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REVIEW

Functionalized Branched Polymers: Promising Immunomodulatory Tools for the Treatment of Cancer and Immune Disorders

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Well-defined synthetic branched nanostructures form an emerging subclass of macromolecular structures whose 3D structure and multivalency offer unique opportunities for fine-tuning internalization and cellular targeting. Dendrimers possess a well-defined 3D-globular backbone with highly versatile functional surface groups and exhibit a range of chemical and biological properties. Branched polymers present unique opportunities for the targeted delivery of diverse bioactive molecules (including targeting ligands, imaging probes, and drugs) via conjugation to multiple sites within the structure. The inherent versatility and multifunctionality of these architectures make them potentially useful for the modulation of multiple immune-related pathways for the treatment of a wide range of disease and disorders, including cancer and human immunodeficiency virus infection. Herein, we describe the key components of the immune system whose targeting can help to overcome immune-related disorders and discuss branched polymers (including dendrimers) as promising delivery systems with unique immunomodulatory properties against cancer and infectious diseases.

1.Introduction

Bioactive molecules, including drugs, peptides, and oligonucleotides, represent promising candidate therapies for a wide range of diseases/disorders. However, poor solubility in water, low permeability across cell membranes, and/or high toxicity often lead to low bioavailability and limited clinical application (1). Novel nanotechnology-based strategies may help to overcome these limitations via the design and synthesis of nanomaterials with versatile loading capacities and targeting functionalities that confer selectivity to bioactive molecule candidates (2, 3).

Among the latest generation of nanotechnological systems, branched polymers, including those highly symmetrical known as dendrimers, have received attention from the scientific community. Dendrimers form organized structures and thereby, unique physical and chemical properties (2, 4). This review explores the potential of such branched polymers as immunotherapeutic tools, providing a critical discussion on the impact of their structures and intrinsic physicochemical properties on immune cell function in cancer and other important immune disorders. We put particular emphasis on tailoring these properties to harness, modulate, and regulate cellular and soluble immune factors towards the generation of specific immune responses.

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1.1. Overview of the critical components of the immune system as potential targets to overcome immune-related disorders

The immune system is a complex cellular network involved in the control of host defenses against pathogens that promptly reacts to foreign pathogens through an effector response (5). However, the immune system must also ignore the presence of self-antigens and innocuous microorganisms from ingested food/drink and the environment (6).

The innate immune system, the first non-specific response against pathogens, is mediated by epithelial barriers, circulating plasma proteins, and diverse types of cells, including epithelial, phagocytic, and natural killer (NK) cells (7), and controls the nature of the induced immune response. Additionally, during human evolution, the innate immune system developed the capacity to activate in response to the recognition of a characteristic "pathogen-associated molecular pattern" (PAMP) by the corresponding pattern recognition receptors (such as Toll-like receptors (TLR) (8). This highly specific system, denominated as the "adaptive immune system", comprises humoral or cell-mediated immune responses. Humoral immunity involves the production of antibodies by B lymphocytes, while cell-mediated immune responses require the activation of T-cells or T-lymphocytes (7).

Antigen-presenting cells (APCs), a heterogeneous cell population derived from bone marrow-resident hematopoietic stem cells (HSCs), represent the link between the innate and adaptive immune systems and constitute an interface between the peripheral tissues and lymphoid organs that stimulates T-cell immune responses (9).

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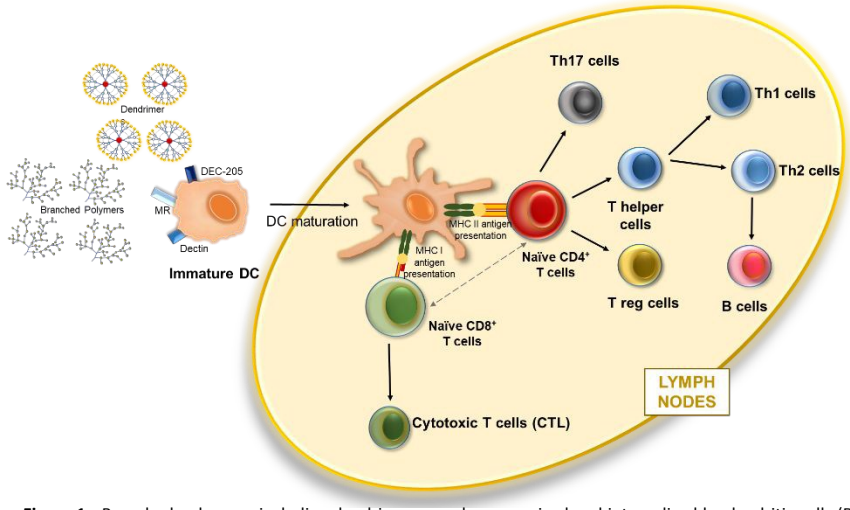

Figure 1 - Branched polymers, including dendrimers, can be recognized and internalized by dendritic cells (DC) by several receptors (e.g., Mannose receptor (MR)/CD206, DEC-205, Dectin 1/2, DC-SIGN). The physicochemical properties of these carriers can be tailored aiming to distinct antigen processing pathways towards DC activation and subsequent expansion of antigen specific effector cells in the lymph nodes.

Dendritic cells (DCs) represent an important subset of APCs, playing a central role in the stimulation of effective antigen-specific responses and the initiation of adaptive immunity (10). In the presence of pathogens, immature dendritic cells (iDCs) process foreign antigens into small peptide fragments. iDCs then migrate to the nearest draining lymph nodes, where their activation into mature DCs (mDCs) initiates an antigen-specific immune response (Figure 1). Antigens are subsequently presented at the surface of mDC membranes complexed with major histocompatibility complex (MHC) I or II molecules. These MHC-antigen complexes are then displayed to specific $CD8^+$ and $CD4^+$ T-cells, respectively (11). Exclusively protein-based antigens can induce an efficient adaptive immune response because only proteins and peptides can engage the required T-cells (6).

Additionally, DCs can recognize, internalize, and present the soluble protein antigen due to its combination with the TLR ligand that thereby enhances the immunogenicity of that immunogen. The activation and expansion of naïve T-cells thus requires three signals provided by DC: i) the presentation of foreign antigen-MHC complexes on the DC surface to T-cell receptors (TCR) (12), ii) the expression of co-stimulatory molecules (CD80/CD86 and CD40) on mDCs that interact with the receptors expressed by T-cells (CD28 and CD40L, respectively) (13), and iii) the release of potent cytokines (interleukin (IL)-2, IL-12) and chemokines (interferon (IFN)-γ) that directly polarize the development of the immune response towards T helper (Th)1 or Th2 cells (14). Pathogens that induce the secretion of IL-12 and other inflammatory cytokines by DCs promote the development of a Th1 cell subset characterized by the production of IFN-γ. Other stimuli that do not induce the production of inflammatory mediators may promote the secretion of IL-4 and IL-13 by Th2 cells. These cytokines, in turn, stimulate B-cells to induce antibody production to neutralize extracellular pathogens and toxins, also improving the recruitment of cells of the innate immune system, such as NK cells and macrophages (5). Effector T-cells migrate to non-lymphoid tissues to carry out their immune functions. After the elimination of pathogens, a subset of T-cells differentiates into memory T-cells to respond quickly to low doses of a given antigen, even without co-stimulation (15).

The process of prophylactic vaccination requires the direct stimulation of the immune system, using attenuated pathogens or synthetic peptide fragments, to avoid the development of an infection/disease after exposure to these harmful agents (16, 17). In response to said agents, vaccination allows the immune system to trigger an adaptive immune response that leads to the production of antibodies that specifically neutralize extracellular pathogens and limit their entry into cells (16, 18). Immunologic memory must be triggered to protect the host against future pathogenic contact and avoid recurrence (16).

