Dendrimer Nanoparticles for Colorectal Cancer Applications

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Abstract

Cancer nanotechnology is a prolific field of research, where nanotools are employed to diagnose and treat cancer with unprecedented precision. Targeted drug delivery is fundamental for more efficient cancer treatments. For this, nanoparticles have been extensively used during the last years in order to improve the specificity, selectivity and controlled release of drug delivery. It holds potential in minimizing systemic toxicity through the development of functionalized particles for targeted treatment. Among all the type of nanoparticles, dendrimers display several advantages, which make them ideal candidates for improved and targeted drug delivery in cancer research. Dendrimers can transport large amount of drug into specific areas. In addition, they can be employed for monitoring the progress of the treatment process, with an unprecedented theranostic capability. Special emphasis is given in colorectal cancer, as well as the preferred employed strategies for producing drug-loaded/functionalized NP's for cancer therapy in the last years.

Keywords: Colorectal cancer; Dendrimer nanoparticles; Targeting.

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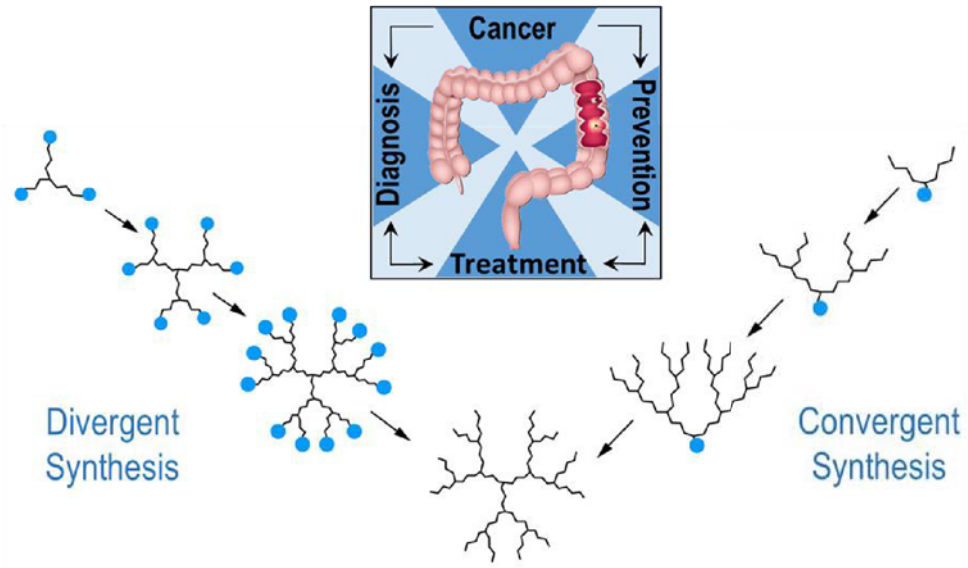
Graphical Abstract



Routes of dendrimer synthesis and the stages of possible applicability of dendrimer nanoparticles in the case of colorectal cancer.

1. Introduction

Nanotechnology is an emerging field of research that is expected to play a definitive role in biomedicine in the near future, particularly for the targeted delivery of drugs at pathological sites inside the human body [1]. Nanoparticles (NPs) can be defined as particulate matter ranging between 1 and 100 nm in diameter, with a high surface area-to-volume ratio, and specialized surface characteristics [2-4]. The use of NPs as drug carriers in oncology started in 1986, when it was reported that NPs showed a tendency to accumulate in tumoral tissues [5]. This passive accumulation, also known as passive or primary targeting, is known as "Enhanced Permeability and Retention" (EPR) effect, and is one of the milestones of cancer treatment using NPs. Therefore, the use of NPs for cancer therapeutics holds very promising due to their high specificity and accumulation in tumor sites, as well as their long blood circulation time **(Figure - 1)**.

However, one of the major problems related to cancer treatment is its intrinsic anti-cancer drug resistance, which can appear prior to chemotherapy as well as acquired resistance due to drug treatment [6]. The selectivity improvement yielded by NPs resulted in a great enhancement in the efficacy of the transported drug, while the occurrence of side effects in the patient was

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reduced. Hence, targeted nanomedicine offers innovative therapeutic strategies to overcome the various limitations of conventional chemotherapy, such as drug resistance, enabling enhanced selectivity, and early and more precise cancer diagnosis [7]. Moreover, it is possible to incorporate targeting moieties selective for cancer cell biomarkers, which improves even more the selectivity and specificity of the treatment [8]. The main types of targeting moieties that are used for decorating and targeting NPs are: i) Antibodies [9]; ii) Peptide-based targeting [10]; iii) Small molecule-based targeting [11]; and iv) Aptamer-based targeting [12, 13].

