# Dendrimers as Drug Nanocarriers: The Future of Gene Therapy and Targeted Therapies in Cancer

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**Abstract**

Synthetic polymers, such as dendrimers, play a critical role in pharmaceutical discovery and development. Advances in the application of nanotechnology in medicine have given rise to multifunctional "smart" nanocarriers that can deliver one or more therapeutic agents safely and selectively to cancer cells, including intracellular gene-specific targeting. Dendrimers with their 3D nanopolymeric architectures are highly attractive class of drug and gene delivery vector. Advances in understanding and manipulating genes gave scientists a tool to make changes in people DNA to prevent or treat diseases. Over the past decade, gene therapy has been in use in clinical trials. The inactivation of the tumor suppressor genes is the main idea of the development of gene therapy in the cancer treatment. Broad spectrum of delivery concepts, including viral vectors, liposomes, cationic polymers and dendrimers, cell-penetrating peptides and gold and magnetic nanoparticles have been investigated. A well-designed vector is the most desirable approach to increase the safety of gene therapy, which is still in its infancy stages in cancer research. More experimental and clinical trials are focused on well-designed and effective doses of vectors that are essential for therapeutic efficacy of gene therapy for its potential in clinical use against a wide variety of cancers.

**Keywords:** nanocarriers, targeted therapy, gene therapy, cancer, drug delivery system, RNA therapeutics, dendriplex, non-viral vectors

## 1. Introduction

Cancer is one of the world's most stressful diseases with no apparent cure in sight for several tumor types and millions of new cases reported every year [1]. Cancer chemotherapy using conventional anticancer agents has been slowed down by several challenges such as severe toxicity, poor membrane permeability, rapid clearance, and narrow therapeutic index. In this regard, a wide range of nanoparticles such as liposomes, polymeric micelles, polymeric nanoparticles, dendrimers, silica nanoparticles, and carbon nanotubes with their structural, physicochemical, and functional diversity can be utilized to enhance drug loading and enable drug internalization in target cancer cells while limiting uptake in normal tissues and cells [2, 3]. The development of smart cancer treatment approaches revolves engineering such unique nanosystems carrying drug and gene payloads that can passively or/and actively target cancer cells [4]. Gene therapy and newer molecular target-based anticancer tactics involve the use of potent but highly labile agents such as monoclonal antibodies, aptamers, siRNA and miRNA that are readily degraded and/or have limited stability *in vivo* [5]. The big limitation of conventional anticancer agents is a poor therapeutic response and adverse side-effects involving healthy organs [6]. To overcome those limitations searching for new effective carrier vectors is very important. They might protect the payload from degradation during the transit, enhance targeting efficiency, optimize drug release profiles, and reduce the adverse toxic effect caused by the non-target organ accumulation of cytotoxic drugs [7–9].

## 2. Dendrimers

Dendrimers are globular macromolecules sized 1–100 nm with an architecture consisting of three distinct domains: a central core, a hyperbranched mantle, and a corona with peripheral reactive functional groups [10]. The high level of control over the synthesis of dendritic architecture makes dendrimers a nearly perfect (spherical) nanocarrier with predictable properties. Many different kinds of dendrimers, including polyamidoamine (PAMAM), poly(propylene imine) (PPI), poly(glycerol-co-succinic acid), poly-L-lysine (PLL), melamine, triazine, poly(glycerol), poly[2,2-bis(hydroxymethyl)propionic acid], poly(ethylene glycol) (PEG), and carbohydrate-based and citric acid-based ones, have been successfully developed for drug delivery [11–14]. The most widely investigated vectors for medical application are two dendrimers: PAMAM and PPI [15, 16]. Those two amine-terminated dendrimers display stimuli-responsive (pH-dependent) drug release behavior. The tertiary amine groups are deprotonated at high pH (alkaline), causing a collapse of the dendrimer on itself, which is named 'back folding' [17]. The utility of dendrimers can be appreciated by their ability to traverse several delivery barriers using two overarching principles, namely active and passive tumor targeting.

