and have shown increased cell permeability and tumor cell death compared to free doxorubicin [43]. Some studies have shown success in optimizing liposome nanocarriers to release the drug in the specific temperature and pH of the osteosarcoma tumor [44]. Others have explored the PEGylation of liposomes, which diminishes nanoparticle re-uptake by the reticuloendothelial system, leading to a longer half-life and lower optimal dosage [45]. Recently, an *in vitro* study demonstrated synergistic benefits of gemcitabine and clofazimine when dually loaded into nanoparticles. This dual loading was achieved by loading hydrophobic gemcitabine into the liposome core and hydrophilic clofazimine between the lipid bilayers [46].

RNAi therapies such as microRNAs and siRNAs have shown promise in downregulating proteins produced by osteosarcoma cells and could be effective in conjunction with chemotherapies. [47, 48] However, potential carriers of nucleic acids are still a barrier to progress, as their poor physicochemical characteristics limit bioavailability and cell uptake. Some research has been published on possible biocompatible carriers, such as Amyg-PLLD [49]. PEGylated liposomes have been studied for the delivery of siRNA, both alone and with doxorubicin [50, 51]. In 2017, one study reported enhanced tumor cell uptake, anti-tumor effects, and improved survival rate in murine models using chito-oligosaccharides to enhance drug delivery [52].

4.2 Ewing Sarcoma

Ewing sarcoma is another common malignant bone tumor that primarily affects adolescents and young adults. While it most frequently presents as a bone tumor, it can also develop in connective tissue and soft tissue surrounding bone. Surgery is the most common method for removing Ewing sarcoma, while chemo- and radiation therapy are usually performed to shrink the tumor before surgery or prevent metastasis and recurrence after surgery. Current protocols include five chemotherapeutic agents (cyclophosphamide, topotecan, etoposide, doxorubicin, and ifosfamide), four of which induce DNA damage, as does radiation therapy [53-57]. At relapse, two additional DNA-damaging agents (irinotecan and temozolomide) are routinely used to re-induce remission. However, the incidence of Ewing sarcoma has remained unchanged for 30 years [58, 59], and there is no treatment available for patients who relapse. The relapse rate in Ewing sarcoma patients is also higher than for any other pediatric cancer, with the 5-year event-free survival rate for such patients at only about 20%.

Some research has suggested that using liposomes to deliver chemotherapy for Ewing sarcoma can decrease toxicity and increase drug circulation. In one report, nanoliposome formulas encapsulating the PARP1 inhibitor talazoparib increased the tolerated dose compared to oral administration [60, 61]. Another study found that liposomes formulated from PLGA increased the half-life of docetaxel [62]. Bisphosphonates such as zoledronic acid can inhibit cancer angiogenesis, and because of their affinity to bone, nanoparticles conjugated with bisphosphonates showed increased uptake and cell toxicity compared to pegylated PGLA nanoparticles [63, 64]. Fontaine *et al.* showed that long-acting PEGylated talazoparib has promising anti-tumor activity in Ewing sarcoma [65]. One approach to Ewing sarcoma involves silencing the miRNA that drives CD99, a hallmark surface antibody in the disease. Exosomes from CD-99-deprived Ewing sarcoma cells can act as "natural" targeted nanocarriers of chemotherapy [66].

The simultaneous delivery of two or more drugs that may further sensitize cancer cells (an approach called synthetic lethality) is also a strategy for cancer treatment and recurrence prevention. The Pediatric Preclinical Testing Program (PPTP) identified this synergistic activity in using talazoparib plus temozolomide to treat Ewing sarcoma [61]. However, the severe toxicity of this combination limits its clinical application. Baldwin *et al.* reported that a nanoformulation of talazoparib plus temozolomide reduced gross toxicity and resulted in a higher tolerated dose than oral talazoparib combined with temozolomide [60].

Another study tested the use of a hydrolyzed galactomannan (hGM)-based amphiphilic nanoparticle for selective intratumoral accumulation in pediatric sarcoma [67]. This self-assembled nanoparticle was created by linking the side chain of hGM with poly (methyl methacrylate) through a graft free radical polymerization reaction, encapsulated with tyrosine kinase inhibitor imatinib with a 7.5% of efficiency. The findings suggest that these nanoparticles can target GLUT-1, as the internalization ratio was 100% in rhabdomyosarcoma and Ewing sarcoma.