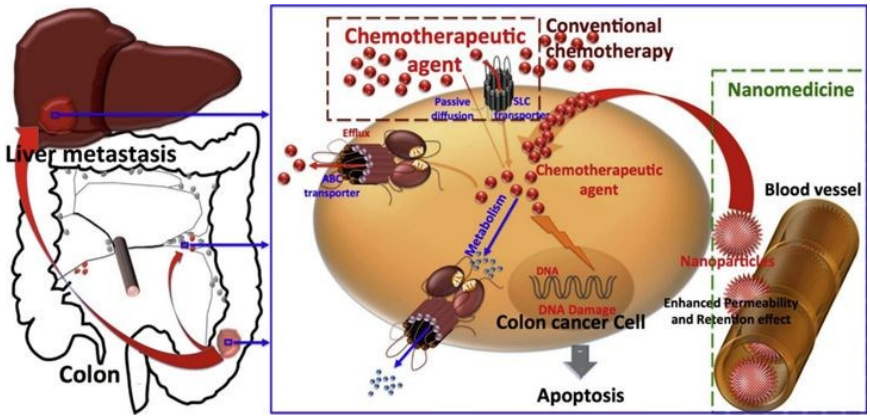

Figure 1 - Rationale for nanomedicine approach in cancer-therapy. Reprinted with permission from [1].

The discovery of non-biodegradable nanoparticles including micelles, nanogels, liposomes, nanoemulsions, polymeric NPs, gold NPs and magnetic NPs, as agents in nano-drug delivery and imaging at pathological sites has enhanced delivery at lower doses and increased aqueous solubility and bioavailability of the drug with reduced side effects [14]. Nevertheless, the clinical application of these NPs to CRC therapy remains limited, as can be seen in **Table 1**.

Table 1 - Current nanotechnology applications in colorectal cancer clinical trials and status.

Study Title	Identifier	Intervention	Status
Targeted polymeric nanoparticles loaded with cetuximab and decorated with somatostatin analogue to colon cancer	NCT03774680	Cetuximab NPs/ Oral approved anticancer drug.	Recruiting
Targeted silica nanoparticles for real time image-guided intraoperative mapping of nodal metastasis	NCT02106598	Fluorescent cRGDY-PEG-Cy5.5-C dots	Recruiting

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TKM 080,301 (Lipid nanoparticles containing siRNA against the PLK1 gene product) in patients with colorectal, pancreas, gastric, breast, ovarian and esophageal cancer with hepatic/ TKM 080,301 for primary or secondary liver cancer	NCT01437007	Drug: TKM-080,301	Completed
Pharmacokinetic, safety and efficacy study of nanoparticle paclitaxel in patients with peritoneal cancer/ A Phase I study of intraperitoneal nanoparticle paclitaxel in patients with peritoneal malignancies	NCT00666991	Nanoparticulate paclitaxel	Completed
Neoadjuvant chemoradiotherapy with CRLX-101 and capecitabine for rectal cancer	NCT02010567	CRLX101/ Capecitabine/ Radiotherapy	Active/ Not recruiting
Liposomal irinotecan, fluorouracil, leucovorin calcium and rucaparib in treating patients with metastatic pancreatic, colorectal, gastroesophageal or biliary cancer	NCT03337087	5-FU/ Leucovorin calcium/ liposomal irinotecan, rucaparib	Recruiting

Moreover, as can be seen from **Table - 1**, none of the ongoing clinical trials using nanotechnology in colorectal cancer comprises the use of dendrimer nanoparticles. After literature research, it was found that there is only one clinical trial using dendrimers, with application in inoperable liver cancers. This dendrimer consists of poly-L-lysine dendrimer as nanovector mixed with complex of 188-Rhenium-ligand (nitro-imidazole-methyl-1,2,3-triazol-methyl-di-[2-pycolyl] amine) [15]. Although a very popular type of nanoparticles, inclusively used in clinical trials for other pathologies such as breast cancer and HIV prevention (VivaGel®), scientific advances are needed for dendrimer applications in the clinical setting.

A variety of dendrimers have been developed and used since the 1980s, but the ones derived from polyamidoamine (PAMAM) are undeniably the most employed. They are generally, biocompatible, and non-immunogenic systems, which favors their use in drug delivery. The core of PAMAM is most commonly ethylenediamine, although more hydrophobic molecules such as diaminododecane, diaminoexane, and diaminobutane may also be used [16].

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Recent literature suggests that dendrimers will be a solid bet in the future of cancer therapeutics [17]. This is because the field of oncology could be transformed by novel diagnostic and therapeutic strategies based on dendrimer nanoparticles. This advanced nanotheranostics area may include improved imaging techniques (such as MRI) by using dendrimers as new contrast agents. Dendrimers will also be applied for targeting a diverse variety of cancers, advancing on their safety and efficacy. In this regard, one of the most interesting targeting strategies involves anti-metabolite drugs, vitamins or hormones that tumors need for growing. Finally, further cancer-related applications of dendrimers in areas such as photodynamic therapy, boron neutron capture therapy, and gene therapy are being studied [18]. This review will cover the fundamentals of research utilizing dendrimers for cancer diagnosis and therapy, with a special focus on colorectal cancer (CRC).

2. Dendrimer nanoparticles

Dendrimers are in the category of polymer nanoparticles. However, they have a very different structure from classical polymers, which makes them unique. They consist of globular molecules made out of branched layers (generations). Such a precise synthesis leads to obtaining monodisperse molecules. On their outer surface, dendrimers can be engineered to have various functional groups such as COOH, COONa, NH_2, or OH [19]. Therefore, after very simple surface modification, dendrimers render intelligent nanoparticles, transporting drugs into specific areas and at the same time can be used for monitoring the state of organs attacked by cancer cells, as well as the progress of the curing process. They can help to limit the anti-cancer drugs delivery to designed goals only, eliminating many side effects of chemotherapy [20]. Adding to this, to visualize the effectiveness of targeting, other moieties – such as imaging agents, can be attached to dendrimers and then assessed by MRI or CT. Because of this chemical multifunctional therapeutic delivery, dendrimer-based nanoparticles have received considerable attention in cancer research [21].