## 3. Cancer treatment and limitations of chemotherapy

Surgery and radiation are the main common treatment in solid tumors as soon as they are recommended to undertake considering the tumor infiltration. These kinds of treatments are

considered as local treatments [18]. As it is well recognized, surgery can be disfiguring and radiation can be damaging to local healthy tissues and organs. Chemotherapy is the third option in cancer treatment, which is called adjuvant therapy to surgery and radiotherapy. It is based on cytotoxic effect (cell-killing therapy). The most desirable effect of chemotherapy is to eliminate cancer completely, which is still in most cases the wishful thinking. If such a cure is not possible, the good result is to even stop the growing tumor [19]. Despite some excellent drugs are available, the efficacy of many existing chemotherapeutics is limited by their inability to reach their therapeutic site of action in sufficient amounts to be effective [20]. In most cases, patients are administered with an excess of medications that are distributed throughout the whole body, and thus, it is extremely difficult to avoid distribution into healthy organs and tissues and the depression of the immune system. It always gives the limitation of dosage that can be given and, in turn, prevents these drugs from achieving the potential cures [21]. Current anticancer drugs often have a poor therapeutic index and they cause a lot of side effects [22]. A major concern is when the medications affect non-cancer cells, causing the adverse reduction of red and white blood cells, and affecting the gastrointestinal tract triggering nausea and diarrhea [23]. To reduce, or even better to avoid such side effects, the drug delivery to the tumor is optimized by preparing carriers containing an active agent associated with a molecule capable to accurately target cancer cells such as antibody drug conjugates (ADC) or nanoparticles [24–26].

## 4. Dendrimers as drug delivery systems

Dendrimers have been engineered as nanodevices, either in nanocarrier drug approaches or as drugs *per se*. The biological effect of dendrimers is caused by terminal moieties and is responsible for the global efficiency. Dendrimers due to their proper, reproducible, and optimized design parameters overcoming the physicochemical limitations of classical drugs (for example, solubility, specificity, stability, biodistribution, and therapeutic efficiency) are successful. They are also able to omit biological problems to reach the right targets such as first-pass effect, immune clearance, cell penetration, and off-target interactions [27]. Polymers are commonly used materials for nanoparticles-based delivery [28], among them dendrimers are the ones more commonly used as a non-viral delivery system. The best drug carrier should meet several requirements such as drug retention, release the drug, unaffecting by the immune system, extending the time in blood circulation, and specific targeting to cells or organs [29]. When a drug carrier is applied to the patient and reaches the level of the blood, it starts an intricate trip before it is able to reach the destination of the target site. When they attach to the target cell membrane, they undergo the endocytosis process. There are several parameters of dendrimers that can facilitate the process. We have to emphasize also the impact of the body structure including size, shape, additional chemistry on the surface, and mechanical flexibility [30]. The nanoparticles, due to their size, have a huge impact on the circulation time if they are applied intravenously (IV), so they are safe for the smallest capillaries and they are not able to clog them [31]. Cellular uptake by phagocytosis and endocytosis is also particle size dependent [32, 33]. The unique uniformity of dendrimers gives them the ability to cross the membrane of cancer cells. The anticancer drug can be either non-covalently encapsulated in the core of the dendrimer or covalently conjugated to its surface, being possible to customize

the drug release profiles by controlled depolarization processes [34, 35]. When amphiphilic dendrimers with a hydrophobic core and hydrophilic branches encapsulate the anticancer drugs, it helps to utilize these dendrimers in local treatments such as intratumoral injections. Such a solution helps to solubilize the hydrophobic drugs and leaves the drug unaltered [36]. The attachment of anticancer drugs to the surface groups of the dendrimer by covalent chemical bonds offers also some other advantages compared to the non-covalent encapsulation. Besides the enhancement of solubilization of the drugs, it is possible to attach many different hydrophobic anticancer drugs, and the controlled release is being maintained [37, 38].

Dendrimers have already been used as passive anticancer nanocarriers [38–41]. There are preclinical promising results *in vitro* as well as *in vivo* with active targeting dendrimers [36]. For example, antibody-dendrimer conjugates showed better efficacy than free antibodies [42–45]. It has been also reported that dendrimers modified with folic acid on the surface generated better tumor accumulations that untargeted controls or free drug, producing a stronger reduction of the tumor mass [46, 47]. Moreover, sugar-modified PPI dendrimers tested by our research team at University of Lodz, Poland, are very attractive and specific for leukemia and lymphoma cells derived from lymphocytes B. Depending on the sugar on the surface and the number of molecules, we can observe the different extend of triggering apoptosis in those cells due to the diversity in affecting particular gene pathways [48–51]. Lysine dendrimers, PAMAM, PPI, and phosphorus have been reported to be able to modulate amyloid peptide aggregation in solution [52–54]. The deposition of amyloid fibrils is characteristic in neurological disorders as well as prion and Alzheimer's diseases. Some of the positively charged dendrimers could even inhibit the growth of amyloid fibrils or even disrupt existing mature of these fibrils. Others could decrease the number of toxic amyloid oligomers [55, 56]. The slow translation of preclinical studies to clinical trials may be due to the toxicity of dendrimers [46, 47], with the aim of the current research in the development of new biocompatible and less toxic alternatives [57, 58].