Jenner developed the first vaccine in 1796 (19) and ever since, vaccination has represented an essential, cost-effective therapeutic approach to control and dramatically reduce the incidence of several diseases. In some cases, vaccination has entirely eradicated diseases, but a large number of infectious diseases, including malaria, tuberculosis, and bacterial and viral diarrhea, remain active (17, 20).

The formulation of antigens as part of inert delivery systems, such as liposomes (21), nanoparticles (22) or dendrimers (23), represents an exciting option to trigger strong, long-lasting, and specific immune responses. The sections below discuss the potential added value of branched polymers, giving a special focus to dendrimers, as vaccine delivery systems against cancer or infectious diseases, including for human immunodeficiency virus (HIV).

1.2. Dendrimers: structure and synthesis

Dendrimers are well-defined, monodisperse, nanosized, synthetic macromolecules with unique three-dimensional hyperbranched architectures (2, 24). The concept of dendrimers is attributed to Flory *et al*., who first published a paper in 1941 that introduced the idea of branched polymers (25). However, Buhleier *et al*. (26) in 1978 pioneered the "repetitive growth with branching" concept. In parallel, Tomalia *et al*. (27) developed a synthetic procedure to create macromolecules that soon took the name "dendrimer", and the first paper that reported the name of "dendrimers" as well-defined branched structures of polyamidoamine (PAMAM) polymers was published in 1985.

Dendrimers comprise three distinct domains: (i) a central core of a functional atom or molecule; (ii) branches that emanate from the core comprising repeated units containing at least one branch junction; and (iii) multiple surface functional groups essential for carrier properties (24). The unique dendrimer architecture affects the internal and surface properties of these macromolecules (28, 29).

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In comparison to traditional linear polymers, dendrimers display unique features that make them ideal drug nanocarriers for selected applications. These include: (i) monodispersity, with a precise molecular weight; (ii) globular shape, with a size range between 1-100 nm that helps them to cross challenging biological barriers; (iii) high multivalency, with numerous functional surface groups; and (iv) reproducibility regarding the scalable size for an industrial production, although this is highly dependent on the synthetic strategy implemented (24, 30).

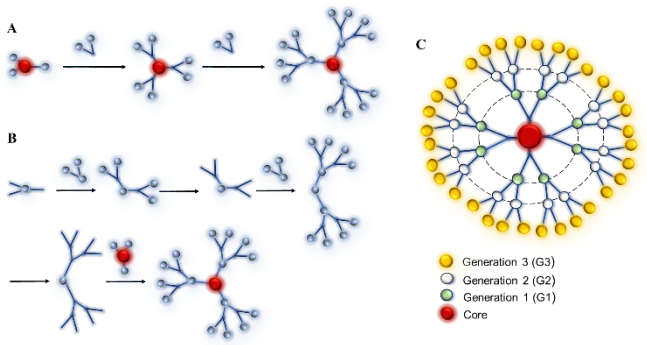

Figure 2: Dendrimers can be synthesized by distinct synthesis routes: A) convergent and B) divergent approaches. In C) Scheme of dendrimer structure and its generations.

The term "generations" defines the number of branching layers of the dendrimer; generation one (G1) has only one layer of branching points, while generation two (G2) displays two layers of branching points, and so on (28). The number of generations influences the size of the dendrimer - the higher the generation, the larger the dendrimer - and strongly affects dendrimer shape. Low generation (up to G4) dendrimers display a planar and elliptical shape, while high generation (G5-G10) dendrimers display a densely packed spherical shape. An increase of one generation doubles the molecular weight of the dendrimer and exponentially expands the number of functional groups at the surface (2) (Figure 2C). For example, G2, G3, and G4 PAMAM dendrimers have 16, 32, or 64 functional surface groups, respectively.

The synthesis of dendrimers employs two main approaches: the divergent and convergent methods (Figure 2).

The divergent method, introduced by Votgle in 1978 (26) and followed by Tomalia and Newkome in 1985 (27), repetitively adds monomer units to a functional core in a two-step process (Figure 2A) that includes a coupling step and an activation step.

The coupling step corresponds to the reaction of the dendrimers' surface functional groups with the reactive group of the branching monomer, thus increasing the number of surface functionalities. To control the reaction, the surface moieties of the monomers do not interact with their reactive group. Therefore, these inert surface functionalities require activation before the next coupling step, by either conversion into a reactive group, coupling with a reactive molecule, or removal of a protection group. The repetition of this process produces an exponential increase in the number of surface functional groups until the desired dendrimer size is reached. This type of synthesis has been widely adopted and employed in the development of a considerable number of dendrimer structures (28).

The PAMAM (31, 32) and poly(propyleneimine) (PPI) pH-responsive amine-terminated dendrimers represent the most widely studied classes (Figure 3) and generally employ the divergent synthesis. Ethylenediamine (EDA) or ammonia constitute the core of the PMAM dendrimers, and several Michael additions of amino groups with methyl acrylate followed by amidation of the resulting esters with ethylenediamine allow the development of final products up to generation ten (24, 33, 34).

PPI dendrimers contain polyamine branches, and their synthesis employs EDA or 1,4–diaminobutane (DAB) as a core with repeated Michael additions of acrylonitrile followed by the catalytic hydrogenation of the nitrile groups (2, 35).

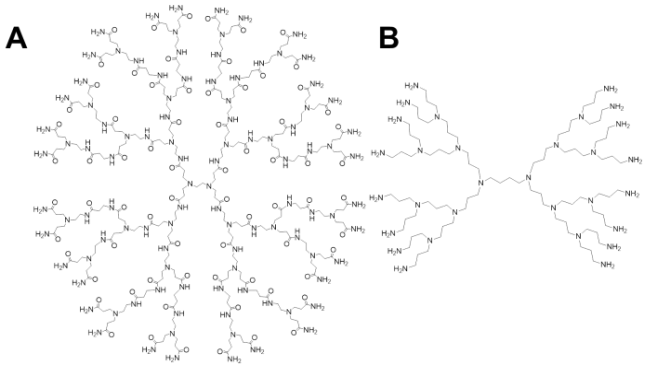

Figure 3: Molecular structures of polyamidoamine (PAMAM) **(A)** and poly(propyleneimine) (PPI) **(B)** dendrimers.

Nevertheless, as the number of coupling steps increases exponentially for each addition, the probability of incomplete functionalization and side reactions also dramatically increases. Unfortunately, this may lead to structural defects in the synthesized dendrimers, thereby constituting a considerable drawback of this method (30). To overcome or at least alleviate these limitations, the development of click chemistry techniques and the acceleration of synthetic approaches has increased final yield (28, 36, 37).

The convergent method was developed by Hawker and Fréchet in 1990 (38) to overcome the disadvantages associated with the divergent approach. In this method, dendrimer construction begins from the end groups and progresses inwards (Figure 2B). The divergent approach involves the synthesis of dendrons (the branched portions of the dendrimer) via successive coupling and activation reactions, followed by anchoring the dendrons to the core. After each coupling reaction, activation of the single focal group of the dendron allows the reaction with the reactive groups of the next monomer. Following the desired number of repetitions, the dendrons are attached to a multifunctional core via their focal groups to yield a multidendron dendrimer (24, 28, 39). The main advantages of this approach include the minimization of defects and the facile purification of the final product. However, the convergent method limits the development of high generation (typically above the sixth) dendrimers due to nanoscale steric problems in the reaction of the dendrons and the core structure (28, 30, 39).