2.1 Properties

Among the innumerous types of NPs available, dendrimers offer multiple advantages. They are highly branched polymers, and therefore specific moieties and drugs can be easily conjugated and encapsulated. PAMAM dendrimers are the most common class of dendrimers,

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suitable for many tissue engineering and regenerative medicine (TERM), materials science, and biotechnology applications [22]. They contain an inner alkyl-diamine core and a peripheral shell made of tertiary amine branches [23, 24]. Therefore, the high level of control over dendritic architectures makes dendrimers ideal carriers in biomedical applications [25]. In addition, the toxicity of dendrimers mainly comes from the high cationic charge density in the periphery, where charges interact with biological cell membrane, resulting in membrane disruption, hurdle that can be easily overcome by surface modification. Dendrimers have the advantages of being biocompatible upon modification and easily eliminated from the body [26] through the kidneys along the same metabolic pathways taken by folate, peptides and antibodies [27-30]. It is well known the impact of size on the in vivo behavior of dendrimers. In general, low generation dendrimers (e.g., G5 or smaller, with hydrodynamic radii of <3.5 nm) display relatively rapid clearance from blood and facile elimination into urine. On the other hand, PEGylated dendrimers or higher generation dendrimers (G6 and above) tend to circulate longer and evenly distribute within major peripheral organs. In addition, only trace amounts were reported to be transported across an intact blood–brain barrier [31]. In addition to urinary excretion, there is also some evidence to suggest that excretion via the feces may play a significant part in dendrimer elimination and that in some cases this is dependent upon dendrimer generation [30]. Limited mechanistic studies for renal retention of PAMAM dendrimers report the localization of these polymers in the lysosomes of proximal tubule cells [32].

In a deeper study, the biodistribution of PAMAM labeled D-Cy5 was studied by injection in healthy rabbits on day 5 of life with the highest accumulation in the bladder and kidneys, confirming its fast renal clearance, even at an earlier age [31]. The present study suggest that more than 90% of the injected dose of the G4 dendrimer is cleared out from the newborn rabbits over 24h, with less than 5% in blood circulation [31]

Since dendrimers possess high density of surface functional molecules, they are easily conjugated with several targeting agents for selective delivery of chemotherapeutics to the tumor tissue. Furthermore, they contain internal cavities for macromolecule encapsulation, being able to transport highly hydrophobic drugs **(Figure 2)** [33].

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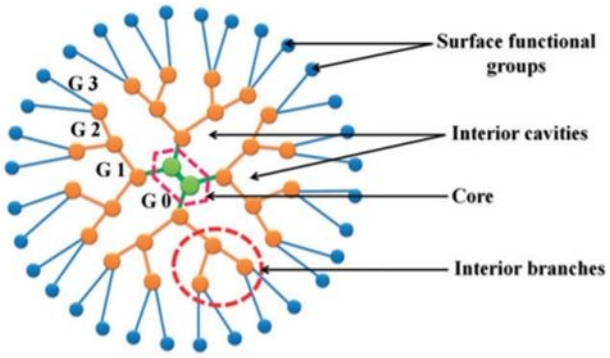

Figure 2 - Dendrimer branched architecture, representing the increasing generations (G). Adapted from [24].

2.2 Synthesis and types of Dendrimers

Dendrimers have been traditionally synthesized by two major routes: the divergent method, introduced by Tomalia *et al.* [24], and the convergent one, developed by Hawker and Fréechet [34]. In the first method (divergent), the final molecule grows radially from a core by the sequential addition of layers of monomers, each layer constituting a new generation (G). The number of surface groups multiplies according to the functionalities in each monomer ramification [35]. It is important that every step of the reaction is fully completed before the addition of a new generation to avoid defects in the branches. One of the main advantages of this approach is that in the final step of the reaction, the surface of the dendrimer can be easily modified with desired functional groups. Moreover, it is a fast synthesis which allows the preparation of large dendrimers. The main drawbacks of this approach is the extensive purification it is required, since the final product and the intermediate reactants have similar molecular weights, charge, and polarity [36]. The main methods of purification used in this type of synthesis are dialysis and precipitation of dendrimers [37]. Also, the higher the generation, the greater the chances are of having branching defects, since the presence of bulky branches creates difficulties in the coupling of new ones. Despite these obstacles, the advantages of this strategy have made it the main route for dendrimer production.

In a reverse way from the divergent synthesis, dendrimers can also be synthesized starting from the surface towards de inner core. The growth of the molecule starts from the ends of the chain, beginning by integrating the various branching points with other monomers that will constitute the dendrimers. Finally, these branches are attached to a central core when they reach the

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desired generation size [38]. In contrast to the divergent growth, this method permits easier purification due to bigger differences between the final products and the initial reagents. The number of reaction steps required for dendrimer synthesis and purification in the convergent method can be reduced, making the preparation of higher generation dendrimers more efficient compared to the divergent method [30, 39]. Also, impurities generated during convergent synthesis can be easily separated as these impurities are usually very different from the synthesized dendrimers in size, structure, and physical properties.