Once these molecular machines arrive at the target site inside the living organism, several barriers must be overcome. Nanocarriers are usually internalized by endocytic processes [59], the processes called vesicular internalization. The most widely studied endocytic pathways are clathrin-mediated endocytosis, caveolae-mediated endocytosis, and macropinocytosis, but other cellular pathways have been recently identified, including clathrin- and caveolae-independent endocytosis and phagocytosis [60]. Molecules, which are internalized by the cell membrane, are endocytosed by the early endosomes pathway. They may progress later to late endosomes and lysosomes. If the loading of dendrimer targets the nucleus, thus the nuclear membrane is another barrier that the dendrimer should come across [61].

We should be very careful designing the drug delivery system because unexpectedly our desired nanovector might have its own power. This is what our genetic research has shown— 4th generation PPI glycodendrimers with maltotriose molecules directly trigger mechanism of apoptosis in mitochondria of lymphocytes B, particularly those transformed to the leukemic cells. That discovery was successfully patented (US 9,877,85) and applied as a potential drug for lymphoproliferative disorders coming from B cells, such as chronic lymphocytic leukemia (CLL) or B-lymphoma. The power of these glycodendrimers relied on the ability to affect several genetic pathways simultaneously, and as opposed to the commonly used drugs or the new ones already proved by FDA, they affect the cell genome very quickly and efficiently according to the natural death process initiation (**Figure 1**).
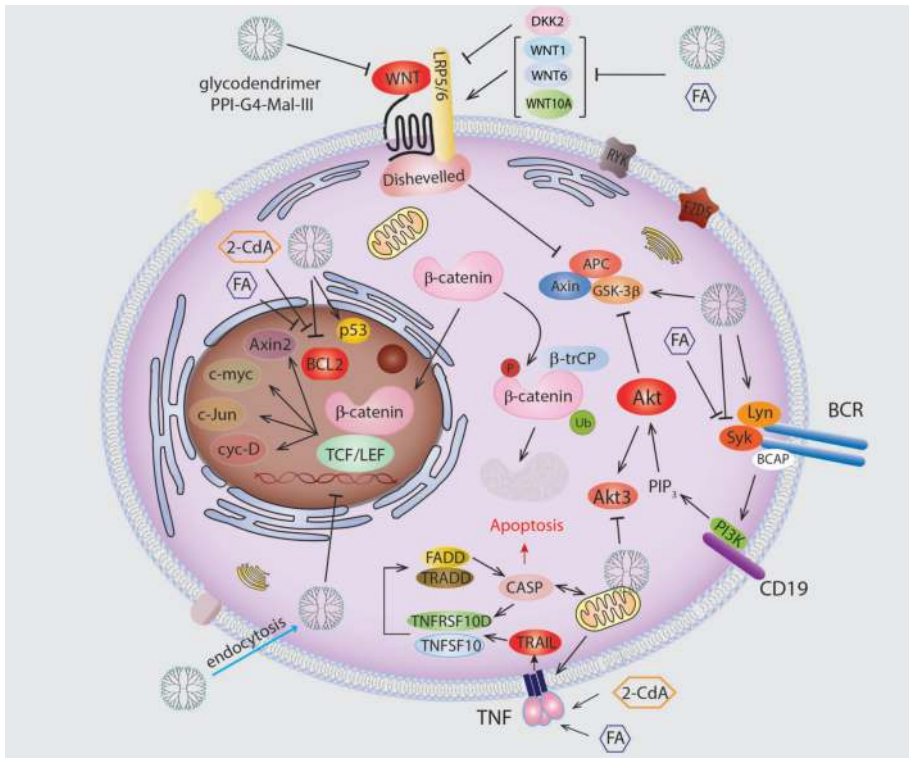
**Figure 1.** Mechanism of action—PPI-G4-OS-Mal3 dendrimers in B-lymphocyte (the illustration prepared by B. Ziemba).