Alhaddad *et al.* reported the use of a novel diamond nanoparticle coated with cationic polymers to deliver interfering RNA to Ewing sarcoma cells [68]. In this strategy, siRNA is absorbed into diamond nanocrystals, and cell uptake is imaged using the nanocrystals' intrinsic fluorescence caused by embedded color-center defects. Cell toxicity of these coated NDs is shown below. The diamond nanocrystal-vectorized siRNA specifically inhibited expression of *EWSR1-FLI1* at the mRNA and protein levels in a serum-containing medium. Diamond nanocrystals also display fluorescence properties that result from creation of a nitrogen-vacancy color center inside the nanodiamond matrix. This property means they could be used for tracking throughout the lifespans of cells and organisms.

Lipoproteins are also appropriate materials for nanoparticle synthesis. Bell *et al.* employed biomimetic high-density lipoprotein (HDL) nanoparticles. These bind to HDL receptors and scavenger receptor type B-1 (SCARB1), which in turn deprives cells of natural

HDL and their cholesterol stores, and blocks cell proliferation. In medulloblastoma and hedgehog-driven Ewing sarcoma tissues, this strategy depleted populations of cancer stem cells. Furthermore, HDL nanoparticles disrupted cell colony formation in medulloblastomas [69]. This study suggests that HDL-mimetic nanoparticles are a promising therapy for the sonic hedgehog subtype of medulloblastoma.

5. Cancers of the central nervous system

5.1 Brain cancer

Brain cancers comprise the second most common cancer in children, making up about 26% of all pediatric cancers [9]. Brain cancers are named based on the cell type from which cancer originated and the tumor location in the brain. They are treated with surgery, radiation, and chemotherapy. However, brain tumors can present a challenge for surgery and delivery of therapeutic agents depending on their location.

5.2 Blood-brain barrier

The blood-brain barrier (BBB) is a dynamic interface that separates the brain from the circulatory system to protect the brain from potentially harmful chemicals and pathogens and regulate the transport of essential nutrition to maintain a stable microenvironment [70]. The BBB is made up of continuous endothelial cells closely sealed by tight junctions and surrounded by astrocytes, pericytes, and the continuous basement membrane. It is a highly selective semipermeable border with a high expression of distinct sets of transporter proteins that only permit the free diffusion of essential small molecules like oxygen. The effectiveness of the BBB means that fewer drugs can be efficacious and the prognosis of pediatric patients with brain cancer is worse [71, 72].

Overwhelmingly, the literature suggests that molecular size plays a vital role in BBB penetration. However, molecular size does not necessarily interfere with BBB permeability [70]. Some small molecules with a molecular weight of around 100 Da, like histamine, do not enter the brain due to the BBB [73]. But if candidate agents interact with the major transporters on the BBB, it can help them pass through it. The inability of many drugs to reach the brain is attributed to the activity of ATP-binding cassette (ABC) efflux transporters, such as breast cancer resistance protein (BCRP) [70].

Nanotechnology has enabled significant progress in delivering therapeutic agents across the BBB. Materials such as gold, lipids, and proteins are being investigated as cytostatic agents or drug carriers to treat or diagnose brain cancer. Nanoparticle size and surface are two main factors that affect ability to cross the BBB. Large particles (over 150 nm in diameter) tend to be blocked by it, whereas a slightly positive surface charge can be favorable for particle-endothelial cell binding [72]. Antibodies with a molecular weight larger than 500 kDa, such as intact IgG typically used to treat various types of cancers, show low penetration of the BBB [74]. Smaller antibodies such as single-chain variable fragments or fragment antigen-binding (Fab) may improve penetration into the central nervous system.

Apart from diffusion, ligand-induced transcytosis is also being tested for delivering drugs across the BBB. While BBB is a highly selective semipermeable endothelial cell border, the unique protein transporters expressed on its membrane that allow necessary nutrients to maintain brain homeostasis provide ideal targets.