Other advantages include higher monodispersity for low generations and fewer branch defects. The main downsides are low yield and difficulties in obtaining higher generations due to steric interferences when the branches are connected to the core [40].

Regarding the available types of dendrimers, Poly(propylene imine) (PPI) dendrimers were the first ones to be reported by in the 70's. Together with PAMAM, they represent the most widely studied type. PPI dendrimers can be based on a 1,4-diaminobutane (DAB) core, but also be synthesized from an ethylenediamine nucleus and other core molecules by a double Michael addition reaction. Hence their interior contains various tertiary tris-propylene amines, and they form full generations with primary amines as surface ends [41].

Another common type of dendrimers are the Poly-l-lysine (PLL) dendrimers, mostly used as gene carriers due to their excellent condensation with oligonucleotides. Among their favorable characteristics are good biocompatibility, water solubility, biodegradability, and flexibility, similar to other dendrimers. With peptide bonds in their structures, both their core and branching units are commonly based on the amino acid lysine PLL dendrimers differ from the general concept of PAMAM and PPI dendrimers since they are mostly asymmetrical [42]. However, they are still precise molecules, with a controlled number of lysines branching out from the core, and terminal amine residues. The lysine in the terminal group of PLL contains two primary amines that are frequently modified for better biological performance [21, 43].

There are many more types of dendrimer nanoparticles, although less used. They can be classified according to their shape, structure, branching, solubility, chirality and attachment, which can be seen in **Figure 3.**

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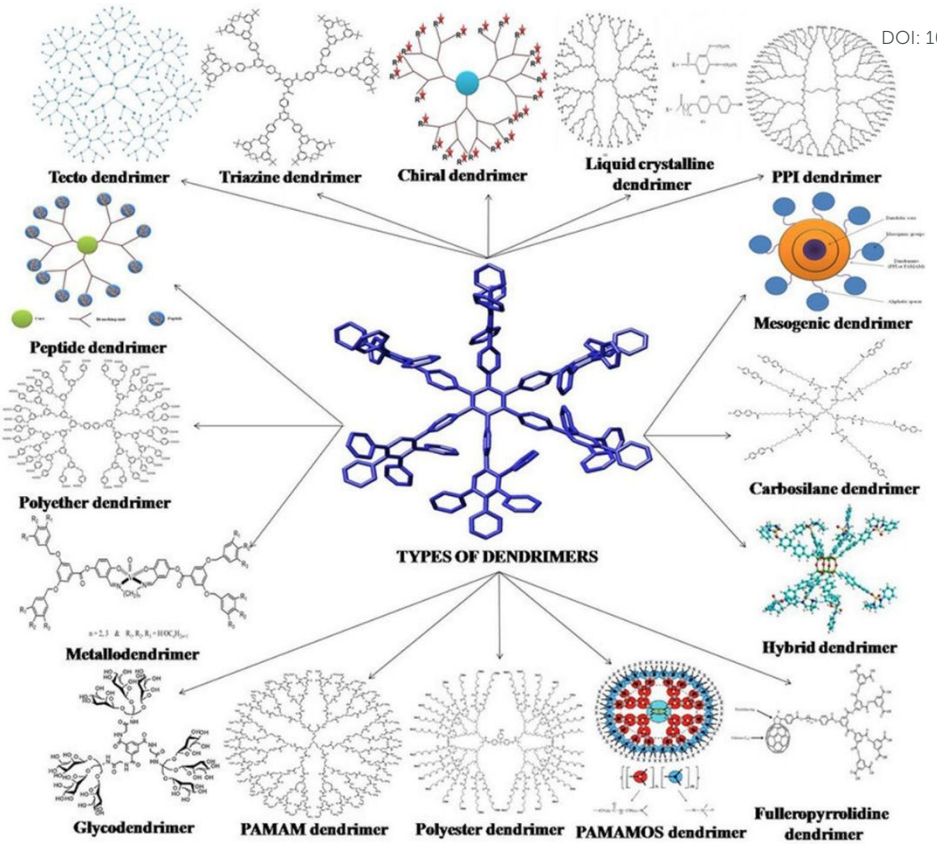

Figure 3- Different types of dendrimers. Reprinted with permission from [44]**.**

When compared to other dendritic NPs, PAMAM surpasses them due to the ease of its preparation, desirable chemical and physical properties, surface functional groups, and comparatively lower toxicity to other dendrimers [45, 46].

The physical and chemical features of dendrimers, which include their high monodispersity, water solubility, encapsulation ability, and large number of functionalizable peripheral groups make these macromolecules suitable contenders as drug delivery vehicles for cancer therapeutics [39]. Moreover, dendrimers are prepared with a level of control unattainable with most linear polymers, leading to nearly monodisperse, globular macromolecules with a large number of peripheral groups [47].

PAMAM dendrimers can be easily prepared using the iterative reaction sequence developed by Tomalia *et al.* [48] and are easily conjugated with various functionalities. The novelty was obtaining these macromolecules by repeating a chemical procedure, as follows: the most applied divergent synthesis of PAMAM dendrimers begins with an amine functional core unit that is reacted with methylacrylate by Michael addition chemical reaction. This results in the synthesis of two new branches per amine group with ester-terminated dendrimer, which is known as a “half-generation” dendrimer [49]. Further amidation of the methyl ester with

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ethylene diamine results in a "full-generation" amine-terminated dendrimers. Reoccurrences of Michael addition and amidation steps yields the next-higher generation dendrimer with additions to its molecular weight, number of terminal functional groups, and size [46].