## 5. Delivery of RNA therapeutics

During the past decades, RNA-based drugs have arisen as good candidates to cure the diseases at the gene and RNA levels. Since 1990, it has been known that nucleic acids can be used to modify protein production in vivo [62]. However, therapeutic RNA delivery has been limited for a long time by many different factors [63]. It is known that naked, single-stranded RNA is easily degraded by nucleases. It can also activate an immune system and is too large to be able to passively cross the cell membrane. Moreover, the negative charge of RNA causes the problem to enter the cell. Therefore, an additional solution should be provided to facilitate cellular entry and escape from endosomes [63, 64]. Typically, cationic polymers (e.g. dendrimers) are used to electrostatically condense the negatively charged RNA into nanoparticles [65]. Very important for effective nucleic acid delivery are modifications made to RNA itself [66], to make it more resistant to degradation and render them unrecognizable by the immune system [67]. RNAs can be modified by means of chemical alterations to the ribose sugar [67, 68], the phosphate linkage, and the individual bases [69–72]. One of such modified RNA is locked nucleic acid (LNA) modification. LNA's ribose moiety is modified with an extra bridge between the 2' oxygen and 4' carbon. The bridge "locks" the ribose in the 3'-*endo*(North) conformation. LNA nucleotides can be mixed with DNA or RNA residues in the oligonucleotide whenever

preferred and hybridize with DNA or RNA according to Watson-Crick base-pairing rules. Due to the high stability of LNA-RNA it started to be used in a biotechnology field in a pharmaceutical business [73]. The multi-valent folate (FA)-conjugated 3WJ RNP constructed to harbor anti-miR-21 LNA sequences (FA-3WJ-LNA-miR21). Specifically targeted anti-miR-21 LNA was delivered to glioblastoma cells. It caused the knock down of miR-21 expression in *in vitro* and *in vivo* models with favorable biodistribution. The results are indicative of the clinical benefit of FA-3WJ RNP-based gene therapy for the successful targeted therapy of developing and even recurring glioblastoma [74]. In the other study, (LNA)-anti-miR was reported as a blockage factor of miR-182-5p in human breast cancer cell line (MCF-7). MTT (3-[4,5 dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay and annexin/propidium iodide staining at different time points after LNA-anti-miR-182-5p transfection were accomplished. The results showed that miR-182-5p inhibition induces apoptosis and thus reduces the viability of MCF-7 cells. These results can be used in translational medicine for future investigation in breast cancer and approach treatment based on antisense therapy.

siRNA is not the only RNA drug to be examined for protein knockdown at the clinical stage (NCT01676259) [75, 76]. Antisense oligonucleotides (ASO) were the first RNA drugs successfully reported in clinical trials. They are able to block protein translation through Watson-Crick base pairing with the target mRNA, similar way to siRNA mechanism, and they can also be modified to improve their stability [77–79]. Despite that the ASOs inhibit protein production through the sterically blocking ribosome attachment or eliciting RNase-H activation, they are also able to promote the exon skipping, which may lead to a deletion of faulty sequences within proteins and thus it can make a protein upregulation, that can be used in diseases where certain genes are repressed [80].

An emerging, but less clinically improved, is microRNA (miRNA) platform for protein knockdown. Endogenous miRNAs are non-coding RNAs that are regulatory factors for a variety of cellular pathways and are often downregulated in diseases [81]. Exogenous mRNAs, or miRNA mimics, delivered therapeutically could make a knockdown of several proteins simultaneously, which might be very useful in cancer, where having a single disease-relevant target is rare [82]. The first miRNA mimic therapy to enter clinical trials was MRX-34—a liposomal-encapsulated miRNA mimic from Mirna Therapeutics meant to treat variety of cancers [83]. Despite the big number of carriers, mRNA molecules are significantly larger than (600–10,000 kDa) than the previously discussed siRNAs (~14 kDa) and ASOs (4–10 kDa), which poses an additional challenge for delivery of mRNA therapeutics [84]. Therapeutic applications based on mRNA are currently being explored as vaccinations against cancer, infectious diseases, and gene editing. Cancer mRNA vaccines have experienced accelerated development in cancer immunotherapy. The majority of approaches tested in clinical trials employ adoptive transfer of DCs transfected with mRNA coding for tumor-specific antigens (TSAs) and immunomodulation of T cells with mRNAs expressing chimeric antigen receptors (CARs) or TSAs [85–87].