The transferrin receptor (TfR) is responsible for transporting iron into the brain parenchyma to maintain appropriate iron levels needed for brain metabolism, neural conductivity, and overall brain function [75]. The TfR is an intriguing and unique target since it is exclusively expressed on the endothelial cells of the brain capillaries and not on endothelial cells lining the vessels in other tissues [76, 77]. This specific property makes transferrin receptor antibody an intriguing concept for delivering drugs through the BBB. When conjugated to nanoparticles, such as liposomes, BBB targeting and penetration will be significantly improved [78-81].

Similarly, cell-penetrating peptides (CPP), which facilitate cellular uptake of molecules ranging from nano-size particles to small chemical compounds to large fragments of DNA, have been used to penetrate the BBB. Wang *et al.* tested the efficacy of CPP and transferrin modified liposomes (Tf-LPs) loaded with doxorubicin to treat glioma. This nanoparticle, which was 120 nm in size and had a zeta potential of 6.81 mV, showed enhanced cellular uptake and reduced toxicity in two types of glioma cells compared to free doxorubicin [82].

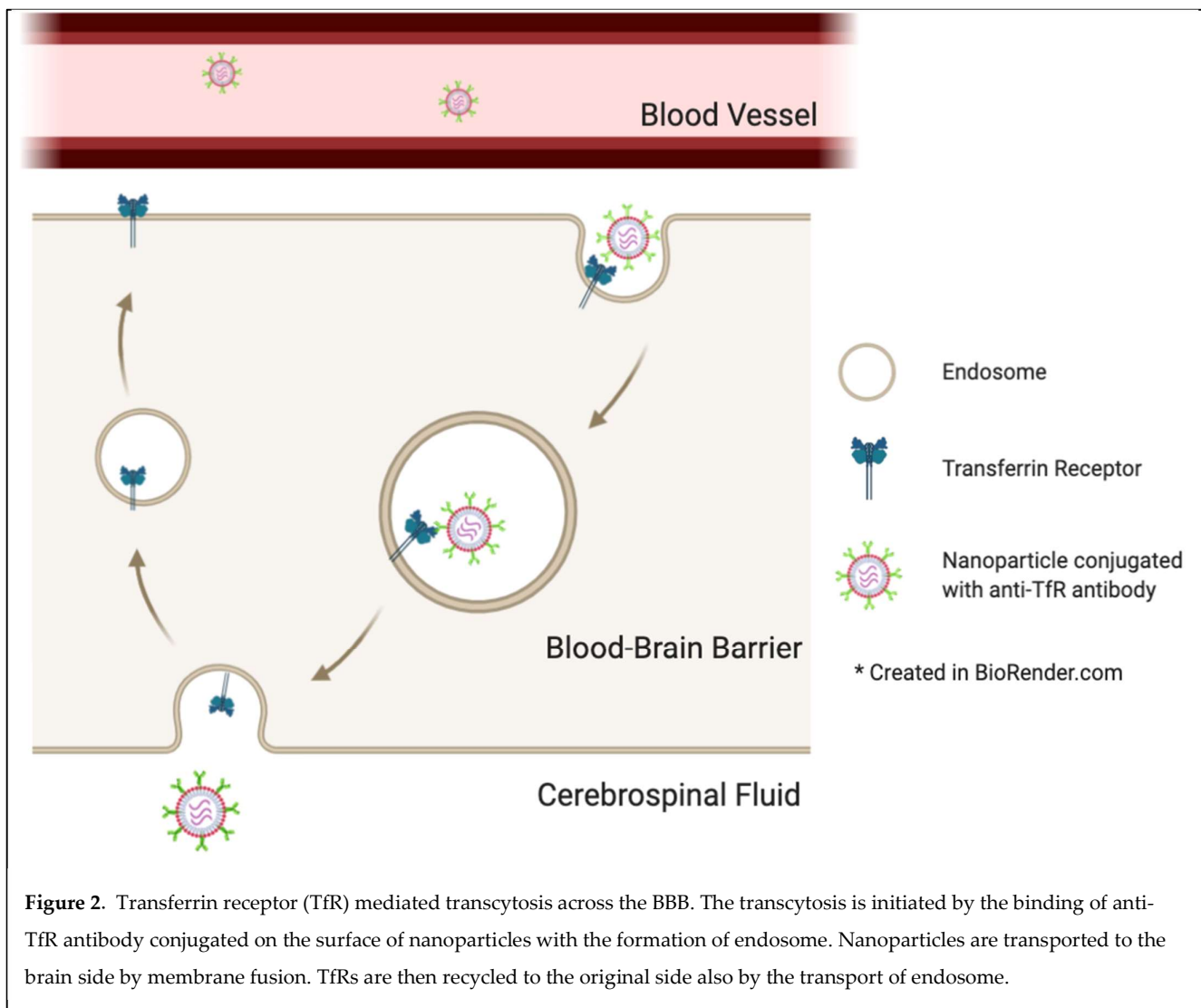
Another promising marker for malignant glioma is amplification of epidermal growth factor receptor (EGFR) expression with a frequency of about 50%. [83, 84] ErbB1 belongs to the ErbB family of receptor tyrosine kinases, including human epidermal growth factor receptors (HER)-2/ErbB2, HER-3/ErbB3, and HER-4/ErbB4. [85] Application of EGF, the natural ligand of EGFR, is a potential strategy to target every subset of tumor cell expressing wild-type EGFR as well as its mutant forms. Conjugation of EGF to the nanoparticle could enable targeted treatment for glioma [86, 87].