Functionalization is an integral part of dendrimer multiple uses. Functionalization is the process of incorporating multiple active sites in dendrimers in order to generate macromolecules with multifunctional architecture [50, 51]. Functionalized dendrimers can have six distinct features known as "Critical Nanoscale Design Parameters" (CNDPs). These parameters consist of size, shape, surface chemistry, flexibility, rigidity, architecture and elemental composition [34]. Depending on the clinical application of the dendrimer nanoparticles, these features can be changed and studied, changing the intrinsic properties, function and performance of the nanoparticles. Surface functionalization of dendrimer nanoparticles usually render increased specificity of dendrimer to improve efficiency of cancer therapy and also an increase in its circulation time [52]. For instance, dendrimer nanoparticles find useful functionalization in the following areas: i) increase biocompatibility (by PEGylating [53] or acetylation [54]); ii) enhance transfection efficiency (usually by aminoacid [55] or lipid functionalization [18]); iii) induce site specific delivery (e.g. aptamer [56], antibody [57], vitamin [58], peptides [59, 60]); or iv) to render stimuli responsive dendrimer nanoparticles (e.g. pH [61], thermo [62], photo [63], redox-responsive [64]).

When it comes to dendrimer targeting, aptamers are comparable to monoclonal antibodies, being well-established therapeutic molecules in terms of specificity and affinity to the target. Aptamers are small enough to enter deep into tumors, showing limited immunogenic effects. Because of these advantages, the use of aptamers is a promising strategy to overcome existing problems of common anticancer therapies. But the advantage of aptamers over antibodies includes their high stability, ease of synthesis, less batch-to-batch variation and facile chemical modifications that allow different conjugation chemistries [56, 65]. Regarding peptide strategies, there are numerous tumor-specific targets, and the peptides that bind them are usually divided into three categories: a) peptides that bind to cell surface receptors; b) peptides that target intracellular receptors; and c) peptides that specifically interact with the extracellular matrix. When comparing peptides to antibodies, the latter presents inherent deficits that may limit their in vivo applications. Their large molecular weight result in slow delivery and diffusion into tumor tissues. Another drawback includes the limited stability of antibodies; their activities depend on their intact spatial conformation, which is problematic for in vivo drug delivery systems and causes storage and transportation problems [60]."

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Efforts can be mane also namely to reduce cytotoxicity and enhance transepithelial transport [37], for interaction with coupling molecules such as natural-based polymers [37], fluorescent probes [37], and an inner hydrophobic core where other molecules can be trapped [66, 67]. Among the myriad possibilities, dendrimers can also find applications as imaging agents [66, 68, 69], and scaffolds for TERM [70].

3. Dendrimer applications in cancer research

A strong body of evidence now suggests that NPs in the form of dendrimers may be of added value in the future of oncology-related theranostics [71-73]. Other than the flexibility for functionalization using diverse ligands and its low-nanometer size, the introduction of stimuli responsive functionality on dendrimers allows the release of payloads in response to specific triggers, as discussed before. These triggers could be endogenous in nature (acid, enzyme, and redox potentials) or it could be applied externally (light and temperature) [74-76]. One of these examples is the recent work developed by Nigam *et al.* [77] on cervical cancer, where iron oxide NPs were modified with Generation 2 (G2) PAMAM dendrimers and loaded with doxorubicin (DOX), therefore combining magnetic chemotherapy and hyperthermia on HeLa cancer cells [77]. When exposed to alternating current magnetic field, results show enhanced cell death as result of fatal synergistic contribution of DOX and high temperatures. Interestingly, the combinatorial treatment reduced cancer cell viability from 100 % to 3.6 % [77].

For targeting purposes, folic acid was conjugated to PAMAM dendrimer NPs [78]. Results showed negligible toxicity towards non-cancerous MRC9 lung fibroblast cells, as well as the ability of dendrimer nanoparticles for targeted co-delivery of siRNA and chemotherapy agents together in lung cancer cells [78].

For breast cancer, one good example of dendrimer application is the work of Chittasupho *et al.* [79]. Having in mind that breast tumors preferentially metastasize to the lung, bone and distant lymph nodes (that secret high levels of CXCL12), the team hypothesized that targeted inhibition of CXCR4 in breast cancer cells should suppress CXCR4-positive tumor cells toward secondary metastatic sites. Results showed enhanced *in vitro* cellular toxicity as compared with non-targeted dendrimers. The modified dendrimers exhibited remarkable reduced migration of BT-549-Luc breast cancer cells towards the used chemoattractant. This report demonstrated the potential utility of LFC131-dendrimer conjugates for breast cancer therapy and metastasis.

In the case of liver cancer, due to lack of cell surface biomarkers and highly metastatic nature, early detection and targeted therapy of hepatocellular cellular carcinoma (HCC) is an unmet clinical need [80]. Galactosamine (Gal) is among the few selective ligands used for targeting HCCs due to its high binding affinity to asialoglycoprotein receptors (ASGPRs) overexpressed in HCC. In a recent work, Yousef *et al.* [80] engineered a nanoscale G4 PAMAM dendrimer NP anchored to Gal and loaded with the potent anticancer curcumin derivative (CDF) as a platform for targeted drug delivery to HCC. Surprisingly, in an *in vivo* xenograft model, cytotoxicity assays in HCC cell lines showed that CDF was more potent as a chemotherapeutic anticancer than the currently in use Doxorubicin, Sorafenib and Cisplatin chemotherapeutic agents [80].