The most recent and the most sophisticated gene delivery is CRISPR-Cas system that also relies on Watson-Crick base-pairing between a single guide RNA (sgRNA) and a corresponding DNA target site followed by a distinct protospacer-adjacent motif (PAM), which is a 3–5 nucleotide DNA sequence required for binding Cas9 and cleavage of the target sequence.

It leads to the double-stranded break (DSB) into a DNA molecule [88]. DSBs can be repaired by cells using non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ results in insertions and deletions causing permanent gene knockout [89]. CRISPR-Cas components based on nanoparticle mRNA delivery are therapeutically attractive due to the temporary ability of mRNA expression. There is also no risk of genomic integration and mRNA cytoplasmic activity, mitigating the need to overcome the nuclear barrier in comparison with pDNA [90]. The major challenges for RNA-based drugs and CRISPR-Cas therapies will be shaping the scope of upcoming clinical trials.

## 6. Clinical studies of dendrimers for targeted cancer therapy

To design the most effective and variety therapies for different kinds of cancer, an effective vector protecting siRNA that is non-toxic and can be targeted at selected cells is necessary [91, 92]. Several classes of dendrimers seem to be good candidates for carriers of oligonucleotides. Cationic carbosilane dendrimers (CBD) characterized by Si—O or Si—C bond and terminated with ammonium or amine groups, form also complexes with siRNAs.

There are many reports presenting promising results in the topic of nucleic acids delivery using complexes called 'dendriplexes' [93–95]. Among variety of proposed candidates, PAMAM dendrimers are the most explored dendrimers type, followed by poly(propylene imine) (PPI) dendrimers, poly(L-lysine) (PLL) dendrimers, and some others [96].

PAMAM dendrimers, hydrophilic, biocompatible, and non-immunogenic particles, are build of ethylenediamine core (most commonly) and methyl acrylate and ethylenediamine branches [97, 98]. They have been successfully used as nucleic acid delivery systems in many *in vitro* and *in vivo* researches of which we present selected examples [107–119].

The transfection efficiency of PAMAM dendrimers largely depends on their generation, which determines the structure of the PAMAM molecule: higher generations are more compact and spherical than the low ones and provide a surface with a high density of primary amines therefore form more stable dendriplexes with higher efficiency [99, 100]. However, dendrimers with high generations results in higher toxicity due to a large number of terminal cationic groups which can interact with negatively charged cell components, e.g. cell membranes causing their disruption [101, 102]. This disadvantage can be diminishing by surface modification with different targeting or shielding moieties providing with not only low toxicity but also enhance the cell uptake and specific accumulation of nucleic acid molecules inside cells [103–106]. For example, novel targeted nanoparticle system consisting of FLT3 ligand-conjugated PAMAM G7 encapsulating a pivotal tumor suppressor and negative regulator of *FLT3* miRNA—miR-150, was developed by Jiang et al. [107] to treat *FLT3*-overexpressing acute myeloid leukemia (AML), a leukemia associated with unfavorable prognosis. The system demonstrated high efficacy significantly inhibiting progression of FLT3-overexpressing AML *in vivo* with no obvious side effects on normal hematopoiesis. In other research, Liu et al. demonstrated that triethanolamine (TEA)-core PAMAM dendrimer is able to deliver Hsp27 siRNA effectively to a castrate-resistant prostate cancer model *in vitro* [108] and *in vivo* [109] and produce potent gene silencing of the heat-shock protein 27 (HSP27), leading

to a notable anticancer effect. To further improve the delivery system, the arginine-terminated PAMAM-G4 dendrimers were developed with the aim of combining and harnessing the unique siRNA delivery properties of the TEA-core PAMAM dendrimer and the cell-penetrating advantages of the arginine-rich motif. The modification led to improved cell uptake of siRNA by comparison with non-modified bearing PAMAM-G4 and to yield potent gene silencing in human hematopoietic CD34+ stem cells [110] and anticancer effects with no discernible toxicity in both *in vitro* and *in vivo* models [110, 111]. Another example of a delivery system where the modification aiming at increasing the efficiency yield is FA-decorated PAMAM G4 (G4-FA) used as a vector for local delivery of siRNA against vascular endothelial growth factor A (siVEGFA) in a xenograft HN12 tumor mouse model of head and neck squamous cell carcinomas. The G4-FA/siVEGFA complex exhibited high tumor uptake, sustained retention properties and pronounced tumor suppression in even single- or two-dose regimen studies [112]. Thioaptamer (TA)-modified PAMAM dendrimers, on the other hand, are proposed as effective miRNA deliver system to breast cancer cells constituting a prototype that it could be safely used in pre-clinical and clinical research [113].

