

## Special Section on Drug Delivery Technologies—Minireview

# Therapeutic Opportunities in Neuroblastoma Using Nanotechnology

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### ABSTRACT

Neuroblastoma (NB) is the most common extracranial solid tumor preferentially occurring in preschoolers. Its characteristic aggressiveness and heterogeneous clinical behavior are especially visible in relapsed or refractory cases and hamper therapeutic success. Although the introduction of novel anti-tumor agents, such as dinutuximab, isotretinoin, irinotecan, or I-131-metaiodobenzylguanidine, has increased survival rates, the situation in high-risk NB remains dismal. Moreover, treatment is particularly aggressive in these patients, leading to short- and long-term toxicities. The extensive research performed

using nanotechnology in recent decades has prompted its application as a therapeutic alternative to overcome some of the common limitations of conventional chemotherapy. Nevertheless, the therapeutic role of nanomedicine in pediatric tumors like NB is not fully elucidated, and to date, only albumin-bound paclitaxel nanoparticles have reached clinic stages. In this review, we summarize the current therapeutic strategies for NB with special attention to the use of nanomedicine. We also highlight the preclinical studies on passive and active targeting nanodelivery of therapeutics in experimental NB models.

### Introduction

Neuroblastoma (NB) is the term that commonly refers to a family of peripheral neuroblastic or neurogenic tumors diagnosed almost exclusively in childhood, classified histologically as ganglioneuroblastoma, ganglioneuroma, and NB (Matthay et al., 2016). This embryonal tumor of the sympathetic nervous system arises from the primitive neural crest cells and normally originates in the adrenal glands or the retroperitoneal paravertebral ganglia (Brodeur and Bagatell, 2014; Luksch et al., 2016). Thus, the most common location of the primary tumor is the abdomen, normally the adrenal medulla, but patients can also have tumors also along the paraspinal or other sympathetic ganglia from the neck to the pelvis (Brodeur, 2003). NB cells are prone to spread and invade other tissues, and metastatic sites are numerous,

including bone marrow, bones, lymph nodes, skin, liver, lung, and sometimes central nervous system (Abbasi et al., 2017). Clinical presentation and symptoms are directly related to the location of the primary tumor and the metastatic sites: from an asymptomatic palpable mass to an abdominal distension, compression of nearby organs, and systemic manifestations.

NB represents 7% of total childhood cancers and is the third most common cancer and most frequently occurring extracranial solid malignancy in childhood (Steliarova-Foucher et al., 2017). It is the most frequently diagnosed cancer during the first year of life, and approximately 90% of cases are diagnosed before the age of 5 years, whereas its incidence is minimal in children older than 10 years (Ward et al., 2014). NB is responsible for 15% of childhood cancer deaths. Although prognosis in infants is favorable, 5-year survival rates remain below 70%, especially for children with high-risk disease (Esiashvili et al., 2009; Trama et al., 2016). The modest progress in survival has been associated with its clinical behavior diversity owing to its biologic complexity and heterogeneity, leading to spontaneous recurrences and relapses.

Regarding the management of NB, diagnosis and staging represent key points in the selection of the best treatment

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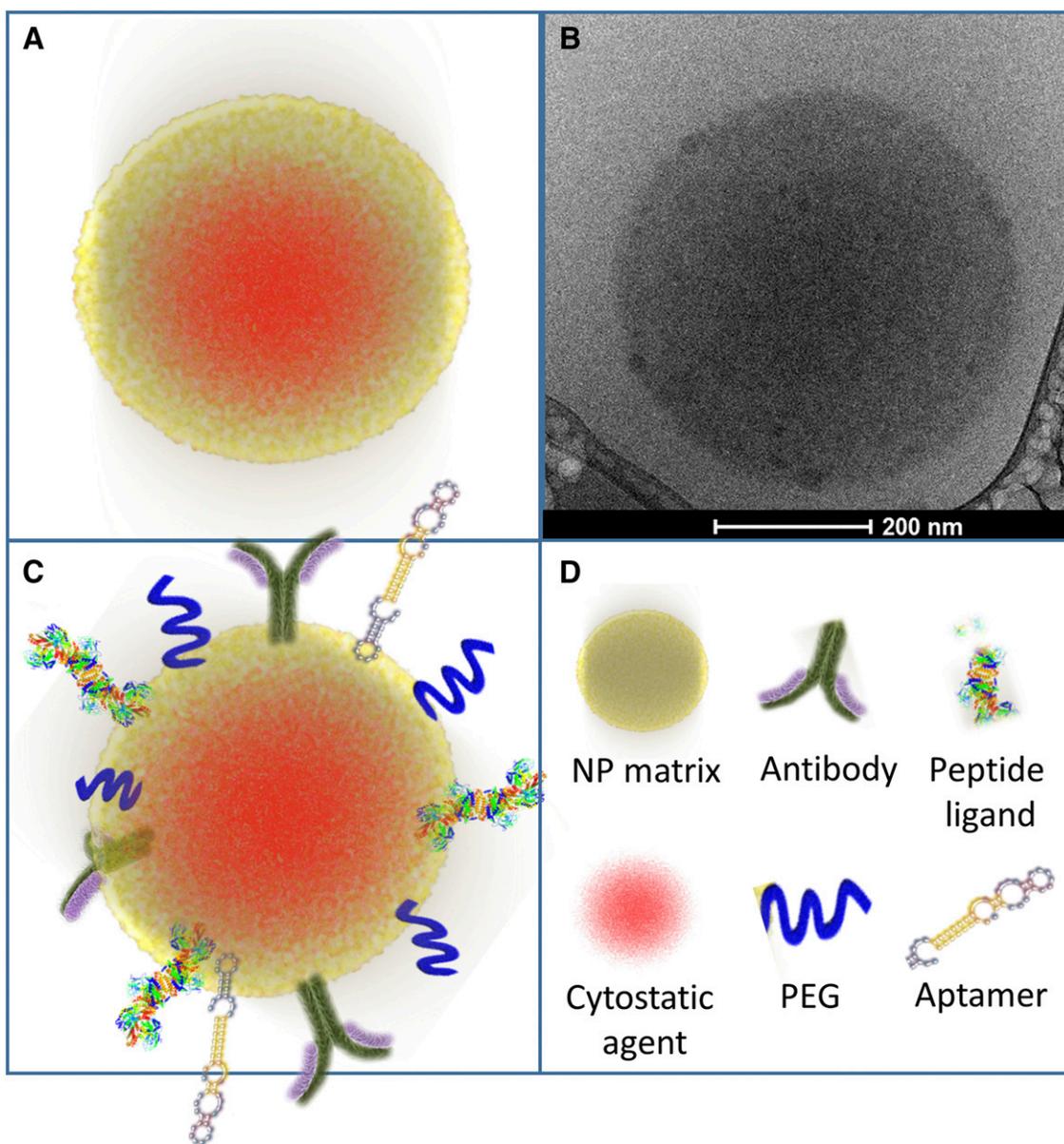
**ABBREVIATIONS:** ALK, anaplastic lymphoma kinase; DOX, doxorubicin; EPR, enhanced permeability and retention; GD2, disialoganglioside 2; HDL, high-density lipoprotein; MIBG, metaiodobenzylguanidine; MRI, magnetic resonance imaging; Nab-PTX, albumin-bound paclitaxel nanoparticles; NB, neuroblastoma; NP, nanoparticle; PEG, poly(ethylene glycol); PTX, paclitaxel; siRNA, small interference RNA.