Glioblastoma is the most common type of malignant brain tumor and one of the deadliest cancers [81]. It has been described that dendrimers have affinity to cross blood–brain barrier after systemic administration [82]. Liu *et al.* [83] utilized a combined chemo- and gene-therapy approach for effective glioma treatment and developed DOX-loaded dendrigraft poly-l-lysine (DGL) dendrimer surface modified with TNF (tumor necrosis factor) related apoptosis-inducing ligand (TRAIL) for the tumor targeting specifically of T7 peptide, a TfR-specific peptide. This approach was based on the knowledge that DOX increased the anticancer effect of TRAIL by regulating the expression of death receptors, as well as stimulation of apoptotic pathways [83, 84].

For example, Langereis *et al.* [85] reported the synthesis of 5–6-nm gadolinium-diethylene triamine penta-acetic acid (Gd-DTPA)-terminated poly(propylene imine) (PPI) dendrimers as MRI agent. Talanov *et al.* [86] reported a PAMAM dendrimer-based nanoprobe with dual MR and fluorescence (Fl) modalities. Gd (III) was covalently attached to a dendrimer to create a fresh macromolecular contrast MRI agent. The authors used 2-(4-isothiocyanatobenzyl)-6-methyl diethylenetriaminepentaacetic acid (1B4M-DTPA) and Cy5.5 as a bifunctional chelating agent. The PAMAM dendrimers covalently attached to the Gd (III)-DTPA chelates and units of the near-infrared (NIR) fluorescent dye, Cy5.5, to form a dual-modality MRI-FI agent [86].

They can be easily functionalized with a diverse variety of ligands to reach the tumor through the different body barriers in the body with minimal loss of activity. This results in the selectively targeting and killing of tumor cells without affecting the normal cells and most importantly, with an actively controlled release mechanism.

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3.1 Dendrimer Nanoparticles in CRC

CRC is the third most diagnosed cancer in the world. It manifests as a malignant neoplasm in the mucosa of the colon or the rectum [87]. Despite notable progress in treatment still leads to significant morbidity and mortality [88]. Colorectal cancer is a heterogeneous group of diseases. These have individual genetic and epigenetic backgrounds. In order to improve clinical management and better predict patient outcome, classification of colorectal cancers based on location, histology, etiologic factors, and molecular mechanisms of tumorigenesis have been made [89]. Their effectiveness of chemotherapy remains limited due to the intrinsic build-up of resistance of cancer cells to chemotherapy drugs, dose-limiting toxicities and other major side effects. New strategies to overcome these issues are being developed, one of which is cancer nanomedicine, a rapidly developing interdisciplinary research field [90].

The chemotherapeutic approach alone has not been found to be very efficient in CRC as the drug molecules may not reach the target site with an effective concentration and suffer a non-specific distribution, with only a small fraction of the drug reaching the tumor [91, 92].

These unique "polymeric compounds" can form intelligent species after modification, transporting drugs into specific areas and at the same time can be used for monitoring the state of organs attacked by cancer cells, as well as the progress of the curing process [14]. Recent advances in nanotechnology have rendered it an attractive approach for designing novel clinical solutions for CRC [14].

When tumor cells are shed from primary tumors or metastatic sites of early-stage cancer patients and enter the bloodstream, they are called circulating tumor cells (CTCs) [93]. CTCs-driven cancer relapse and metastasis are the leading causes of cancer-related death worldwide. Owing to the importance of CTCs as an indicator of poor prognosis, various approaches were exploited to efficiently isolate and capture CTCs from large populations of interfering cells. Although several technologies (such as microfluidics-based, size-based filtration, etc) have been developed, they were not truly successful. So, a team developed a nanotechnology-based cell detection and capture method [93]: Purified anti-Slex (aSlex) antibody and FITC labeled-second antibody (IgG/IgM-FITC) were used for the following synthesis of aSlex-coated dendrimer conjugates. By doing this, dendrimers would capture (attach to) saliva acidifying Louis oligosaccharides X (Sialyl Lewis X, Slex, a type II carbohydrate antigen for mediating the colorectal cancer metastatic process. The colorectal CTCs were not only captured in artificial blood samples but restrained in cell activity by the conjugates, which means, according the to the referred paper, that there was a specific interaction between antigen (present in CTCs) and

antibody (present in dendrimers). Xie *et al*. [93] reported an effective approach to specifically bind and capture colon cancer HT29 cells by using multiple Sialyl Lewis X antibodies (aSlex)-conjugated with PAMAM dendrimers **(Figure - 4A)**. Results indicated that the conjugate showed the enhanced capture of HT29 cells in a concentration-dependent manner and the maximum capture efficiency of 77.88 % was obtained within 1 hour exposure [93]. This work provided a novel conceptual guidance for the effective prevention of cancer metastasis.