A frequent way of using dendriplexes in anticancer therapy is to provide them in conjunction with approved anticancer agents [114–116]. Researchers from Virginia Commonwealth University used nanoplexes of PAMAM dendrimer with polyethylene glycol and lactobionic acid complexed with AEG-1 siRNA against hepatocellular carcinoma (HCC), a fatal cancer with no effective therapy. Applied in the combination with all-trans retinoic acid (ATRA), the complex developed a profound and synergistic inhibition in tumor growth in human HCC xenografts model suggesting, that combinatorial approach might be an effective way to combat resistant types of cancer [117]. Liu et al. used PAPMAM dendrimers as a nanoparticle delivery platform for a MDR1 gene targeting siRNA to reverse multidrug resistance (MDR) in human breast cancer MCF-7/ADR cells. This PAMAM-siMDR1 complex decorated additionally with phospholipid demonstrated high gene silencing efficiency and enhanced cellular uptake of siMDR1 resulting in rising of cellular accumulation of doxorubicin (DOX), inhibition of the tumor cell migration, and due to synergistic work with paclitaxel (PTX), increase of cell apoptosis, and cell phase regulation [118]. More complex system designed in order to achieve effective treatment to MDR breast cancer is PAMAM functionalized graphene oxide (GO-PAMAM) which can load DOX and MMP-9 shRNA plasmid at the same time [119].

It is still a challenging task to deliver the anticancer drugs to brain tumors and overcome the restriction of blood-brain barrier (BBB). He et al. [12] have proposed recently an interesting approach. G4.0 PAMAM dendrimers have been conjugated with two targeted ligands—transferrin and wheat germ agglutinin. Such conjugates were used for crossing the BBB and incorporation drugs to brain tumor cells. That dual-targeting drug carrier system allowed to deliver successfully DOX inside the brain tumor and provided a potential therapy for brain cancer [12].

Dendrimers have been investigated for ophthalmic drug delivery since it offers a number of advantages as a carrier system. They may improve effective delivery of therapeutic agents to intraocular tissues, such as the retina or choroid, using non-invasive delivery methods. Eye cancers are not among the most common but also in this area, scientists have started to look for inspiration in nanoparticles [120]. Kang et al. made a successful single injection of subconjunctival

G3.5 PAMAM dendrimer to transgenic murine retinoblastoma with no associated toxicity. The higher dose of nanoparticle even could reach and decrease the tumor burden in the untreated, contralateral eye [121].

Poly(propylene imine) (PPI) dendrimers are constructed from a 1,4-diaminobutane core and propylene imine branches [122]. Positively charged surface of PPI dendrimers provides an interaction with nucleic acids, enabling the dendritic scaffold to be used as a vector for gene transfection [123].