option. Exploratory surgery, followed by histologic confirmation and ultrasonography, is the mainstay for a primary diagnosis, whereas computed tomography and magnetic resonance imaging (MRI) have become essential, especially to detect tumor spread. Although the complete detection of metastases remains a challenge in aggressive NBs, the application of nuclear medicine has shown high specificity and improved the accuracy of tumor localization (Swift et al., 2018).

Therapeutic modalities include surgery, radiotherapy, and chemotherapy. Sometimes, surgery is the only treatment needed, but chemotherapy and radiotherapy are mandatory in metastatic and locally advanced disease. Current chemotherapeutic protocols are occasionally insufficient to effect cures and may endanger survivors' health as they grow up (Robison and Hudson, 2014; Elzembely et al., 2018). This problem denotes the need to search for novel

therapeutic strategies with the aim of beating the disease, as well as minimizing chemotherapy- and radiotherapy-associated toxicity. In this context, drug-delivery systems have demonstrated many benefits by improving drug bioavailability and biodistribution (Sieswerda et al., 2011; Ravi Kumar et al., 2013). Cancer nanomedicine emerged two decades ago to offer solid therapeutic alternatives to common chemotherapy, as illustrated by the market launch of liposomal doxorubicin (DOX) (Doxil) in 1995 (Shi et al., 2017).

Nanomedicines include several types of colloid systems at the nanoscale size (1–1000 nm) (Fig. 1). They are constructed on polymers, lipids, or metals, among other materials, that allow for encapsulation of multiple therapeutic agents (e.g., small molecules, peptides, or nucleic acids) (Smith et al., 2017; McClements, 2018). A wide range of nanoformulations is available based on liposomes, micelles, solid/porous nanoparticles (NPs), dendrimers, carbon nanotubes, and many



**Fig. 1.** General features of nanomedicines. (A) Nontargeted NP for cytostatic drug delivery. (B) Image of cryo-transmission electronic microscopy (Cryo-TEM) showing a squalenoyl-gemcitabine nanoassembly under investigation for pediatric cancer treatment by our group. (C) Targeted NP for cytostatic drug delivery. (D) Components and moieties of nanomedicines.

other nanocarriers, including prodrug squalene-based nano-assemblies (Fig. 1B), that have shown encouraging outcomes in the therapeutic index improvement of several antitumor compounds (Peer et al., 2007). An added reason for success has been associated on some occasions with an enhanced permeability and retention (EPR) effect in tumor tissues (Maeda et al., 2000). Furthermore, nanocarriers can be functionalized at their surface with monoclonal antibodies to deliver drugs selectively toward tumor tissues (targeted nanomedicines) or with hydrophilic molecules [e.g., poly(ethylene glycol) (PEG)] to increase their blood circulation time in the body (Fig. 1C). Nanotechnology has also been proposed as a promising tool for cancer detection because of the physicochemical properties of NPs in which the superparamagnetism of metallic NPs for MRI is noteworthy (Chen et al., 2018a).

This framework fosters the introduction of nanomedicines in current NB protocols to improve therapeutics, especially in high-risk or relapsed NB patients for whom common treatments are ineffective. The goals of this review are to describe the current state-of-the-art NB therapeutics with special attention on the incoming role of nanomedicines for the management of this childhood cancer. The outcomes concerning the research and development of nanoformulations that have been tested in *in vitro* and *in vivo* models of NB in the last 5 years are discussed in depth.