1.3. Physicochemical properties of dendrimers and their biological impact

Dendrimers can be classified according to various physicochemical properties: structure, shape, branching, solubility, chirality, and attachment (40). Dendrimer characterization usually employs a wide range of complementary analytical techniques, as reviewed elsewhere (41-43). The chemical identity of the dendrimers can be confirmed via nuclear magnetic resonance (NMR), mass spectrometry (MS), infrared radiation (IR), UV-VIS spectrometry, and/or circular dichroism (CD). Final dendrimer conjugate sizes can be explored by atomic force microscopy (AFM), transmission electron microscopy (TEM), or dynamic light scattering (DLS). Finally,

the complexation of dendrimers with DNA or RNA can be studied by isothermal titration calorimetry (ITC) as well as various chromatographic and electromigration methods (e.g., electrophoresis or capillary electrophoresis).

Surface decoration of dendrimers can modulate biodistribution, endocytosis, and dosage, thus controlling the release of drugs and final conjugate biocompatibility (44). Therefore, the high surface functionality of dendrimers has been explored to extend their applications (45) as drug delivery systems (for anti-cancer, anti-viral, and anti-microbial purposes), gene transfection reagents, and vaccine candidates (39, 46-48).

1.3.1. Surface charge

The charge of the surface end groups (positive, negative, or neutral) also influences the release of the active agent from the dendrimer at the desired site of action.

Cationic dendrimers, such as PAMAM and PPI, with a high density of amine surface end groups, can form complexes with DNA (49) or siRNA (50) and then interact with biological membranes to promote intracellular delivery (51, 52). The highly condensed structure of high generation dendrimers formed after complexation may protect nucleic acids from degradation. This, in addition to the electrostatic interactions established between positively-charged dendrimers and most cellular membranes (6), potentiates the application of dendrimers as gene delivery systems and non-viral gene transfection reagents (53, 54).

Although beneficial for complexation, the drawbacks of cationic dendrimer multivalency relate to the disruption of membrane integrity, cell death, and toxicity following their uptake by endocytosis (51, 55). Cytotoxicity constitutes a significant limitation regarding their *in vivo* application (56).

The surface functionalization of PAMAM and PPI dendrimers with bioactive molecules may overcome these toxic effects and provide the system with targeting properties (4). The primary amino groups on the surface of these dendrimers can be readily derivatized under various reaction conditions (i.e., solvents, concentrations, temperatures, and catalysts). The application of N-hydroxysuccinimide (NHS) (57) or members of the carbodiimide family, which includes N,N'-diisopropyl carbodiimide (DIC) (58), dicyclohexyl carbodiimide- (DCC) (59), or 1-(3-(dimethylamino)propyl)-3-ethylcarbodi-imide (EDC) (60) as coupling agents represent the most popular strategies employed to decorate dendrimers. Furthermore, the exploration of bioorthogonal groups for the conjugation of bioactive molecules may allow the development of new dendrimeric structures, while the application of stimuli-responsive linkers (i.e., hydrazone (61) and disulfide bonds (62) supports the controlled release of bioactive molecules.

Following these synthetic strategies, agents such as poly (ethylene glycol) (PEG) (63), folate (64), carbohydrates (such as mannose (65) and fucose (66)), or nucleic acids can be employed to modify the surface of the dendrimer. PEG is a non-toxic, non-immunogenic, and water-soluble polymer and its conjugation to PAMAM dendrimers enhances the transfection efficiency and reduces cell cytotoxicity compared to non-modified PAMAM (67). Folic acid (FA) is a natural B-vitamin that exhibits high affinity for the folate receptor (KD ≈ 10^{-10} M) and displays tumor-specific properties. The covalent conjugation of FA to G6-PAMAM improved tumor-cell selectivity and subsequent uptake when compared to non-modified dendrimers (68). The conjugation of carbohydrates to dendrimers can aid the development of immunotherapeutic delivery systems, as these saccharides can trigger DC activation via glycan receptors (69). Motoyama *et al*. developed a novel thioalkylated mannose-modified G3 PAMAM dendrimer with α-cyclodextrin as an APC-selective siRNA carrier (70). Moreover, Ciolkowski *et al.* functionalized the amino groups of G4 PAMAM with pyrrolidone moieties to improve the biocompatibility of the unmodified PAMAM, by reducing their interaction with biological membranes (71).

In contrast to higher generation dendrimers with neutral and cationic surface groups, low generation dendrimers presenting anionic or neutral polar terminal surface groups display robust and biocompatible profiles (44). Low generation dendrimers have been explored, for example, as non-viral vectors of chemical agents and genes (72) for HIV treatment (73). Dendrimers presenting terminal carboxylate and sulfonate functional groups prevented HIV replication by impairing the interaction between the HIV envelope proteins and receptors on the host cells, thus suppressing HIV-host cell communication (74). Moreover, dendrimers carrying anti-HIV drugs into HIV-infected cells can inhibit HIV replication (75). In addition to these polyanionic dendrimers that have been explored for the prevention of HIV replication, unmodified polycationic dendrimers also inhibited the binding of the trans-activator of transcription (Tat) protein (trans-activator of all HIV genes) to trans-acting responsive element (TAR) RNA of HIV (76).

1.3.2. Mean average size

An increase in the generation number of dendrimers results in changes from a few to tens of nanometers in diameter (77, 78). The size of dendrimers defines their overall three-dimensional shape; lower generation dendrimers display a more open and amorphous structure, while higher generations present a spherical structure that may be suited to carrying molecules, such as drugs, metals, or probes in their internal void space (24, 79). The structural properties of dendrimers enable the entrapment of hydrophobic molecules, thus increasing molecule solubility. Dendrimers of higher generations (above G4) present even larger internal volumes allowing for high loading capacities (80). However, the use of a polymer-drug linker could allow better control of drug release kinetics (81).

In general, dendrimers display sizes like those of important biomacromolecules, such as proteins and DNA, which inhibits their excretion from the body (44, 82). For example, the kidneys readily eliminate PAMAM dendrimers below G5 via glomerular renal filtration, but as dendrimer size increases, the elimination rate will instead depend on the hepatic clearance (83).

In tumor tissues, dendrimers of a few nanometers in size tend to extravasate from defective tumor-associated blood vessels, thus accumulating in different organs or being excreted by renal filtration (84). PEG-surface modified dendrimers can overcome these unfavorable pharmacokinetic and biodistribution profiles. Zhong *et al.* studied the effect of PEGylated G3 PAMAM dendrimers on the systemic and lung cellular biodistribution, demonstrating that high-density PEG surface modification enhanced pulmonary absorption and plasma concentration upon pulmonary delivery without cytotoxic effects (85).

The size and surface characteristics of dendrimers also strongly influence the selective accumulation in lymph nodes and lymphatic vessels. Studies using a PAMAM dendrimer decorated with a chelating agent to complex gadolinium as a common contrasting agent in magnetic resonance imaging (MRI) demonstrated that larger sizes led to lower systemic clearance (less renal elimination) and higher lymph node accumulation. Additionally, G6 PAMAM dendrimers (≈10 nm) exhibited higher selective lymphatic uptake compared to G2 and G4 generations, which suffered rapid clearance (86). The conjugation of fluorescent probes to G6 PAMAM dendrimers further confirmed these findings.

The hydrophobicity of dendrimers also affects their selective accumulation in the lymph nodes. Kobayashi *et al.* compared the effect of G5 PPI dendrimer derivatized with DAB with a G4 PAMAM dendrimer with a similar molecular weight, finding that DAB conferred higher hydrophobicity to the structure and led to the more extensive accumulation of the G5 PPI into the lymph nodes and lymph vessels (87).