As in the case of the PAMAM dendrimers, the surface of PPI dendrimers can be freely modified to reduce their toxicity and increase their uptake by target cells. A small library of alkanoate-modified PPI G5 dendrimers was developed and tested for their ability to transfect DNA to neuroblastoma Neuro-2a cells. It was shown that a balanced hydrophobic surface modification results in improved transfection, low cytotoxicity, and hemotoxicity [124]. Much larger modifications of PPI dendrimers in order to increase their efficiency as gene carriers have been made by a team of researchers from The State University of New Jersey [123–126]. In 2009, Taratula et al. modified PPI G5-siRNA complex with dithiol-containing cross-linker molecules followed by PEG coating. To direct the complex specifically to the human ovarian and lungs cancer cells, an analog of luteinizing hormone-releasing hormone (LHRH) peptide was conjugated to the end of PEG. The modification and targeting approach confers the complex stability in plasma and intracellular bioavailability, promoted its tumor-specific uptake and accumulation in the cells, and efficient gene silencing. Moreover, in vivo study confirmed high specificity of the proposed targeting delivery approach [125]. A year later, the same team developed a novel way to compact and deliver nucleic acids with lower, third-generation PPI dendrimers by using gold nanoparticles (AuNP) as a "labile catalytic" packaging agents. The AuNP helped dendrimers to compact siRNA but were not included in the final complex. The efficiency of mRNA silencing by this approach was even higher than that with PPI G5 dendrimers [126]. To further improve the efficiency of investigated delivery systems, the authors developed siRNA vectors based on PPI G5 dendrimers and superparamagnetic iron oxide nanoparticles, together with incorporation of PEG coating and LHRH conjugation. This novel multifunctional siRNA delivery system improved selective internalization into cancer cells and increased the efficiency of targeted gene silencing *in vitro* and sufficiently enhanced *in vivo* activity of anticancer drug—cisplatin [127]. In further studies the team designed a drug delivery system (DDS) containing a PPI dendrimer as a carrier and a LHRH peptide as a tumor-targeting moiety, siRNA targeted to CD44 mRNA and anticancer drug—PTX. The proposed DDS was tested in vitro and in vivo using metastatic ovarian cancer cells. The treatment resulted in suppression of CD44 mRNA and protein expression, induction of cell death and tumor melting, and moreover, it was free from adverse side effects [128]. The potential of PPI dendrimers as a core of delivery complexes was also investigated in the combination therapy against multidrug-resistant breast cancer cells (MCF-7/ADR). Copolymer consisting of PPI dendrimer, Pluronic P123 and anti-CD44 monoclonal antibody (anti-CD44-P123-PPI) loaded with pDNA-iMDR1-shRNA against MDR1 protein demonstrated high efficiency of transfection contributing to increased sensitivity of cancer cells to the DOX. The results demonstrated that the administration of anti-CD44-P123-PPI/pDNA-iMDR1-shRNA nanocomplexes combined with DOX inhibit tumor growth more efficiently than DOX alone [129]. Poly(propylene imine) (PPI) dendrimers with surface modification with maltose have been

tested as drug carriers for nucleoside analog (NA) 5′-triphosphates. The study showed the interactions between PPI dendrimers of 3rd (G3) or 4th (G4) generation and cytidine-5′-triphosphate (CTP) measured by Isothermal Titration Calorimetry method. CTP was used as a good representative molecule of pyrimidine nucleoside analog (NA)—cytarabine (ara-CTP) commonly used in leukemia treatment. Dendriplexes made of PPI dendrimers and NAs may help to improve NA limitations such as low solubility and stability or resistance in leukemia cells. The study depicted that dendrimer generation is responsible for the efficiency of complex formation. Also a type of surface modification of dendrimer with maltose residues and a type of solvent used to prepare dendriplexes were evaluated. The results of PPI dendrimers creating complexes with CTP were highly efficient that makes them promising candidates for a drug delivery system [130]. As soon as we know that cationic nature of PPI dendrimers makes it possible to form complexes with nucleotide Ara-C triphosphate forms (Ara-CTP), the authors went further to test the concept of applying PPI glycodendrimers as a drug delivery system. They wanted to facilitate the delivery of cytarabine to cancer cells to overcome metabolic limitations of the drug. As a leukemic cell lines models they used 1301 and HL-60 as well as peripheral blood mononuclear cells. The enhanced activity of Ara-C triphosphate forming (Ara-CTP) complexes with PPI-M dendrimers had been shown. An enhanced uptake and cytotoxicity of Ara-CTP-dendriplexes toward 1301 cells with blocked human equilibrative nucleoside transporter, hENT1, suggested that it might be a multipurpose candidate for resistant acute lymphoblastic leukemia chemotherapy with lower expression of hENT1 [131]. It has been also reported that PPI-Ma-DS did not impact THP-1 cells (monocytic cell line model of innate immunity effectors) viability and growth even at high concentrations (up to 100 μM). They also did not induce expression of genes for important signaling pathways: Jak/STAT, Keap1/Nrf2 and ER stress. The high concentrations of 4th generation PPI-Mal-DS (25–100 μM) induced nuclear translocation of p65 NF-κB protein and its DNA-binding activity. It leads to NF-κB-dependent increased expression of mRNA for NF-κB targets: *IGFBP3*, *TNFAIP3*, and *TNF*. The 3rd generation of PPI-Mal-DS dendrimers did not exert the same effect. There was observed no increase in pro-inflammatory cytokine secretion which is a very promising result [132]. PPI-5G dendrimers, similar to PAMAM, also possessed the ability to deliver anticancer drugs to brain tumors. Gajbhije and Jain reported polysorbate-80-conjugated PPI dendrimers for targeted delivery of docetaxel (DTX) to the brain tumor [133]. This complex reduced the tumor volume more than 50% after 1 week of treatment. It is because this formulation owing the higher BBB permeability of polysorbate-80-anchored dendrimers [134]. The other report showed that PPI-5G dendrimers conjugated with thiamine exhibited improved delivery of PTX across the BBB and the preferential brain uptake of PTX by the nanoconjugates might be attributed to the association with the thiamine transporters or increased passive diffusion secondary to an improved concentration gradient of the dendrimers located at the BBB interface [135].