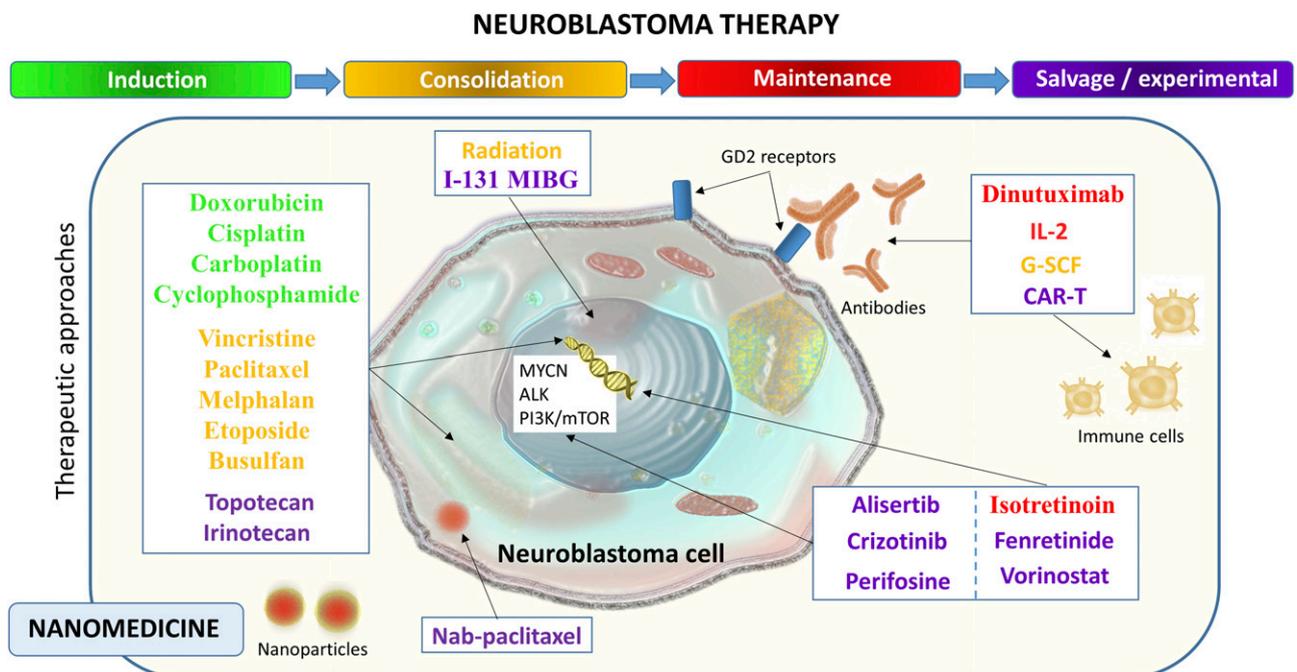
### Therapeutic Strategies in Neuroblastoma: Targeting High-Risk Disease

At diagnosis, patients with NB can be classified according to clinical and biologic characteristics into three different risk groups: low, intermediate, or high (Cohn et al., 2009). The mainstay of treatment of low-risk patients involves a complete surgical resection but in some cases, the tumor undergoes a spontaneous regression (Brodeur and Bagatell, 2014). Intermediate-risk group patients receive from two to

eight cycles of chemotherapy together with surgical resection. Low- and intermediate-risk patients have an overall survival rate >90%; however, survival of high-risk NB patients is still <40% (Pinto et al., 2015). They frequently show an early inadequate treatment response so that therapy must be intensified. Sadly, treatment is, in many cases, limited and unable to eradicate completely the metastatic cells (Speleman et al., 2016). Considering this dismal situation, the search for novel therapeutic strategies (including cancer nanomedicine) should draw our attention.

**Conventional Chemotherapy.** NB protocols include vincristine, cyclophosphamide, cisplatin, DOX, ifosfamide, carboplatin, topotecan, irinotecan, and taxoids, among many other cytostatic drugs (George et al., 2010). The regimen selection varies according to risk stratification and phase of treatment, which is defined as induction, consolidation, and maintenance therapy or postconsolidation (Fig. 2). The induction stage entails multiple cycles of anthracyclines and alkylating agents, aimed at reducing the tumor area before surgical resection and arresting metastatic spread. Afterward, a myeloablative chemotherapy regimen is given in the consolidation phase, followed by autologous stem cell rescue, bone marrow transplantation, and granulocytic cell-stimulating factor administration (Sung et al., 2013). The establishment of this strategy has considerably improved outcome and holds the key to achieving tumor remission in high-risk NB patients. Thus, the optimal conditioning of this regimen is currently under investigation to lower toxicities and prolong survival. These upgrades include a strategic choice of the stem cell-collection stage and the anticancer drug cocktail, based on a combination of busulfan/melphalan or carboplatin/etoposide/melphalan or carboplatin/etoposide/cyclophosphamide (Smith and Foster, 2018).

In the maintenance phase, treatment is supplemented with isotretinoin, together with immunotherapy. Salvage or experimental protocols have recently introduced topotecan,



**Fig. 2.** Management of neuroblastoma. Current therapies in clinic (chemotherapy, radiotherapy, immunotherapy, molecular therapy, and nanomedicine) versus treatment phases (induction, consolidation, maintenance, salvage/experimental).

irinotecan, temozolomide, and fenretinide in clinical settings to prolong survival (Villablanca et al., 2011; Di Giannatale et al., 2014; Mody et al., 2017). Interestingly, the synthetic retinoid derivative fenretinide, currently in phase 2 clinical trials (NCT00053326), has been reported to trigger not only an antitumor effect similar to that of isotretinoin but also an immune-modulatory effect (Applebaum et al., 2017a).

**Radiotherapy.** NB is considered a radiosensitive tumor, and radiation is given mainly in the consolidation phase, although it can be restricted because of its adverse effects (Li et al., 2017). On the other hand, functional imaging with nuclear scintigraphy (computed tomography or MRI) is fundamental, particularly for accurate staging, treatment planning, and detection of metastatic disease (Chen et al., 2018b). The most widely used radioisotope for this purpose is the I-123 metaiodobenzylguanidine (MIBG), with a sensitivity of approximately 90% (Sharp et al., 2016). Bearing this in mind, the I-131 MIBG, a radio-metabolic epinephrine analog, has been used alone or combined with chemotherapeutics as an alternative radiotherapeutic approach in refractory cases and salvage therapy (Kayano and Kinuya, 2018). With the same localizing properties of I-123 MIBG, but with a longer half-life, it delivers a focal dose of radiation to all the tumor sites with promising response rates in refractory NB (Matthay et al., 2012). A phase 3 study has now been launched for newly diagnosed high-risk NB patients to test whether the addition of I-131 MIBG at induction chemotherapy improves survival (NCT03126916).

**Immunotherapy.** The high heterogeneity of NB derives from its transient embryonic structure, leading to a wide range of cellular phenotypes, sometimes present even in a single tumor (Aygun, 2018). The phenotypic analysis has uncovered miscellaneous types of highly expressed surface markers that can also act in tumorigenesis or contribute to progression of the oncologic process [e.g., CD117, CD133, CD114, CD57, CD171, or disialoganglioside 2 (GD2)] (Malone and Stegmaier, 2017; Kholodenko et al., 2018).