Capecitabine is one of the most used anticancer drugs in CRC, and is converted into 5-FU by various metabolic enzymes involved in DNA damage and tumor growth inhibition [94]. However, capecitabine has multiple adverse side effects affecting the blood, hair cells, bone marrow, and liver. In this context, Nabavizadeh *et al.* [92] induced colon adenocarcinoma in mouse models with azoxymethane, a carcinogen agent, and then investigated the potentiality of non-modified G4 PAMAM dendrimers to improve capecitabine therapeutic index and decrease its adverse side effects on liver and bone marrow [92]. Although no targeting was performed, the team compared the effects of free and conjugated capecitabine form on tumor size and blood cell lines abnormalities. Results showed reduced side effects in the liver and blood along with decreased tumor size when compared with the free form [92].

In a recent work, Aliboland *et al.* [12] encapsulated gold (Au) NPs inside PAMAM dendrimer NPs. The aim of this study was to investigate the theranostic capability of curcumin-loaded dendrimer-gold hybrid structures **(Figure - 4B)**.

The obtained results confirmed higher cellular uptake, internalization and cytotoxicity of Apt-PEG-AuPAMAM-CUR in comparison with PEG-AuPAMAM-CUR in C26 and HT29 colorectal cancer cell lines. Moreover, the system worked as an effective anti-tumor therapy and accurate computed tomography imaging of C26 tumor-bearing mice due to gold accumulation [12].

Similarly, Alibolandi *et al*. [95] developed camptothecin-loaded pegylated PAMAM dendrimer **(Figure - 4C)**. For targeting purposes, the team functionalized the system with AS1411 anti-nucleolin aptamers for site-specific targeting against CRC cells, which overexpresses nucleolin receptors [95]. Remarkably, aptamer AS1411 has now entered clinical trials (phase II/III trials) for acute leukaemia therapy [96]. Comparative *in vitro* cytotoxicity experiments demonstrated that the targeted camptothecin loaded-pegylated dendrimers had higher anti-proliferation activity towards nucleolin-positive HT29 and C26 colorectal cancer cells than nucleolin-negative CHO cell line. The same system was tested *in vivo* on C26 tumor-bearing mice with promising results.

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Another anti-cancer drug currently used in the clinics is irinotecan [97]. However, its utility is limited by its narrow therapeutic index. Theoretically, a possibility to improve therapeutic index consists on increasing drug exposure in the diseased tissue, without accumulation in the healthy tissues. Dendrimer nanotechnology offers this possibility, as shown by England *et al.* [97]. They modified generation 5 L-lysine dendrimer with a polyoxazoline as a drug delivery vehicle for improving the therapeutic index of SN-38, the active metabolite of irinotecan **(Figure - 4D)**. This extensive study comprised different linker technologies to obtain diverse pharmacokinetic profiles of drug release. Three conjugates with plasma release half-lives of 2.5 h, 21 h, and 72 h were tested for efficacy and toxicity using a mouse SW620 xenograft model. The linker with a plasma release half-life of 21 h achieved sustained SN-38 exposure in blood, above the target concentration. Overall, these extensive studies allowed to identify a linker, a dose and dosing regimen for SN-38 conjugated to polyoxazoline-modified dendrimer that maximized efficacy and minimized adverse side effects [97].

Narmani *et al.* [98] studied the anti-cancer efficacy of oxaliplatin (OX) using a nanocarrier system with enhanced targeting efficacy towards folic acid receptors (FAR) expressing CRC cells *in vitro* [98]. This system consisted of PAMAM dendrimers G4 imprinted with polyethyleneglycol (PEG) and folic acid. Polyethelen glycation of polymeric NPs is frequently applied to increase stability, and thus the half-life, as well as the non-immunogenic and non-antigenic properties [99, 100]. The PEG-PAMAM nano-complex containing OX was shown to have a superior cellular uptake in SW480 cell line. The cell viability tests clearly demonstrated the cancer cell growth inhibition effects of PEG-PAMAM-FA-OX. These fundamental scientific advances **(see Table 2)**, coupled with practical methods to covalently conjugate a wide range of bioactive molecules to the surface of a dendrimer or encapsulate them as guest molecules within void spaces, provide a highly versatile and potentially extremely powerful technological platform to fight colorectal cancer.

In another innovative approach, an *in vivo* pretargeting using radionuclides (that are usually incompatible with antibody-based vectors (e.g., ^{18}F, ^{68}Ga, ^{11}C, and ^{64}Cu)), thus helping imaging after the administration of the radiotracer and intensely decreasing the dose of radiation doses to healthy tissues compared to traditional protocol [101]. For this, athymic nude mice bearing subcutaneous SW1222 human colorectal cancer xenografts were. Indeed, pretargeted PET and biodistribution experiments in a murine model of colorectal carcinoma revealed that sshuA33-DEN-TCO (TCO-bearing dendrimers to huA33) produced dramatically improved tumoral activity concentrations compared to an analogous, dendrimer-lacking immunoconjugate (sshuA33-PEG12-TCO- nondendrimeric control immunoconjugate). Surprisingly, the attachment of the G

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0.5 dendrimeric structures didn't hinder the *in vivo* performance of the immunoconjugate, suggesting that this bifunctional scaffold may have applications past pretargeting [101].

Table 2 - Summary of different strategies using dendrimer NP for CRC therapy.