Immunotherapy is more commonly used for brain tumors than for other tumor types because of the brain's unique environment, which can be regarded as an immune-privileged site separate from the rest of the body that prohibits immune cells from entering [88-91]. Therefore, the microglia take a predominant position inside the brain and tend to be pro-tumorigenic under certain conditions, such as intensive secretion of growth factors and lack of appropriate T-cell regulation. Moreover, most brain tumors have a unique extracellular structure that provides inhibitory regulation of T cells to prevent their migration and activation [92]. This suggests that immunotherapy should be adjusted based on the specific cancer type or even certain gene mutations and their surrounding micro-environments. Specifically, the selected tumor antigens should have a tumor-unique expression pattern, the ability to activate T cells and elicit the ensuing immune response, and ideally should downregulate tumor functions to minimize tumors' ability to bypass the immune system. Short peptides or antibodies are the most frequently used immunostimulating agents for this purpose due to their specific binding capacity [93]. They are commonly used together with a drug delivery platform (nanoparticles loaded with a combination of drugs) as a dual treatment method [94-96].

5.3 Glioma

Glioma is one of the most common brain cancers in children and adolescents, and originates from glial cells, which support and nourish nerves in the brain [97]. A folacin-modified poly(ϵ -caprolactone) micelle was designed to deliver luteolin – a xanthone extracted from vegetables with broad spectra anti-cancer effects – to treat glioblastoma [98]. Folate acids were conjugated onto the surface of this nanoparticle to allow binding to the folate receptor, a type of glycoprotein with increased expression in many tumor tissues. Compared to free luteolin and micelles without folacin modification, luteolin-loaded folate acid-modified micelles to glioma tissues induces a significantly higher cell inhibition and increased apoptosis in glioma [98].

TfR are overexpressed in both BBB endothelial cells and gliomas. Fan *et al.* reported a trans-BBB delivery strategy that used Human H-Ferritin and L-Ferritin protein-covered iron oxide nanoparticles (HFn) to target TfR of the BBB endothelial cells and induce transcytosis [99] (**Figure 2**). The nanoparticles demonstrated adequate loading capacity for various drugs and excellent dual tumor-targeting prospects. They were carried through the BBB in the endosome by TfR-mediated transcytosis, recognizing and entering the glia cells by human H-ferritin receptor-mediated tumor targeting.



5.4 Medulloblastoma

Medulloblastoma is a common pediatric brain tumor located in the cerebellum, the lower back part of the brain that controls coordination, movement, and balance. Once established, medulloblastoma tends to spread to other parts of the brain through the cerebrospinal fluid, but rarely spreads to other body tissues. It occurs at any age, but most likely to happen in childhood, and is rare in adults.