The reproducible monodisperse nature of dendrimers represents one of the principal factors that has driven their application in diverse biomedical applications. By understanding the exact nature of dendrimers, one can establish a correlation between the biological impact of the dendrimer and their specific structure (28) and, as a result, predict *in vivo* biological effects and avoid failures at later stages of clinical trials.

2. Dendrimers as immune modulators: challenges and possibilities

Dendrimers themselves do not induce adverse host responses, such as immune or inflammatory reactions (88), and most proteins and T-cell epitope peptides present low intrinsic immunogenicity (89, 90). Due to safety concerns and progress in biotechnology and molecular biology, most new vaccines comprise recombinant subunit proteins or peptides, or non-viral vectors to deliver DNA that encodes for a single or multiple antigens (small subunits of pathogens) (91-93).

The delivery of small antigens and/or immune-regulators towards an effective modulation of adaptive and innate responses represents a particularly promising application of dendrimers (18, 94). Dendrimers carrying small antigens allow the formation of multi-antigenic conjugates with well-defined structures (46), conferring greater exposure and better presentation to the immune cells, and improving overall immunogenicity (95). An example of a dendrimer for immune application is the Multiple Antigenic Peptide (MAP), first described by Tam *et al*. in 1988 (96). This commercially available dendrimer was synthesized via a solid-phase or convergent strategies using the R- and ε- amino groups of lysine as branching points. The branched structure obtained demonstrated robust immunogenicity and can be surface-decorated with many different ligands (i.e., hydrazones, disulfide bonds, iminothiolane, or amino-acidic sequences) to stabilize the molecule antigen and thus improve the induction of specific immune responses (97).

The modification of dendrimers with antigens and/or adjuvants can impart immunostimulatory properties to the nanocarrier (6, 23). As an example, targeting specific glycan-binding lectin receptors overexpressed on dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) with glycans relies on glycan density, as well as on the spacer and nature of the carrier molecule employed (98).

Cationic dendrimers such as carbosilane dendrimers can induce changes in gene expression in different cell types and may be used to treat autoimmune processes. Perisé-Barrios *et al*. (99) verified that carbosilane dendrimers limited the M2-like polarization of tumor-associated macrophages (TAMs), therefore constituting a potentially efficient tool to overcome tumor-immune evasion.

2.1. Peptide- and glyco-dendrimers for cancer immunotherapy

Peptide-dendrimers and glyco-dendrimers have been explored as potential modulators of cancer immune responses (Figure 4). Peptide-dendrimers are highly branched macromolecules that possess a peptide bond in the branching core and/or in the peripheral chains (100). Peptide-dendrimers can be divided into three groups: i) "grafted" peptide-dendrimers, with peptides functionalizing the surface), ii) dendrimers entirely composed by amino acids, or iii) dendrimers that have amino acids in the core and functionalized groups at the surface, but not in the branching units (24, 101).

Apart from the typical characteristics of general dendrimers, peptide-dendrimers have properties similar to proteins, such as biocompatibility, solubility in water, and resistance to proteolytic digestion (101, 102). Given these advantages, peptide-dendrimers have been explored as drug and gene delivery systems (103, 104).

For example, *in vivo* testing of a mPEGylated doxorubicin (Dox) peptide-dendrimer conjugate indicated low toxicity, a lack of unwanted side effects, and improved antitumor efficacy in a 4T1

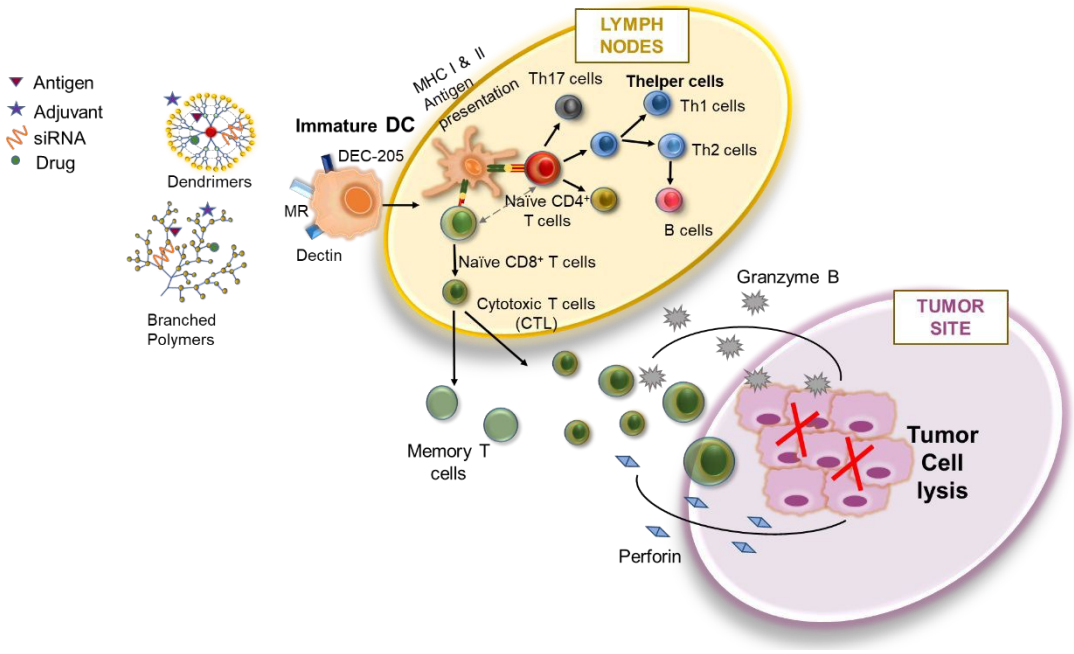

Figure 4: Representation of the interaction between antigen- and/or adjuvant-conjugated branch polymers with dendritic cells and their impact on the activation and expansion of different subpopulations of T cells, towards the induction of a long-term immunity and destruction of tumor cells.

breast cancer mouse model (103). Pan *et al.* tested mPEGylated diaminocyclohexyl platinum (II) (DACHPt) peptide-dendrimer conjugates for the delivery of the antitumor drug DACHPt as an ovarian cancer therapy, discovering reduced cell proliferation and increased apoptosis of tumor cells *in vivo* (105).

One of the advantages of using peptide-dendrimers in immunotherapy relates to the spatial disposition of the ligands. The unique spatial conformation of dendrimers and branched peptides, in general, was used by Heegard *et al.* (106) and Tsikaris *et al.* (107) to develop a polypeptide with α-helical content and differential spatial distribution of the antigen. Compared to free peptides, the reduction of conformational freedom of the antigenic moieties increased the immune response.

Glyco-dendrimers possess carbohydrate-binding moieties with a broad affinity (10^{-5}–10^{-6} M range) to a class of proteins called lectins (108). Suitable sugar moieties include glucose, mannose, and galactose (24), which all display interesting characteristics towards immune applications. Immune cells, such as DC, exhibit a wide range of surface carbohydrate receptors (i.e., lectin receptors) that mediate the immune response (Figure 1). As binding efficiency increases when multiple sugar moieties interact with the receptor, glyco-dendrimers may provide exciting opportunities (109). Glyco-dendrimer synthesis employs the convergent and divergent strategies, although the derivatization of pre-existing dendrimers with desired sugar moieties is the preferred approach. In many cases, coupling uses two steps: (i) sugar derivatization with a spacer and (ii) dendrimer derivatization, while the application of protecting groups on hydroxyl sugar moieties avoids side reactions and facilitates purification (110).