Type of Dendrimer	Target	Anti-cancer drug	Reference
PAMAM G4	N/A	Capecitabine	[92]
Gold NPs inside PAMAM	MUC-1 aptamer	Curcumin	[12]
Pegylated PAMAM	AS1411 anti-nucleolin aptamers	Camptothecin	[95]
L-lysine dendrimer G5 modified with polyoxazoline	N/A	SN-38 (active metabolite of Irinotecan)	[97]
PAMAM G4	Folic acid	Oxaliplatin	[98]
PAMAM G5	8 trans-cyclooctene	N/A	[101]

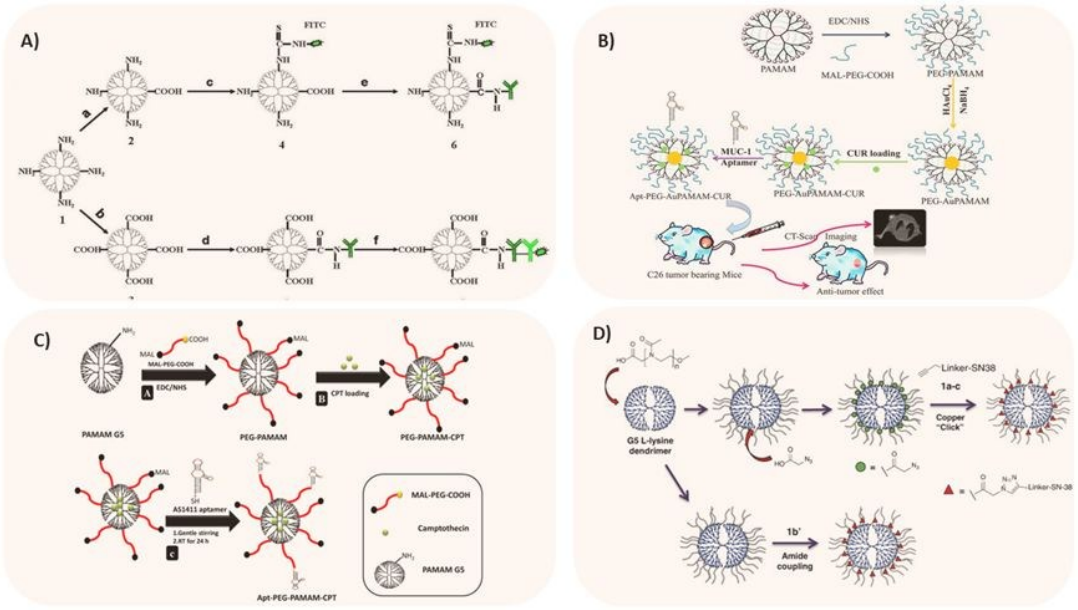

Figure 3 - Different strategies for employing dendrimer NPs in CRC research. A) Two synthetic procedures of aSlex-conjugated dendrimers with FITC labeling. Reprinted with permission from [93]. Copyright © 2015 Nature. B) Dendrimer-gold hybrid structure synthesized by complexing $AuCl_4^-$ ions with PEGylated amine-terminated generation 5 PAMAM dendrimer. The resultant hybrid system was loaded with curcumin. The curcumin-loaded PEGylated Au dendrimer was further conjugated and tested, *in vitro* and *in vivo*, to MUC-1 aptamer for targeting colorectal adenocarcinoma. Reprinted with permission from [12]. Copyright © 2018 Elsevier. C) Schematic representation of (A) synthesis of pegylated PAMAM dendrimer (PEG-PAMAM); (B) camptothecin (CPT) loading in the cavities of PEG-PAMAM; (C) conjugation of thiolated AS1411 aptamers to the maleimide groups of MAL-PEG-

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PAMAMCPT and preparation of Apt-PEG-PAMAM-CPT. Reprinted with permission from [95]. Copyright © 2017 Elsevier. D) Synthesis of polyoxazoline-modified dendrimers. Reprinted with permission from [97]. Copyright © 2017 Elsevier.

4. Conclusions

The inefficacy of conventional chemotherapeutic methods has led to the development of new strategies, which can be used to improve the efficiency of anti-cancer drug delivery into tumors while minimizing distribution and toxicity in healthy tissues as well as novel imaging tools. These novel strategies based on the use of NPs loaded with drugs offer unprecedented opportunities both at the preclinical and clinical levels. However, some challenges still remain, such as improving the localization, biodistribution, biocompatibility, and efficacy of these nano-drug systems *in vivo*, to meet the requirements of precision cancer diagnosis and therapy. In the current scenario, among the new nanotechnology platforms, dendrimer-based chemotherapeutics have emerged as one of the most promising nanotools over the available conventional chemotherapies for the treatment of a variety of tumors. However, their application in colorectal cancer is still in its infancy.

An ideal therapeutic must have the ability to target cancer cells, image the extent of the tumor and sense its signatures, deliver a therapeutic, and monitor cells for their response. Although we are not there yet, this is the goal for our nanotherapeutics.

Nanotechnology, and specially dendrimer nanoparticles with all of the above unique features, will support medical products to develop beyond a single mode of action into multifunctional platforms performing several functions such as nanotheranostics. Researchers around the world are enthusiastically incorporating nanotechnology in CRC treatment.

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Routes of dendrimer synthesis and the stages of possible applicability of dendrimer nanoparticles in the case of colorectal cancer.