At present, tumor-associated gangliosides have become the most important targetable epitopes in the clinical setting (Suzuki and Cheung, 2015; Sait and Modak, 2017). These molecules participate in cellular communication, adhesion, growth, and differentiation. In fact, the Food and Drug Administration approved dinutuximab (Unituxin) in 2015, the first approved anti-disialoganglioside 2 (GD2) specific monoclonal antibody, for the treatment of high-risk NB patients (Ploessl et al., 2016). This immunotherapeutic approach is in many cases administered along with interleukin-2 and isotretinoin in the maintenance therapy. Clinicians have reported that the combination of this anti-GD2 antibody with other therapies increased the 5-year survival rates of NB patients by 20% (Kholodenko et al., 2018). An incoming Children's Oncology Group trial is planning to include immunotherapy in the induction stage to evaluate the benefits when combined with chemotherapy.

Some cell therapy and genome-editing approaches have recently reached clinical trials. For instance, the infusion of haplo-identical natural killer cells in combination with other therapies is currently being investigated in phase 2 clinical trials (NCT00698009). One goal of this approach is to determine whether the cytolytic action of natural killer cells against NB cells could lower the doses of the concomitant treatments required and, thus, reduce their toxicity (Nguyen et al., 2018a). In addition, the next generation of chimeric antigen receptor

T cells for adoptive cell therapy will be directed against GD2-positive tumors (Tesfaye and Savoldo, 2018). Another strategy currently in phase 1/2 studies is based on gene modification via virus of autologous NB cells separated from the patient to secrete lymphotactin, an interleukin-2 (NCT00062855). Once reinjected into the patient, these gene-modified NB cells can attract the immune system, helping the body kill the malignant cells. The results of these studies will determine whether this immune response can overcome active, recurrent NB.

**Next-Generation Antitumor Therapies.** In recent years, extra efforts have been made to define the molecular landscapes of NB (Matthay et al., 2012; Cheung and Dyer, 2013). The use of next-generation sequencing approaches led to the identification of new molecular targets, such as *ALK*, *PHOX2B*, *BARD1*, *TERT*, *ATR*, and *PTPRD* genes, in addition to *MYCN* gene (Peifer et al., 2015; Schulte and Eggert, 2015; Cao et al., 2017). Among them, *MYCN* amplification is reported to be present in around 25% of cases and is associated with aggressive NBs (Tonini et al., 1997).

NB relapse is reported to present an increased mutational burden and, hence, a greater pool of targetable aberrations that translate into novel therapeutic opportunities (Fletcher et al., 2018). For instance, serine/threonine kinases Aurora A and Aurora B inhibitors, such as alisertib, are crucial regulators of the cell cycle and have also shown to be efficient blockers of MYCN/MAX interactions (Mossé et al., 2012). The only druggable target clearly validated in this tumor to date is the anaplastic lymphoma kinase (*ALK*) mutation. In favor of the proposed *ALK* inhibitors were the findings that crizotinib holds promise for treatment of patients with deregulated *ALK* function (Esposito et al., 2017). Epigenetic regulation has also been reported to be a decisive factor in tumor development (Gröbner et al., 2018). Recently, the histone deacetylase inhibitor vorinostat reached phase 2 clinical trials to treat high-risk NB patients (NCT02559778). Within the multiple signaling pathways, several AKT/PI3K/mTOR inhibitors have been postulated. Among them, the AKT inhibitor perifosine is noteworthy, administered in combination with the mTOR inhibitor temsirolimus (Becher et al., 2017).

Improvement of conventional chemotherapy protocols is reaching its limit, and some of the novel compounds investigated have displayed a low half-life, poor penetration, or solubility issues that hinder their use in the clinic. Also, cancer cells develop resistance to chemotherapy, although via many different mechanisms. Multidrug resistance is one of the major events that limit the use of cytostatic agents. The most important associated mechanism is the increased efflux rate of drugs from cancer cells through the ATP-binding cassette transporters. In this sense, cancer stem cells are reported to be the master regulators of chemoresistance (Alisi et al., 2013; Fruci et al., 2016). Moreover, the inclusion and promotion of novel therapeutic candidates in clinical trials are hampered as a result of the small and heterogeneous patient population (Fletcher et al., 2018). With this in mind, cancer nanomedicine is thought to represent an alternative therapeutic approach that can provide novel opportunities for the treatment of pediatric cancers (Rodríguez-Nogales et al., 2018).

## Nanomedicines for Neuroblastoma

Current therapeutic failure reported, especially in aggressive disease, has encouraged researchers to design novel

nanoformulations for treating NB patients. Encouragingly, albumin-bound paclitaxel NPs (nab-PTX) (Fig. 2) reached phase 1 clinical trials for refractory NB and other pediatric solid tumors (Moreno et al., 2018). These studies have reported a manageable toxicity profile and a suitable preliminary clinical activity of nab-PTX (Abraxane) in monotherapy, which allowed for promotion to phase 2 trials (NCT01962103). One possible reason for success is that nab-PTX has shown enhanced cell transport, better penetration, and lower elimination of PTX compared with nonencapsulated PTX (Chen et al., 2014). The next generation of nontargeted and targeted nanomedicines for NB is reviewed and discussed in the following sections.