Surface carbohydrates mediate the interaction between cells and antibodies, viruses, bacteria, and other carbohydrates (34). However, carbohydrates by themselves do not induce immune cell proliferation and maturation, being denominated T-cell independent antigens (111).

Dziadek *et al.* (112) developed a mucin (MUC)-1 glyco-peptide dendrimer to bind to the immunoglobulins of B-cells and trigger the production of IgM antibodies to induce a humoral immune response specific to MUC1-glycopeptide antigen transgenic mice. Sheng *et al.* (113) developed a novel mannose-based antigen delivery system employing an ovalbumin (OVA) PAMAM dendrimer (MDO) whose potent immune-mediated effect resulted from enhanced antigen presentation and improved DC maturation. Thus, mice immunized with MDO produced extensive $CD4^+$ and $CD8^+$ T-cell-mediated immune responses and demonstrated an OVA-specific IFN-γ effect. Additionally, in a B16-OVA melanoma model, pre-immunization with MDO delayed tumor growth 11 days post-immunization, proving the prophylactic efficacy of this vaccination strategy.

To overcome the drawbacks of conventional cancer therapeutics, Lee *et al.* (114) developed a novel approach combining chemotherapy and immunotherapy (chemo-immunotherapy) drugs into delivery systems based on aptamer-dendrimer bioconjugates. *In vitro* studies demonstrated that Dox-loaded aptamer G4-dendrimer-CpG bioconjugates (Dox@Apt•dONT-DEN) increased IL-1β, IL-12, IL-6 and tumor necrosis factor (TNF)-α secretion by the macrophage RAW 264.7 cell line. Evaluating the antitumor activity of Dox@Apt•dONT-DEN in a 22RV1 xenograft tumor model of prostate cancer demonstrated a tumor volume reduction of 78% at 36 days post-injection when compared to PBS. Overall, it constitutes a promising strategy to combine cancer-targeting ability, immune-stimulating function, and improved drug delivery profiles.

Perisé-Barrios *et al.* (115) demonstrated that a carbosilane dendrimer (2G-03NN24) led to the reduction of tumor size and level of intratumoral blood vessel formation in the MC38 mouse tumor model. Additionally, treatment significantly reduced TAM number, thereby disrupting the microenvironment of the tumor niche.

In an intracranial rodent gliosarcoma model, hydroxyl-functionalized G4-PAMAM dendrimers became selectively and homogeneously distributed throughout the solid tumor (~6 mm) and the peritumoral area within fifteen minutes after systemic administration, with subsequent accumulation and retention in tumor-associated microglia/TAMs. These hydroxyl- functionalized G4-PAMAM dendrimers constitute a promising strategy for the delivery of immunomodulatory molecules to TAMs.

Table 1 summarizes different dendrimer structures/cargos/strategies employed to modulate the immune response as a cancer therapy.

2.2. Functionalized dendrimers as potential anti-HIV agents

Preventive and therapeutic vaccinations that elicit strong and specific cellular and humoral immune responses represent a promising strategy to eradicate long-term HIV infection by inhibiting HIV replication. While preventive HIV vaccines aim to protect patients from HIV infections, therapeutic HIV vaccines are designed to control HIV infection in persons harboring the virus (116).

Functionalization of dendrimers with HIV-derived peptides, viral proteins, or inactive viral particles can enhance host-specific immune responses (95). The functionalization of dendrimers with CD4 receptors, glycosphingolipids, or DC-SIGN (73), can impede HIV binding to host cells and thereby function as anti-HIV agents (Figure 5). As an example, Dutta *et al.* (117) developed a mannosylated PPI dendrimer loaded with the anti-HIV drug lamivudine to target macrophages, which function as reservoirs for HIV viruses.

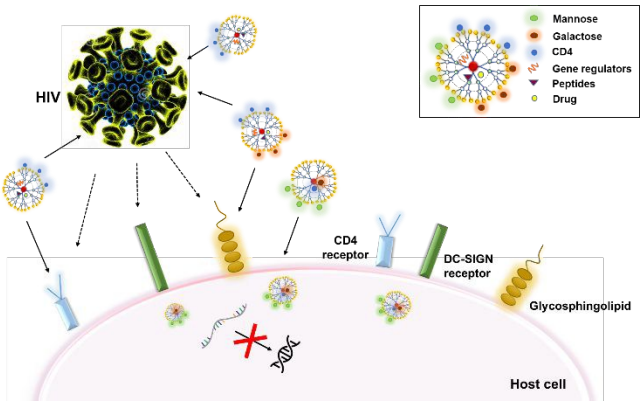

Figure 5: Examples of the use of functionalized dendrimers as anti-HIV agents. Adapted from (118).

Materials Horizons

REVIEW

Table 1. Examples of dendrimers as promising tools to modulate host immune responses against cancer.

Disease/Target	Application / Strategy	Dendrimer composition	Cargo	Immune biological effect	References
-	Cancer vaccine	MUC1-glycopeptide dendrimer	Two-component glyco-peptide vaccine comprising MUC1 tandem repeat (sialyl-TN antigen+PDTRPAP motif) and Tetanus toxoid T-cell epitope peptide.	Proliferation of $CD3^+$ and $CD8^+$T-cells.	(119)
Melanoma	Natural killer (NK)-targeted vaccine	N-Acetyl-D-glucosamine-coated PAMAM dendrimer (PAMAM-GlcNAc8)	Glyco-dendrimers as NKR-P1 ligands (non-classical MHC-recognition).	Delayed B16F10 murine melanoma tumor growth and prolonged survival after intraperitoneal (IP) administration. *Ex vivo* enhanced NK cell activity.	(120)
-	Multivalent cancer vaccine/multiple antigen presentation	MUC1-glycopeptide dendrimer	OVA-T-cell peptide and MUC1 tandem repeat (sialyl-TN antigen+PDTRPAP motif) mixed with Freund's adjuvant (CFA).	Induced humoral immune response specific to MUC1-glycopeptide antigen in DO11.10 transgenic mice (CD4 receptor specific for OVA peptide sequence).	(121)
Melanoma	DC-targeted cancer vaccine	Mannosylated PAMAM dendrimer ovalbumin (MDO)	OVA	Induced OVA-specific T-cell response *in vitro*, due to higher antigen uptake and DC effective maturation. MDO induced robust *in vivo* OVA-specific $CD4^+$/$CD8^+$ T-cell and antibody responses and $CD8^+$ T-cell proliferation. Pre-immunized animals challenged with B16-OVA tumor cells and MDO-treated animals displayed slower or no tumor growth for 11 days.	(122)
-	NK-targeted vaccine	N-Acetyl-D-glucosamine-coated PAMAM dendrimer (PAMAM-GlcNAc8)	Soluble antigens: T-independent (2,4dinitrophenylated lipopolysaccharide, DNP-LPS), T-dependent soluble (keyhole limpet hemocyanin, KLH) or corpuscular	No significant changes in B cell, helper T-cell, cytotoxic T-cell (CTL), and NK T-cell (NKT) number; PAMAM-GlcNAc8 induced higher antigen-specific IgG2a levels, decreased IgG levels, and unaltered IgM levels. Overall,	(123)