Choi *et al.* explored the strategy of suicide gene therapy for pediatric brain cancer medulloblastoma. Poly(beta-amino ester) nanoparticles were developed to deliver plasmid DNA encoding the suicide gene of herpes simplex virus I thymidine kinase [100]. Delivery of the virus suicide gene induced controlled apoptosis of transfected cancer cells and prolong overall survival in mice. These results suggest that these biodegradable nanoparticles could be a safe and effective method for treating pediatric CNS malignancies.

Kim *et al.* engineered high-density lipoprotein-mimetic nanoparticles with enhanced stability and targeting ability for treating the sonic hedgehog (SHH) subtype of medulloblastoma [101]. Apolipoprotein A1 was incorporated into the shell of the nanoparticle and provided better structural stability while keeping LDE225, an SHH inhibitor, encapsulated in the hydrophobic core of the nanocarrier. Anti-CD15 was conjugated on the surface of engineered high-density lipoprotein-mimetic nanoparticles (eHNPs) for receptor-

facilitated delivery. eHNPs can serve as stable drug carriers while also providing a therapeutic effect through SR-B1-mediated intracellular cholesterol deletion in SHH medulloblastoma.

Radiotherapy is an integral component of cancer treatment. However, the radiation-induced adverse sequelae and resistance create clinical limitations. Thus, combining radiotherapy with different therapeutic agents that block specific DNA repair pathways could achieve a better therapeutic efficacy than monotherapy with a lower radiation dosage that minimizes potential adverse effects. Kievit *et al.* reported a strategy to sensitize pediatric tumor cells, including medulloblastoma and ependymoma cells, to radiotherapy by nanoparticle-based delivery of siRNA; this knocks down the expression of Ape1, an enzyme involved in the base excision repair pathway [102]. This superparamagnetic iron oxide nanoparticle was coated with chitosan, PEG, and polyethyleneimine and can bind to siRNA and protect it from degradation. The treated medulloblastoma and ependymoma cells exhibited over 75% reduction in Ape1 expression and 80% inhibition of Ape1 activity, which indicates the potential for siApe1 as an efficacious delivery strategy.

6. Less Common Cancers

6.1 Retinoblastoma

Retinoblastoma makes up 3% of all childhood cancers, and in severe cases, it can cause blindness and metastasis beyond the eye if not treated promptly and effectively [103, 104]. Ocular malignancies pose unique challenges, and monotherapies must provide enhanced permeation through the retinal pigment endothelial layer [105]. A variety of nano-applications have been explored in recent years to surpass these challenges. One study found that introducing biodegradable nanoparticles into tumor tissues during laser irradiation treatment allowed for selective damage to retinal cancer cells by decreasing the heat capacity and increasing the thermal conductivity of cancerous tissue. Sensitizing the cancerous tissue refined the treatment and increased the lethal zone area by 51% [106]. A similar study found that cytotoxicity of retinoblastoma cells treated with ultrasonic hyperthermia increased with the concentration of gold nanoparticles present. The same cell viability (50%) was achieved in half the time when gold nanoparticles were present. [107] Another study evaluated the efficacy of injecting conjugated gold nanorods into the eye (using femtosecond pulse lasers) to selectively accumulate in retinoblastoma cells and induce ablation of only cells containing the nanorods. In combination, gold nanorods and femtosecond pulse lasers decreased viability of retinoblastoma cells to about 10%, compared to 100% viability in untreated cells [108].

Researchers have explored the delivery of natural therapeutics through nanoparticles to treat retinoblastoma. One study utilized polymeric nano-micelles as a delivery method to improve the water solubility of celestrol, a Chinese herb that displays inhibition of angiogenesis mediated retinoblastoma growth in murine models [109]. Similar to many other pediatric cancers, nanoparticles have been used for drug delivery to reduce cytotoxicity of chemotherapies. A study explored the targeted co-delivery in lipid nanoparticles of miR-181a, a microRNA, and melphalan, a currently approved chemotherapy for retinoblastoma. The nanoparticles were outfitted with a cationic lipid that changes conformation at the acidic pH of retinoblastoma tissue to trigger endosomal release, as well as several structural lipids to improve the structure, fluidity, and colloidal stability. *In vivo*, the combination therapy reduced retinoblastoma cells by 72% compared to treatment with free melphalan alone [110].