### Nontargeted Nanomedicines for Neuroblastoma

#### Delivery of Anthracyclines and Alkylating Agents.

Chemotherapy-associated toxicity is reported to provoke not only abandonment of treatment but also late sequelae and chronic complications, making long-term monitoring necessary (von der Weid, 2008). In this sense, endocrine deficits, as well as secondary malignant neoplasms or chronic renal failure, have been frequently described (Applebaum et al., 2017b). The aimless cytotoxicity that leads to these issues often derives from the use of potent alkylating agents and anthracyclines (Fulbright et al., 2010). For example, cardiac dysfunction induced by use of DOX contributes to early morbidity and mortality among childhood cancer survivors. In the same way, it has been reported that NB survivors have an increased risk of developing congestive heart failure, myocardial infarction, pericardial disease, or valvular abnormalities (Mulrooney et al., 2009; Friedman and Henderson, 2018). Nanographene oxidated NPs were coated with gelatin for carboplatin and DOX delivery (Makharza et al., 2015; Vittorio et al., 2018). In vitro studies demonstrated that the effect of gelatin coating is essential for the biocompatibility of graphene NPs, making possible its future administration in animals. DOX-Nano graphene oxide was coated with a dextran-catechin conjugate to form nanohybrids. This combination approach between DOX and the anticancer polyphenol catechin reported both a reversion of the DOX resistance mechanism (via P-polyglycoprotein downregulation) and a synergistic effect in vitro. Although surface modification of graphene improved the biocompatibility of this nanomaterial, the safety of the nanoformulation is questionable. Given that graphene material is not strictly biodegradable, more in vivo experiments are required to confirm the feasibility of this type of nanocarrier. Conversely, Zhen et al. (2013) proposed a safer approach by using milk-derived proteins as scaffolds to formulate casein NPs cross-linked by transglutaminase to encapsulate cisplatin. The in vitro experiments assayed on the SH-SY5Y cell line did not show conclusive differences between bulk cisplatin and cisplatin-NP treatments. Nonetheless, the in vivo experiments determined a significant efficacy and toxicity improvement with encapsulated cisplatin. These results denote that the clinical success of nanomedicines relies on complex biologic interactions with the tumor tissue. Thus, more data about the NPs' interaction with the tumor microenvironment is needed for nanomedicines to progress in clinical practice. A preclinical in vivo study designed in 2014 provided a kidney capsule model of NB to evaluate the cell uptake and behavior of liposomes and their interaction with vascular leaks in the tumor area (Ghaghada

et al., 2016). Using a liposomal contrast agent as a nanoprobe, high-resolution computed tomography imaging results indicated that the liposome cell uptake in NB was not governed by tumor volume or area. It is important to bear in mind that NB is a solid tumor, and the potential therapeutic benefit of nanomedicines could rely on a hypothetical EPR effect. Even though the EPR effect of nanomedicines has been well established and documented, these results have been postulated based on preclinical models. The outcomes in clinical settings are still modest or unknown and deserve further attention (Taurin et al., 2012).

**Delivery of Camptothecins and Synthetic Retinoid Derivatives.** SN-38, the active metabolite of irinotecan, is unable to reach the market owing to its poor solubility and high toxicity (Hahn et al., 2018). Given that it is considerably more active than irinotecan, SN-38 was conjugated with tocopherol succinate to form a prodrug and was afterward encapsulated in biodegradable poly(lactide)-PEG-based NPs (Alferiev et al., 2015). Administered NPs in a xenograft model of NB provided rapid tumor regression and a prolonged animal survival, confirming the feasibility and therapeutic efficacy improvement of the formulation. In a subsequent preclinical study, this formulation was tested in a mouse model of NB using an irinotecan treatment regimen as control (Iyer et al., 2015). SN-38 prodrug-NPs regimen was reported to be more effective at restraining NB tumor growth and recurrence. According to this, the nanoformulation increased exposure of tumor tissue to the cytotoxic effects but with no evidence of chemotherapeutic toxicity. More recently, a similar strategy was proposed by linking tocopherol oxyacetate to SN-38 to enhance NP retention and to improve drug delivery (Nguyen et al., 2018b). Afresh, compared with irinotecan treatment polymeric NPs showed a greater tumor response and prolonged event-free survival in SH-SY5Y and IMR-32 NB xenografts. Although the irinotecan group controlled tumor for 45 days, the nanoparticulated SN38-prodrug achieved a tumor remission and control for at least 180 days. Therefore, nanotechnology might be crucial for the rescue of abandoned drugs that were not able to reach the market because of their troublesome formulation.

In the case of topotecan, another camptothecin inhibitor of topoisomerase I, the main limitation is its low half-life (<30 minutes). Topotecan undergoes a pH-dependent, reversible hydrolysis into a nonactive carboxylate at physiologic pH (Devriese et al., 2015). With this in mind, a liposomal topotecan formulation was prepared to protect the cytostatic drug from this pH-induced hydrolysis (Chernov et al., 2017; Gilabert-Oriol et al., 2017). Pharmacokinetic studies showed that the nanoformulation exhibited a 10-fold increase in plasma half-life values in comparison with equivalent doses of topotecan (Hycamtin); however, encapsulated topotecan only modestly increased the life span of NB mice. This finding reminds us that the ability of nanomedicines to effect cures is sometimes controversial and that a further optimization is sometimes needed to demonstrate a strong benefit over conventional treatments. Nevertheless, the evidence concerning avoiding toxicities is more solid and well documented. For example, fenretinide was incorporated in reconstituted high-density lipoprotein (HDL)-based NPs before their administration to NB cell lines SMS-KCNR and SK-N-SH (Sabnis et al., 2013). Although in vitro assays showed only a slight improvement in efficacy, the novel formulation proved to be nontoxic in nonmalignant cells, unlike free fenretinide administration. Synthetic and reconstituted HDL-based NPs have been

suggested to be an ideal carrier for the delivery of drugs in NB because tumor cells and their cancer stem cells overexpress the scavenger receptor class B, type 1 receptor that can bind to HDL (Basha et al., 2014; Subramanian et al., 2018).

**Nonviral Gene Delivery.** The use of gene therapy was proposed with the aim of downregulating *MYCN* expression by means of small interfering RNA (siRNA). The use of drug delivery systems is a suitable nonviral alternative approach to overcome the poor stability in biologic fluids and the low intracellular penetration of siRNAs (Ravi Kumar et al., 2004). The MYC-associated factor X dimerization (MYC-MAX) protein 3 transcription factor siRNA was nanocomplexed in superparamagnetic iron oxide NPs (Duong et al., 2017). Our purpose was to treat NB cell lines with the developed formulation either as a single therapeutic agent or in combination with common cytotoxic drugs used in NB treatment. Cells exposed to siRNA nanocomplexes underwent apoptosis caused by the oncogene knockdown. Moreover, the additive effect of these siRNA-NPs with other cytostatic drugs supports a hypothetical combinatory strategy.