			antigen (sheep red blood cells, SRBC).	modulation of anti-tumor responses occurred via NK cell stimulation.	
Melanoma	NK-targeted vaccine	N-Acetyl-D-glucosamine-coated polyamidoamine dendrimer (PAMAM-GlcNAc8)	PAMAM-GlcNAc8 by itself present high affinity to NKR-P1A and NKR-P1C activating proteins.	Increased serum anti-B16F10 melanoma IgG and IgG2a levels led to antibody dependent cell-mediated cytotoxicity (ADCC), plasma cells, and antigen-presenting B cells (increased CD80 and CD86). Up-regulation of IFN-γ by NK1.1 expressing cell population. Overall enhancement of tumor-specific antibody formation triggered by NK cell activation.	(124)
Melanoma	DNA vaccine - Dendrimer platform for DNA delivery	G5-PAMAM	DNA and MHC class II–targeting peptides (gp70-derived AH1, SPSYVYHQF; TRP2180–188, SVYDFFVWL; PAn DR epitope (PADRE), aKXVAAWTLKAAaZC-modified hemagglutinin- derived H2-I-Ed- restricted HA110–120, SFERFEIFPKEC).	Enhanced transfection of murine and human APC *in vitro*; DNA-peptide-PMAM dendrimer administration (N:P ratio 10:1) through subcutaneous route showed high transfection efficiency of mainly DC in lymph nodes (LN) and elicited the generation of T-cells and reduced tumor growth in a B16F10 melanoma model.	(125)
Prostate cancer	Chemo-immunotherapy	Doxorubicin (Dox)-loaded aptamer dendrimer-CpG bioconjugate (Dox@Apt•dONT-DEN):	Single-strand DNA-A9 PSMA (prostate-specific membrane antigen) RNA aptamer as targeting agent and CpG and Dox conjugated to PAMAM dendrimer CpG–doxorubicin (Dox).	*In vitro* increased levels of the interleukin (IL)-1β, IL-12, IL-6, and tumor necrosis factor (TNF)-α secreted by macrophages Raw264.7 cells; decreased tumor growth by 78% in a 22RV1 xenograft tumor model following repeated intravenous treatment. Anti-tumor effect attributable to cancer-targeting, immune-stimulating function, and prolonged blood circulation.	(114)
-	Dendritic cell (DC)-targeted cancer vaccine	Dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN)- using Lewis-type epitope Le^b glyco-peptide conjugated to PAMAM dendrimer	OVA Peptide sequences SIINFEKL for Le^b OTI glyco-peptide dendrimers and ISQAVHAAHAEINEAGR for Le^b OTII glyco-peptide dendrimers.	*In vitro* internalization by bone marrow-derived dendritic cells (BMDC) was the highest for 3-4 Le^b glyco-peptide dendrimer, which co-localized with lysosomes/early endosomes. Strong induction of antigen-specific $CD8^+$ and $CD4^+$ T-cell proliferation by 2-7 Le^b dendrimer-treated DC. Generation 0-1 Le^b OT-I glyco-peptide dendrimer induced highest antigen-specific T-cell proliferation.	(98)

Cervical cancer (Human papillomavirus (HPV))	Therapeutic vaccine	Polyacrylate amphiphilic 4-arm star-dendrimers conjugated HPV epitopes	HPV-16 E7 protein epitope 8Q (QAEPDRAHYNIVTFCCKCD; E744−62) contains cytotoxic T lymphocyte (CTL), T helper and B-cell epitopes.	One single dose allowed reduction of tumor growth and eradication in E7-expressing TC-1 tumor mouse model by day 21 post-tumor inoculation.	(126)
-	Tumor immunotherapy	2G-03NN24 carbosilane dendrimer	-	*In vitro* reduction of LPS-induced IL-10 secretion by monocyte-derived M2 macrophages and STAT3 phosphorylation; switch to M1-like polarization of tumor-associated macrophages (TAM) inhibiting the proliferation of K562 tumor cells.	(99)
Myeloid leukemia (Hematopoietic stem cell transplantation (HSCT))	NK cell-based immuno-therapeutic treatments	Poly (phosphorhydrazone) dendrimers capped with amino-bis(methylene phosphonate) end groups	-	After 18 days of incubation, dendrimers activated the peripheral blood mononuclear cells (PBMC), which were then destroyed by NK cells. To evaluate the *in vivo* antitumor effect of the human NK cells expanded *ex vivo*, dendrimer-treated PBMC were injected in a myeloid leukemia mouse tumor model. Tumor weight was significantly decreased by dendrimer-expanded NK cells.	(127)

Table 2- Dendrimers as regulators of HIV- immune responses.

Disease/Target	Application / Strategy	Dendrimer composition	Cargo	Immune biological effect	References
HIV-1	DC-based vaccine	Third-generation mannosylated poly-L-lysine dendrimer-peptide conjugates	Segment 544-549 of HIV gp41.	The HIV-1 gp41(541-555) conjugate (8a) (containing the LLSGIV motif) elicited polyclonal antibodies and a weaker immune response than control. Multiple mannose groups stabilized the antigenicity of the peptide gp41(541-555) by protecting it from proteolysis and enhancing interaction with receptors on immune cells.	(128)
HIV	DC-based vaccine	Water-soluble carbosilane (CBS) dendriplex	Three different HIV-derived peptides were synthesized: peptide from Nef sequence - HIV-HXB2 location Nef (172-191) (NHGMDDPEREVLEWRFDSRLAF- COOH), peptide from Gag-P24 sequence -HIV-HXB2 location P24 (NH-DTINEEAAEW-COOH) and peptide from envelope Gp160 - HIV-HXB2 location Gp160 (634e648) (NH-EIDNYTNTIYTLLEE- COOH).	HIV-derived peptides enhanced *in vitro* capture by immature and mature DC (iDC and mDC) when formed complexes with 2G-NN16. The Gp160/2G-NN16 showed the earliest and strongest uptake by DCs. The uptake of 2G-NN16 and 2G-NN16/Gp160 had no significant effect on the iDC phenotype, mDC maturation ability, and migration of DC, which is crucial for presenting antigens to T-cells. No impact on allogeneic T helper cells stimulation via mDC and cytokines (IL-12p70 and TNF-α) secretion *in vitro*.	(129)
HIV	DC-based vaccine	Maltose functionalized positively charged G4 poly(propylene imine) (PPI) glyco-dendrimers	Three peptide structures were used: HIV-HXB2 location Nef (172– 191), peptide derived from Gag-P24 sequence (HIV-HXB2) and peptide derived from envelope Gp160.	HIV-P24-derived glyco-dendrimer had higher uptake rates compared with other peptide-dendrimeric structures. More than 80% of iDC were positive for P24-peptide presence after 2 h of incubation.	(130)
HIV	DC-based vaccine	Anionic PEG-citrate G2 dendrimer	Multi-polytope (gag/pol/env/tat) of HIV genome.	After two weeks of BALB/c mice immunization with a mixture of polytope-dendrimer, the levels of IgG1 and IgG2a specific antibody significantly increased compared with control groups. The IFN- γ level increased in immunized mice.	(88)

Table 3 - Different strategies using dendrimers and cargos to modulate the immune response against pathogen infections