6.2 Wilms Tumor

Wilms tumor is the most common form of pediatric kidney cancer and accounts for 5% of all pediatric cancers [111]. Nanomedicine applications are sparse in Wilms tumor. One *in vitro* study targeted neural cell adhesion molecule (NCAM) expressed by Wilms tumor stem cells with a nanosized conjugate of paclitaxel bound to a biodegradable

polyglutamic acid polymer. The conjugate reduced tumor size by roughly five times compared to untreated cells [112].

6.3 Other Pediatric Cancers

Nanomedicine is still a relatively new field, and new oncology applications are being developed at exponential rates. The applications discussed above could be modified to treat several rare yet consequential pediatric cancers. Hepatoblastoma, hepatocellular carcinoma, pleuropulmonary blastoma, and tracheobronchial tumor together account for less than 2% of all pediatric cancers and require further exploration into nano-applications and diagnostics and treatment.

7. Conclusions

With the maturity of novel anti-cancer therapy, more and more nanoparticle-based drugs are also being FDA-approved, improving outcomes for adult cancer patients. Nonetheless, effective strategies for treatment-resistant pediatric cancer remain elusive and only a few drugs are now approved for pediatric patients. Roadblocks to progress include ethical challenges unique to pediatric patients, such as the need to involve parents of younger patients in the informed consent process and the possibility that adolescents will not wish to share their patient information with others. Additional concerns surround genetic testing of newborns and the possibility of later discriminatory practices or other legal issues for themselves and their families.

Currently, pediatric cancer is commonly treated with chemotherapy, which triggers potential severe side-effects and causes toxicity to normal tissues. With different metabolic rates and immature organs, the tolerated dose is also different in children and adults, making it more challenging to identify the optimal dosage. To avoid drug resistance often brought about by monotherapy, the combination of two or more chemotherapy agents changed little over the past two decades. However, organs and tissues grow rapidly in children, which means that they can respond differently to the medication at different developmental stages. As a result, the pediatric cancer survival rate remains low. What is worse, a large portion of survivors suffers from short- or long-term adverse effects, such as nephrotoxicity, cardiotoxicity, infertility, and deafness. Targeted therapy is studied extensively and believed to be a promising strategy to overcome off-target toxicity. Nanoparticles can easily achieve this goal by conjugating the antibodies or specific peptides against the target proteins. This emerging field is of high interest to researchers in academia and pharmaceutical companies, leading to tremendous development progress. All the nanoformulations discussed in this review are shown in table 1. However, most novel nanomedicine milestones have been achieved for adult cancers, while pediatric cancer treatment is still in the early stage. For example, the biocompatibility of several liposome formulations is well-known, and many nanodrug delivery systems have reached the clinical phase in adults. However, information regarding their safety in children is very limited [113]. The main obstacles for pediatric nanomedicine development are the current dearth of clinical trial protocols and the ethical issues mentioned above. However, as nanotechnology is more broadly accepted and more nanotechnology-related products are developed, greater use of nanomedicine applications (also as part of the RACE for Children Act) for childhood cancer therapy is expected in the near future.

FUNDING

This work is supported by Army Breast Cancer Research Program (W81XWH-16-1-0731) (SY, MW, AK, SS) and Greehey Children's Cancer Research Institute at the University of Texas Health at San Antonio (RK).

Table 1. Nanoformulations discussed in this review.