**Other Strategies.** Less-aggressive modalities are also gaining interest as anticancer therapies in NB. They are characterized by drug activation after a given external stimulus, and combination with nanotechnology may reinforce their potential (Abrahamse and Hamblin, 2016). For instance, a sonodynamic strategy was designed by incorporating a porphyrin into polymeric NPs (Canaparo et al., 2013). In this study, SH-SY5Y cells were exposed to poly-methyl-methacrylate core-shell NPs loaded with meso-tetrakis (4-sulfonatophenyl) porphyrins. The formulation was reported to ameliorate the sonosensitized activation of porphyrin, generating reactive oxygen species and leading to NB cell death in vitro.

Cano-Mejia et al. (2017) described a photothermal immunotherapy approach using Prussian blue NPs. NPs injected in the tumor area triggered a cancer cell death response via photothermal activation. Moreover, to boost the immune

response elicited by the Prussian blue NPs tumor ablation, anti-CTLA-4 antibodies were administered as an immune checkpoint, unleashing the immune response of infiltrated T-lymphocytes against the remaining tumor cells. Treated mice exhibited long-term survival, and the authors underlined the utility of this strategy to ameliorate the outlook in high-risk NB. Of note, the same group recently established a thermal therapeutic window for immunogenic cancer cell death triggered by these NPs that resulted in a long-term survival in vivo (Sweeney et al., 2018).

The highlighted approaches herein are part of a longer list of nontargeted nanomedicines that have been recently proposed for the treatment of NB patients (Table 1). Some of these have demonstrated solid evidence in vivo, which supports their future implementation in clinical practice to high-risk NB patients. Special attention should be given to the best-in-class biodegradable and biocompatible nanocarriers that have exhibited a great capacity in the minimization of associated anthracycline and alkylating agent toxicity. Furthermore, some of these investigated NP-based strategies can overcome efflux-mediated resistances that often hamper the use of common chemotherapeutic agents in the clinic. In that sense, the inclusion of Abraxane (nab-PTX) in clinical trials endorses the implementation of similar nanosystems. With respect to irinotecan or topotecan, their nanoencapsulation has been shown to overcome bioavailability issues of classic formulations that frequently hinder their therapeutic success. In the case of nanomedicines for gene therapy delivery, the current data are encouraging but limited. Considering the high genetic burden involved, especially in high-risk NB, more experiments are warranted to elucidate their therapeutic feasibility. On the other hand, we believe it is too early to estimate the real impact of NPs used in alternative therapies, such as sonodynamic therapy or photothermal immunotherapy. Future experiments will assess their clinical utility at least as adjuvant or complementary treatments.

TABLE 1  
Nontargeted nanomedicines for neuroblastoma (NB)

Formulation	Type of Nanoparticle (NP)	Drug	Size	Assays	References
			<i>nm</i>		
Cisplatin-casein NPs	Protein-based	Cisplatin	257	In vitro/in vivo	Zhen et al. (2013)
Fenretinide rHDL NPs	lipoprotein	Fenretinide	40	In vitro	Sabnis et al. (2013)
TTPS poly-methyl methacrylate NPs	Polymeric	TTPS	72	In vitro	Canaparo et al. (2013)
Carboplatin-gel-NGO	Graphenic	Carboplatin	100	In vitro	Makharza et al. (2015)
SN38-TS-HO-PLA-mPEG NPs-	Polymeric	SN38	<100	In vitro/in vivo	Alferiev et al. (2015), Iyer et al. (2015)
Iodixanol-liposomes	Lipid	Iodixanol	135	In vivo	Ghaghada et al. (2016)
PAMAM Anthocyanin dendrimer NPs	Dendrimer	Anthocyanin	134	In vitro	Yesil-Celiktas et al. (2017)
MXD3 siRNA iron oxide NPs	Metallic	MXD3 siRNA	56	In vitro	Duong et al. (2017)
Prussian blue NPs	Inorganic	—	10–100	In vitro/in vivo	Cano-Mejia et al. (2017), Sweeney et al. (2018)
Liposomal topotecan	Lipid	Topotecan	100	In vitro/in vivo	Chernov et al. (2017), Gilabert-Oriol et al. (2017)
Pyrazolo[3,4-d] pyrimidine HAS NPs	Protein-based	Pyrazolo[3,4-d] pyrimidine	100–200	In vitro	Fallacara et al. (2017)
Dextran-nanoceria curcumin NPs	Metallic	Curcumin	14	In vitro	Kalashnikova et al. (2017)
GO-AgNPs	Inorganic	Silver	15	In vitro	Yuan et al. (2017)
Fe <sub>3</sub> O <sub>4</sub> Prussian blue NPs	Metallic	—	164	In vitro/in vivo	Kale et al. (2017)
Dex-CT-rGO DOX nanohybrids	Inorganic	DOX	300	In vitro	Vittorio et al. (2018)
PL-block-PEG SN-38 TOA NPs	Polymeric	SN-38	50–70	In vitro/In vivo	Nguyen et al. (2018b)
sHDL WGA-TA NPs	Lipoprotein	WGA-TA	8–12	In vitro/in vivo	Subramanian et al. (2018)
C@HSA-MNPs@rGO-DOX	Inorganic	DOX	100	In vitro	Lerra et al. (2018)

DOX, doxorubicin; HDL, high-density-lipoprotein; NP, nanoparticle; PEG, poly(ethylene glycol).

## Targeted Nanomedicines for Neuroblastoma

To avoid systemic toxicities, oncologists demand strategies to increase cytostatic drug exposure in the tumor area. NP-surface engineering has broken new ground in novel therapeutic opportunities with the objective of carrying drugs selectively toward tumor tissue (Bazak et al., 2015). Moreover, the latest findings in the biology and epitope recognition of NB cells have supplied improved knowledge of substance to technologists, prompting the construction of several targeted nanomedicines against NB. In the attempt to overcome tumor relapses, surface-modified nanomedicines are also intended to detain distant metastases or circulating cancer stem cells.