Poly(ʟ-lysine) (PLL) dendrimers, amino acid-based macromolecules characterized by high biocompatibility and low toxicity, have also been developed as non-viral vectors for gene delivery [136–139]. In example, in 2002 it was reported that dendritic PLL G5 and G6 transfected DNA into several different cell lines with high efficiency and without any cytotoxic effects [139]. The results of more recent studies confirm previous reports. Newly synthesized

siRNA carriers containing amphiphilic PLL dendrons exhibited not only the siRNA binding properties but also the ability to inhibit the proliferation of glioblastoma cells while being non-toxic for cell types that share the anatomical space with tumor cells during the course of the disease [140]. Research on the use of PLL dendritic structures as gene carriers in combination with traditional anticancer drugs also yields promising results. PLL G3 dendrimers with a silsesquioxane cubic core were conjugated with a c(RGDfK) peptide through PEG spacer for codelivery of DOX and siRNA to glioblastoma U87 cells. The complex showed high transfection efficiency and gene silencing and was more toxic to U87 cells than free DOX [141].

# 7. Concluding remarks

Although conventional chemotherapy has been the cornerstone in the fight against cancer, is far from being totally satisfactory due to the problems related with their formulation, pharmacokinetics, and the last but not least, severe side effects of such a therapy. Last past decades the huge progress has been made in the understanding of the disease, its molecular background and development of newer targeted therapies. Unfortunately, an effective treatment of several forms of cancer still remains a major challenge. Recent advances in drugs based on dendrimer and gene delivery using dendrimers as a vector has appeared as a great option to overcome the limitations of conventional chemotherapy. Currently, more than 50% of the cancers are not curable and drug nanocarriers might help to decrease this percentage. Nanomedicine represents one of the fastest growing research areas and is regarded as one of the most promising tools for cancer treatment. Several solutions based on nanoparticles have been developed and many are used in clinical cancer care. Liposomes and polymer conjugates were the first nanocarriers to be approved by FDA; however, only five liposomal drugs, two polymer-protein conjugates, and two dendrimers are in the market up to date. Abraxane®, an albumin-bound paclitaxel nanoparticle, has been approved by FDA in 2005 for the treatment of metastatic breast cancer. In 2012 the same drug has been approved for the first-line treatment of advanced non-small lung cancer and in 2013 for the metastatic pancreatic cancer. There was an absence of evidence and guidance, regulatory decisions on nanomedicine therapeutics. The FDA collaborates with the Nanotechnology Characterization Laboratory (NCL) to facilitate the regulatory review and in-depth characterization of nanodrugs in medicine. The European Technology Platform on Nanomedicine (ETPN) set up a European Nano-Characterization Laboratory (EU-NCL) as the part of the Horizon2020 project. The regulatory problems seem to be finally overcome since FDA published the Guidance for Industry ('Drug Products, Including Biological Products, that Contain Nanomaterials') in December 2017 (https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/ Guidances/default.htm). Looking into the future, the use of cancer theragnostics, combining anticancer targeted therapy and diagnosis by multifunctional nanoparticles, that combine the therapeutic and imaging agent, might be a revolution in the cancer treatment because they allow to diagnose, visualize, and kill the cancer cells simultaneously and both treatment and diagnostic in the real time. This is a future of medicine, right now it still seems to be a science-fiction movie, but proudly we are coming closer every year to such an amazing progress in diagnostic and treatment thanks to the broad usage of nanoparticles and nanotechnology.

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