Disease/Target	Application / Strategy	Dendrimer composition	Cargo	Immune biological effect	References
Foot-and-mouth disease virus (FMDV)	Viral-based vaccine	Dendrimeric peptide of T-cell epitope N-terminally elongated with a Lys tree branching out into four copies of the B-cell epitope(B_4T(thi))	3A(21-35) T-cell epitope and VP1 (136–154) B-cell epitope from FMDV isolate C-S8c1.	Induced high titers of FMDV-neutralizing and IgA antibodies in both pigs and outbred mice (Swiss CD1 strain), activated T-cells, and induced IFN-γ release.	(131)
Schistosoma japonicum	DNA vaccine	G4 PAMAM modified with lysine	Plasmid DNA	DNA vaccines combined with PAMAM-Lys produced higher levels of protection compared to naked DNA vaccines against *S. japonicum* infection in a mouse model. Antibodies from mice immunized with PAMAM-Lys combined DNA vaccines were higher than those of mice immunized with the naked DNA vaccines. PAMAM-Lys vector induced an IgG2a antibody response and an increase in IL-2 and IFN-gamma production.	(132)
Rabies virus		G4 amine-terminated poly(ether imine) dendrimer	Plasmid DNA	Protective levels of rabies virus neutralizing antibodies titer (≥0.5 IU/mL) were observed. At day 14 after immunization with the dendriplex (dendrimer-DNA vaccine), protective levels of rabies virus neutralizing antibodies in mouse significantly increased.	(133)
H1N1 influenza, Toxoplasma gondii, and Ebola virus	RNA based vaccine	PAMAM dendrimer with an ethylenediamine core (Dendritech)	Antigens encoded by encapsulated mRNA replicons.	A modified dendrimer nanoparticle (MDNP)-delivered RNA encoding the hemagglutinin protein (HA) of an H1N1 influenza virus (A/WSN/33) or the Ebola virus (EBOV) glycoprotein (GP). MDNP protected mice against lethal viral infection and did not generate a systemic increase in inflammatory cytokines when using doses 500-fold higher than that required for Ebola and *T. gondii* protection.	(134)

Foot-and-mouth disease virus (FMDV)	Viral-based vaccine	Dendrimeric peptide consisting of four copies of a B-cell epitope (VP1(136-154)) linked through thioether bonds to a T-cell epitope (3A(21-35)) of FMDV (B4T(thi))	B and T epitopes of FMDV O-UKG 11/01 (presently the most prevalent FMDV serotype).	Two copies of a B-cell epitope from a type O isolate with thioether (B_2T(thi)) or maleimide (B_2T(mal)) elicited similar/higher B and T-cell specific responses than tetravalent B_4T(thi) in a pig model. While partial protection was observed in animals immunized with B_4T(thi) (60%) and B_2T(thi) (80%), B_2T(mal) conferred full protection against FMDV challenge, with high levels of circulating IgG2 and mucosal IgA.	(135)

Table 4 – Dendrimers Currently in Clinical Trials

Disease / Target	Brand name	Composition	Immune / Biological effect	Status / Phase	Reference
Liver Cancer	IMDENDRIM	[188Re] rhenium complex coupled to an imidazolic ligand and associated with a dendrimer	-	Phase 1	(136)
-	OP-101	Dendrimer N-Acetyl-Cysteine	-	Phase 1	(137)
Lung / Prostate cancer	DEP® docetaxel	Docetaxel attached to a DEP® dendrimer scaffold	Preclinical studies evidenced the improved anti-tumor effect obtained for DEP® docetaxel against breast cancer when compared to docetaxel. The improved efficacy resulted from a longer circulating half-life, an extended release of the docetaxel, and the ability of the dendrimer-docetaxel construct to target the tumor tissue.	Phase 2	(138)
Colon cancer	DEP® irinotecan combination	Irinotecan (Camptosar®) and cetuximab (Erbitux®) attached to a DEP® dendrimer scaffold	High dose DEP irinotecan and Erbitux® combination showed complete suppression of tumor growth in HT-29 xenograph mice model	Phase 1	(139)

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Materials Horizons

REVIEW

Carbosilane (CBS) dendrimers present a C- and Si-structure, with a highly apolar central core and flexible branches, making them a promising approach to target HIV (140). Pion *et al.* (129) assessed carbosilane dendrimers (2 G-NN16) displaying HIV-derived peptides Gp160, discovering that the elevated levels of internalization into mDC increased cell migration.

A more recent strategy for HIV treatment employs multi-epitope vaccines to induce immunity against multiple antigenic targets. Abdoli *et al.* (88) developed a polyepitope vaccine using gag/pol/env/tat sequences of the HIV genome and an anionic PEG-citrate G2 dendrimer. Two weeks after BALB/c mice immunization, the antibody specific levels of IgG1 and IgG2a were significantly increased compared to the control group, demonstrating the induction of a robust immune response.

Despite these efforts, the development of an efficient HIV vaccine suffers from the diversity and high mutation rate of HIV, as well as the hyperactivation of the immune system by HIV itself (116). Table 2 presents a review of the strategies employed to induce an immune response against HIV, based on the conjugation of distinct cargos to dendrimer structures.

2.3. Dendrimer-based prophylactic vaccination approaches against pathogen infection

The mechanisms used by pathogens to infect host cells involve multiple receptors and signaling pathways (141), and numerous dendrimer-based approaches have been used to modulate host immune response against pathogen infection (Table 3).

For example, modified dendrimer nanoparticles (MDNPs) functionalized with mRNA replicons as antigens generated protective immunity against a broad spectrum of lethal pathogen challenges, including H1N1 influenza, Toxoplasma gondii, and the Ebola virus (134).

BALB/c mice pre-immunized with RNA encoding the influenza A/WSN/33 HA protein carried by MDNPs for 14 days survived without major clinical signs of infection. The same dendrimeric platform conjugated with RNA expressing full-length Kikwit EBOV GP (VEEV-GP MDNP) (viral GP is the primary target antigen from Ebola virus) induced a GP-specific T-cell response demonstrated by the secretion of IFN-γ and IL-2 by $CD8^+$ and $CD4^+$ T-cells present within the splenocytes of immunized mice (134). The authors also developed a multiplexed MDNP vaccine with six T. gondii-specific antigens (GRA6, ROP2A, ROP18, SAG1, SAG2A, and AMA1) that were encoded into VEEV replicons. The animals were immunized with a single dose of this vaccine and then challenged with a lethal dose of T. gondii type II strain Prugniaud. The control animals died after 12 days, while the mice vaccinated with hexaplex MNDP survived for more than six months.

B-cell and T-cell epitope virus have also been used as antigens in a specific dendrimeric structure, conferring protection in immunized pigs suffering from foot-and-mouth disease virus (FMDV) disease (131).

3. The emergence of branched polymers as potential immunomodulators

Branched polymers include star, hyperbranched with a dendritic-like architecture, and graft polymers that can be readily prepared on a massive scale due to their rapid and cost-effective synthesis, which comprise a one-pot reaction and simplified purification steps. While branched polymers cannot be constructed with the same degree of precision as dendrimers, their conformation in solution, size, shape, and multivalency, provide advantages when compared to linear counterparts. In solution, branched polymers exhibit a 3D structure with a small hydrodynamic radius and enhanced solubility, and they display unique self-assembly properties. Their multivalency provides tunable thermal, mechanical, or rheological features and stimuli-responsiveness and enables the conjugation of a large number of bioactive molecules. The combination of these characteristics leads to enhanced biodistribution, pharmacokinetic, biological barrier crossing, and cell trafficking (142-144).

Compared to linear polymers, branched polymers with similar molecular weight have a different impact on the biological environment; increased cell uptake and improved pharmacokinetics have been reported in the literature (144-147). Lynn *et al.* (147) explored these different behaviors for immune applications – the conjugation of the small molecule TLR-7 and TLR-8 agonist (IMDQ) to N-(2-hydroxypropyl) methacrylamide (HPMA) polymers of varying shapes and compositions demonstrated that polymers with branched morphology increased local and reduced systemic cytokine production, thereby reducing morbidity and drug toxicity.

Very few examples have been reported with other branched systems apart from dendrimers in immunotherapy. Recently, Guan *et al.* (148) employed a branched PEI to yield a ternary complex with unmethylated cytosine-phosphate-guanine (as an adjuvant) and OVA (antigen) to enhance uptake of the complex, permit adjuvant recognition of the TLR, and, in parallel, promote the escape of ovalbumin from the endosomes to the cytosol.