**Antibody Targeting.** Children with high-risk NB currently undergo anti-GD2 monoclonal antibody therapy (dinutuximab) (Ploessl et al., 2016). Targeting nanomedicines against cell-surface gangliosides has attracted researchers' attention also as an immune-targeted strategy. As shown in Table 2, several experiments have been performed by linking anti-GD2 antibodies or their fractions to NPs. In the case of iron (Xu et al., 2014; Baiu et al., 2015) and gold anti-GD2 NPs (Jiao et al., 2016), the authors concurred with high tumor specificity targeting and suggested the use of anti-GD2 NPs not only as anticancer nanomedicines per se but also as suitable diagnostic nanodevices in NB therapy. Theragnosis (simultaneous diagnosis and treatment) is nowadays gaining relevance in nanotechnology (Lee et al., 2015). Given that metallic NPs can be monitored by MRI, they might be very useful in NB, where diagnosis and staging are crucial (Chen et al., 2018a). Recently, SN-38 was loaded into anti-GD2 poly(lactic-co-glycolic) acid PEG NPs (Monterrubio et al., 2017). When this targeted nano-drug delivery system was administered to mice bearing GD2-high expression NB, the extent of tumor penetration by SN-38 was considerably greater in comparison with nontargeted NPs or just the free drug. This group performed a

microdialysis technique to confirm a sustained exposure to the targeted drug in the interstitial fluid of the tumors without any of the interference from the unreleased drug that can be found in common pharmacokinetic studies. Thus, the authors suggested that their antigen-specific delivery system using monoclonal antibodies could be administered in NB GD2-positive patients to enhance the antitumor activity while reducing toxicity of SN-38.

NB expresses common tumor epitopes, receptors, or moieties that have also been investigated for targeting nanomedicines with antibodies. Such is the case of vascular endothelial growth factor (VEGF), which is involved in the angiogenesis process and cancer progression. Bevacizumab selectively binds to this protein, so it was conjugated to SiO<sub>2</sub>-layered double-hydroxide DOX loaded nano-composites with the following goals: 1) improving the cellular uptake and the targeting effect of DOX and 2) inhibiting angiogenesis (Zhu et al., 2017). In vitro and in vivo assays demonstrated that this combinatory approach considerably alleviated DOX side effects and enhanced the anti-NB and antiangiogenesis efficiency mediated both by the targeted DOX delivery and the inhibition of the VEGF-related signaling pathways. These promising approaches, based on antibody-NPs, suggest that they may well be applied in the near future; furthermore, the fact that dinutuximab or bevacizumab are already commercialized reinforces their use in clinical practice; however, again, safety might be a matter of concern since anti-GD2 therapy is reported to provoke acute toxicities, including severe neuropathies that gravely affect these patients (Anghelescu et al., 2015). Bearing this in mind, antibody-NPs will have to show considerable benefits over common treatments and nontargeted nanomedicines in future preclinical evaluations.

**Peptide Targeting.** Peptide-based ligands engineered to NPs have been designed from the neuronal signaling pathways

TABLE 2  
Targeted nanomedicines for neuroblastoma (NB)

Targeting Moiety/Formulation	NB Cell Target	Type of Nanoparticle (NP)	Drug	Size	Assays	References
Anti-GD2/microRNA-34a-silica NPs	GD2	Organic-inorganic	MicroRNA-34a	74	In vitro/in vivo	Tivnan et al. (2012)
HSYWLRS Peptide/DOX-liposomes	Neuropilin-1	Lipid	DOX	130	In vitro/in vivo	Loi et al. (2013), Cossu et al. (2015)
Anti-GD2/iron oxide NPs	GD2	Metallic	—	99	In vitro/in vivo	Xu et al. (2014), Baiu et al. (2015)
AntiGD2/ALK siRNA liposomes	GD2	Lipid	ALK siRNA	135–165	In vitro/in vivo	Di Paolo et al. (2015)
NGR peptide/bortezomib liposomes	aminopeptidase N	Lipid	Bortezomib	165	In vitro	Zuccari et al. (2015)
Anti-GD2/gold NPs	GD2	Metallic	—	50	In vitro	Jiao et al. (2016)
RVG/PLGA-CaCO <sub>3</sub> NPs	Nicotinic acetylcholine receptor	Polymeric	DOX	200–220	In vitro/in vivo	Lee et al. (2016), Jang et al. (2018)
NACA/CDC20 siRNA-PTX-liposomes	Gaba receptor	Lipid	CDC20 siRNA + PTX	130–150	In vitro/in vivo	Bhunja et al. (2017)
PGA/PTX NPs	Neural cell adhesion molecule	Polymeric	PTX	9	In vitro/in vivo	Markovsky et al. (2017)
Anti-GD2/PLGA-PEG SN-38 NPs	GD2	Polymeric	SN-38	272	In vitro/in vivo	Monterrubio et al. (2017)
Bevacizumab/SiO <sub>2</sub> LDH DOX NPs	VEGF	Inorganic	DOX	253	In vitro/in vivo	Zhu et al. (2017)
Dendritic PG/NTP-PTX-PEG NPs	Neural cell adhesion molecule	Polymeric	PTX	70	In vitro/in vivo	Vossen et al. (2018)
Anti-GD2/YM155 liposomes	GD2	Lipid	Sepantronium bromide	170	In vitro/in vivo	Gholizadeh et al. (2018)

ALK, anaplastic lymphoma kinase; DOX, doxorubicin; GD2, disialoganglioside 2; PTX, pertussin; VEGF, vascular endothelial growth factor.

of the peripheral nervous system. The decoration of poly(lactico-glycolic) acid NPs with rabies virus glycoproteins, which can target the nicotinic acetylcholine receptor of NB cells, has been described (Lee et al., 2016). NPs were loaded with calcium carbonate and administered intravenously to tumor-bearing mice. Once the NPs reached the tumor, facilitated by specific-tumor targeting, calcium carbonate converted into microbubbles of CO<sub>2</sub> triggered by the acidic tumor environment. In vivo results determined that gas generated from NPs was a safe and tumor-specific contrast agent for ultrasound imaging, allowing the authors to propose this formulation as another interesting tool for NB theragnosis. Next, the same group took a step forward and loaded DOX in a similar nanoformulation before its administration in a NB-bearing mouse model (Jang et al., 2018). The CO<sub>2</sub> gas generated in the tumor microenvironment accelerated the release of DOX, attaining an enhanced therapeutic efficacy in vivo compared with free DOX. Of note, this approach combines active NP tumor targeting with a stimuli-responsive strategy that facilitates the delivery of drugs in a spatial-, temporal- and dosage-controlled manner (Mura et al., 2013).