Formulation	Drug loaded	Targeting anent	Size (nm)	ZP (mV)	Diseases	Animal model	Route	Reference
Solid Lipid Nanoparticle	Mitoxantrone β -element	-	124.6	0.162	Leukemia	K562/DOX xeno-grafts tumor model mice	IV ¹	[31]
Ionizable Lipid Nano-particle	mRNA	-	70	-	Leukemia	-	-	[32]
Magnetic nanoparticles	Hyperthermia effect	Epithelial cellular adhe-sion molecule	5	-	Leukemia	AKR mice	IP ²	[33]
Aptamer-equippeded pro-tamine nanoparticle	dsDNA/ Doxorubi-cin complex, siRNAs	Oligonucleotide ap-tamers	103	-	Lymphoma	-	-	[35]
PEG-PLGA nanoparticle	BEZ235	Anti-CD20, anti-Lym1	70	-	Lymphoma	CD-1 mice	IV	[36]
Lipid nanoparticle	siRNA	-	100	-	Lymphoma	-	-	[38]
Liposome	Doxorubicin	-	93.61	-23	Osteosarcoma	-	-	[44]
PEGylated-liposome	Doxorubicin	-	-	-	Osteosarcoma	Phase II trial	IV	[45]
Liposome	Gemcitabine, Clo-fazimine	-	135	-9.3	Osteosarcoma	-	-	[46]
Polysaccharide nanopar-ticle	siRNA	Folic acid	270	10	Osteosarcoma	Osteosarcoma 143B cells. Tumor-bear-ing mice models	IV	[49]
PEGylated liposome	siRNA	-	100	19.24	Osteosarcoma	-	-	[50]
PEGylated liposome	Doxorubicin, JIP1 siRNA	YSA peptide	108.9	18.47	Osteosarcoma	-	-	[51]
Chitooligosaccharides Modified Liposome	Doxorubicin	Chitooligosaccharides	100	33.9	Osteosarcoma	MG63 cell-bearing nude mice	IV	[52]
Liposome	Talazoparib	-	74.5	15.3	Ewing sarcoma	NCr-nu/nu and scid CB17 mice	IV	[60]
Galactomannan-based nanoparticle	Imatinib	Monosaccharide and disaccharide residues	84	-0.5	Ewing sarcoma, rhabdomyosarcoma	Mice bearing PDX models	IV	[67]

Diamond nanoparticle	siRNA	-	50	27	Ewing sarcoma	Ewing sarcoma mouse model	IV	[68]
High-density lipoprotein nanoparticle	High-density lipoprotein	High-density lipoprotein	10	-	Medulloblastoma, Ewing sarcoma	-	-	[69]
Liposome	Doxorubicin	Cell-Penetrating Peptide, Transferrin	128.64	6.81	Glioma	Intracranial U87 glioma-bearing mice	IV	[82]
PEGylated micelle	Luteolin	Folic acid	34.7	-9.2	Glioma	C57 mice	IV	[98]
H-ferritin nanoparticle	Doxorubicin	H-ferritin	12	-	Glioma	U87MG orthotopic tumor-bearing mice	IV	[99]
PBAE nanoparticle	Plasmid DNA	-	100-200	12	Medulloblastoma	Athymic nude mice with 5e5 BT-12 cells	IP	[100]
High-density lipoprotein nanoparticle	LDE225	Apolipoprotein A1, anti-CD15	15	-	Medulloblastoma	SmoA1+/+;Math1-GFP+/+ SmoA1 MB tumor-bearing mice	IV	[101]
PEGylated iron oxide nanoparticle	siRNA	-	40	15	Medulloblastoma, ependymoma	-	-	[102]
Magnesium oxide nanoparticle	Hyperthermia effect	-	-	-	Retinoblastoma	-	-	[106]
Gold nanoparticle	Ultrasound hyperthermia	-	89	38.6	Retinoblastoma	-	-	[107]
PEGylated gold nanoparticle	-	Anti-EpCAM	11	-	Retinoblastoma	Squamous cell carcinoma xenografts in nu/nu mice	IV	[108]
Celastrol nanomicelle	Celastrol	-	48	12	Retinoblastoma	Female NOD-SCID mice	IP	[109]
Lipid nanoparticles	miR-181a, melphalan	-	171	24.5	Retinoblastoma	-	-	[110]

PGA nanoparticle	Paclitaxel	NCAM targeting pep- tide	10	-	Wilms Tumor	NOD/SCID mice	IV	[112]
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¹IV: intravenous administration. ²IP: intraperitoneal administration

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