β-cyclodextrin (β-CD) has been employed as the core for multi-branched triblock polymers in the development of a combination therapy targeted at DCs (149, 150). The polymeric corona is composed of an inner section of poly(lactide acid) (PLA), which is hydrophobic and can encapsulate the immunomodulatory drug IMDQ. A middle section of poly [2-(dimethylamino) ethyl methacrylate], pDMAEM possess pH-responsive properties and can complex pDNA, while an outermost block composed of poly[oligo(2-ethyl-2-oxazoline) methacrylate], pEtOxMA, stabilizes the system and avoids non-specific interaction with systemic proteins in the blood. *In vitro* studies demonstrated a high cell uptake of the developed polymeric nanocarrier and a combination therapy/drug synergism-driven increase in therapeutic output.

Zhang *et al.* (151) transfected TAMs with poly(aspartic acid)-based star polymers bearing folic acid or mannose. TAMs represent an interesting targeting for cancer treatment, as they can promote the development of malignant disease by facilitating tumor growth, angiogenesis, and metastasis. In the presence of DNA (pEGFP), poly(aspartic acid)-based star polymers assembled into polyplexes with a narrow size distribution (50–80 nm). Branched polymers decorated with folate or mannose targeting ligands displayed

improved *in vitro* transfection and gene expression in RAW 264.7 macrophages when compared to polymers lacking the targeting ligand.

Another study described the conjugation of the MUC1 glycopeptide in association T-cell epitope P2 derived from tetanus toxoid to a hyperbranched polyglycerol through alkine-azide copper-mediated click chemistry. The final conjugate displayed higher solubility in water when compared to the free glycopeptide. Assessments of immune response induction in MCF-7 tumor-bearing mice demonstrated robust immune stimulation, as evidenced by measuring the IgG antibody levels (152).

4. Currently marketed dendrimers and branched polymers

Despite the advantages of dendrimers and the concerted efforts of researchers within this field, few examples have met market approval or are in clinical development (Table 4). PAMAM and PPI are already commercially available for research uses only.

VivaGel® (Starpharma), a water-based vaginal gel (3% wt/wt) comprising a naphthalene disulfonic acid conjugated to a PLL dendrimer via amine linkers of SPL7013 (active drug) formulated using Carbopol®, was the first FDA-approved dendrimer on the market. The formulation comprises a dendrimeric system with 32 amino groups constructed by the addition of four generations of L-lysine and prevents the spread of sexually transmitted diseases and bacterial vaginosis (2, 153).

DEP® docetaxel and DEP® cabazitaxel, currently in phase 1/2 clinical trials, represent conjugated PLL dendrimers. Clinical trials led by StarPharma reported that DEP® docetaxel possesses significant tumor targeting (40-fold compared to the free drug) and superior anticancer effects in a range of cancer types when compared with free docetaxel (Taxotere®). Furthermore, DEP® docetaxel also reduced neutropenia/thrombocytopenia related to the use of Taxotere® alone (123, 124). Indeed, DEP® cabazitaxel received regulatory and ethics approval to start phase 1/2 clinical trials. More dendrimers currently in clinical trials are described in Table 4.

The presence of highly reactive surface terminal amino- or carboxyl- groups also make dendrimers a versatile tool for immunodiagnostics with Stratus® CS (Siemens Healthcare), Dimension® RxL (Dade Behring) and Elecsys® (Roche) reaching the market. As an example, Singh *et al.* (154) employed an anti-CKMB antibody (a cardiac marker) functionalized with dithiothreitol coupled to a PAMAM dendrimer using an iodoacetamide derivative to detect myocardial injury. Comparisons of the final dendrimeric complex Stratus® II, a commercially-available automated enzyme immunoassay system, revealed a shorter assay time and higher sensitivity with no biochemical changes observed in the antibody structure. Similarly, the detection of disease could also employ myoglobin, cardiac troponin I, cortisol, and thyroid markers, such as thyroxine, as predictive markers (155, 156). The USA Research Army Laboratory developed similar technology for the detection of anthrax (Alert ticket™).

Most human vaccines present on the market are prophylactic; however, therapeutic vaccines can be developed to act against established infections or diseases through the activation of T-cell immune responses (157-159).

Conclusions and Future Perspectives

The important progress occurred on materials science and nanotechnology, in general, has started to significantly impact immunotherapy approaches towards more effective and safe therapeutic strategies (160). Branched synthetic polymers, and dendrimers in particular, present architectures that exhibit promising properties for application in several biomedical applications. The translation of dendrimers into the clinic has been boosted by the development of synthetic tools suitable for high batch-to-batch reproducibility and controllable functionalization of specific sites at the dendrimer backbone with a known number of bioactive molecules, but still there remains room for improvement to promote other types of dendrimers, apart from those reported above, to reach the clinical scenario. Advances in the understanding of the impact of mean average size, architecture, and surface charge on the *in vivo* behavior of branched polymers have enabled their application as anti-cancer, anti-viral, and anti-infectious strategies; but still a much more exhaustive physicochemical characterization (including solution conformation, viscosity, deformability) could benefit the identification of descriptors that would allow speeding up the design of adequate polymeric carriers in immunotherapeutic approaches.

On the other hand, further in-depth knowledge regarding disease pathogenesis and immune system biology at the molecular level will help to unlock the full potential of such architectures as immune-modulatory agents, expanding their application in the prevention and treatment of multiple immune-related disorders. The surface of branched polymers can accommodate multiple functionalities, thereby amplifying their properties. The functionalization of dendrimer surface groups with HIV-derived peptides, viral proteins, or inactive viruses has shown enormous potential in the battle against HIV by both inhibiting cell entry and preventing viral replication.

The application of branched systems as vaccines also has a promising future. Due to their multivalency and well-defined 3D-architecture, dendrimers, in particular, are extensively cited as a potentially exciting approach to the generation of improved vaccines. Alterations to the surface carbohydrates on tumor cells have allowed the development of glyco-dendrimers as modulators of cancer immune responses.

However, robust large-scale synthesis processes for dendrimers and acceptable reproducibility are still necessary to exploit their full potential in immunotherapy. Also, we must establish a correlation between product quality (e.g., the degree of surface modification) and biological behavior (e.g., therapeutic efficiency, toxicity, and pharmacokinetic profile) to ensure safety and efficacy. Dendrimers that cannot meet these criteria will be impossible to translate to the clinic. Other types of branched polymers with a less demanding synthetic approaches could even bypass the expectancy achieved by dendrimers. If rationally designed and through one-pot controlled polymerization techniques, multivalent systems presenting self-assembling characteristics with very low polydispersity can be achieved. Larger sizes with more flexible branches (less compactness than dendrimers) could allow a better presentation of antigens enhancing the response of DC towards a greater overall immunogenicity to attack, for example, tumor cells.

Importantly, for chronic administrations polymer biodegradability would significantly facilitate to achieve the requested safety: benefit ratio and polypeptide-based materials, such as PLL and others are already making his path towards market approval.

Finally, one could consider that to improve immune efficacy, combination therapy approaches would be most probably required. For example, adequately functionalized branched polymers may be combined with established immunotherapeutic strategies, such as checkpoint blockade antibodies and adoptive T-cell therapy, to finally see the full potential of these clinical therapeutics and allow them to impact human health in the coming years significantly

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Conflicts of interest

The authors declare no conflicts of interest.

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Materials Horizons

REVIEW

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