Other targeting ligands are currently being explored. Loi et al. (2013) performed a combined in vitro-ex vivo phage display screening to validate NB-targeting moieties and were able to recognize and isolate novel peptide ligands. Selected peptide ligands were coupled to DOX-loaded liposomes, and their administration in preclinical NB animal models led to a significant decrease in tumor volume and survival enhancement. Then, other multitarget approaches, including the endothelial cell marker aminopeptidase A, the perivascular cell marker aminopeptidase N, and the aforementioned GD2, were investigated (Cossu et al., 2015). The authors validated a novel peptide ligand called HSYWLRS. The conjugation of this peptide to DOX-liposomes increased their tumor vascular permeability and perfusion in vivo and thus boosted the therapeutic response of DOX-targeted liposomes. In another study, synthesized proteasome inhibitor bortezomib-loaded liposomes were decorated with targeting-ligand peptides for the tumor endothelial cell marker (Zuccari et al., 2015). These tumor vascular-targeted liposomes were evaluated in vivo, exhibiting lower toxicity and a better therapeutic index than free bortezomib.

**Other Targeting Moieties.** NP functionalization with drugs, aptamers, or surfactants is also frequently described in the literature. Instead of using peptides, CDC20siRNA and PTX were coencapsulated in a cationic amphiphilic liposomal formulation decorated with nipecotic acid-derived moieties. Bhunia et al. (2017) postulated that nipecotic acid-derived molecules attached to NPs may competitively inhibit the GABA transporter, overexpressed in NB cells, attaining a liposome-targeted delivery. Intravenously administered targeted liposomes considerably inhibited tumor growth of xenografted human NB mice. This is a striking example of a combinatorial approach that harnesses cancer nanomedicine to merge targeting and gene/cytostatic drug delivery. Beyond doubt, the use of peptides or small molecules represents an interesting alternative to antibodies. Of note, some of these nanoformulations probably display less specificity toward tumor tissue than antibody-decorated NPs. Nonetheless, it may be taken as an advantage rather than a hurdle to progress in the clinic. Unlike antibodies, these molecules do not show an inherent immunotoxicity, so further investigations are needed to elucidate the best strategies.

The studies described have shed light on the possibilities of targeted nanomedicines in the field (Table 2). Some studies reported lower toxicities and enhancement of the antitumor efficacy in preclinical models, which may be optimal in relapsed NB; however, its extreme variability and our poor understanding of the disease oblige us to be cautious until a suitable candidate is validated. The experience gained in clinical practice with immunotherapy supports the option of anti-GD2-targeted nanomedicines loaded with cytostatic drugs to progress in clinical settings in the future. Sadly, to date, there are more challenges than opportunities to effect total cures in NB children with a poor prognosis. The effectiveness of targeted nanomedicines in detaining metastases is nowadays a matter of debate. The metastatic process is not merely a cellular-centered phenomenon but a response to supracellular alterations linked to tissue disorganization. The complexity of tumor biology is not only determined by cell-cell interaction but also by cell-matrix interplay (Tadeo et al., 2016a). Nonetheless, the demonstration that the components of the tumor microenvironment decisively mediate clinical aggressiveness, prognosis (Tadeo et al., 2016b, 2018), and response to treatment (Johnsen et al., 2018) opens up a new field of knowledge and clinical application, especially in new and promising nanomedicines that are much more respectful toward the bodily integrity of NB children.

## Conclusions and Future Directions

Current NB protocols range from conventional chemotherapy administration to up-to-date immunotherapy regimens. Even if the constant optimization of these therapies has ameliorated the perspectives and prognosis of these children, high-risk NB is still incurable in approximately 50% of cases. In addition, some of the novel targets that have been proposed, such as actionable genetic aberrations, often meet with insufficient patients to validate therapies. This quandary has prompted the advent of cancer nanomedicine for improving therapeutics in NB.

Efforts have been made in the last few years to attain the best nanoformulations to replace current treatments. We have highlighted some preclinical studies on nanomedicines that have proven to alleviate chemotherapeutic-associated toxicity, suggesting that their use would avoid treatment abandonment and late sequelae. Abraxane (nab-PTX), currently in phase 1 and 2 for NB treatment, is at the head of these outlined approaches and leads the pathway for the implementation of analogous nanomedicines. Likewise, we anticipate that other biocompatible and biodegradable nanocarriers carrying anthracyclines, alkylating agents, or taxoids might reach the clinic soon. The outcomes gathered concerning targeted nanomedicines are quite valuable. Surface-decorated nanomedicines with anti-GD2 antibodies have demonstrated an enhanced antitumor efficacy over non-targeted therapies that establish preclinical consolidation for designing future experiments.

Cancer nanomedicine is gradually gaining relevance in adult cancer as well. To extend this approach to a pediatric cancer such NB, proposed nanoformulations must show strong benefits over current treatments like reduction of the cardiotoxicity associated with DOX treatment.

Some aspects, such as the safety profile, drug cargo capacity, or biodistribution of drug-delivery systems, are crucial. A suitable

validation of candidates in standardized orthotopic models of NB that correctly reproduce the disease is mandatory. The complex NB biology still represents the main obstacle; so collaboration between pediatric oncologists, biologists, and technologists is essential for achieving therapeutic success. We are confident that the knowledge acquired will uncover the real potential of nanomedicines for the management of NB.

#### Authorship Contributions

Wrote or contributed to the writing of the manuscript: Rodríguez-Nogales, Noguera, Couvreur, Blanco-Prieto.

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