Dendritic Nanoparticles for Immune Modulation: A potential next-generation nanocarrier for cancer immunotherapy

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Abstract

Immune activations, whether they occur from direct immune checkpoint blockade or indirectly as a result of chemotherapy, is an approach that has drastically impacted the way we treat cancer. Utilizing patients' own immune systems for anti-tumor efficacy has been translated to robust immunotherapies; however, clinically significant successes have been found in only a subset of patient populations. Dendrimers and dendritic polymers have recently emerged as a potential nanocarrier platform that significantly improves the therapeutic efficacy of current and next-generation cancer immunotherapies. In this paper, we highlight the recent progress in developing dendritic polymer-based therapeutics with immune-modulating properties. Specifically, dendrimers, dendrimer hybrids, and dendronized copolymers have demonstrated promising results and are currently in pre-clinical development. Despite their early stage of development, these nanocarriers hold immense potential to make profound impact to cancer immunotherapy and combination therapy. This overview provides insights into the potential impact of dendrimers and dendron-based polymers, offering a preview of their potential utilities for various aspects of cancer treatment.

Keywords: Cancer immunotherapy, Immune checkpoint blockade, Dendritic nanoparticles, Dendrimers

1. Introduction

Cancer immunotherapy has revolutionized cancer treatments.[1] When cancer develops, tumor cells commonly overexpress various proteins to evade immunosurveillance, hindering the body's ability to recognize and eliminate malignant cells.[2] Consequently, the primary approach of cancer immunotherapy revolves around stimulating and modulating the immune system to re-target cancer cells.[3] To date, various immune checkpoint blockades, including monoclonal antibodies (mAbs) targeting cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed cell death-1 (PD-1), and programmed cell death ligand-1 (PD-L1), have been developed and approved for multiple cancer types such as lung cancer, melanoma, ovarian cancer, and renal cell carcinoma.[4-7] Despite the significant breakthroughs in immunotherapy, it comes with its own set of side effects, like inconsistent outcomes among patients. For example, in Hodgkin's lymphoma, only a minor subset of 17% showed a complete response to nivolumab, a PD-1 antibody; similarly, metastatic breast cancer patients experienced a mere 3% objective response rate to avelumab, an anti-PD-L1 treatment.[8, 9] In addition, mAb-based immunotherapy has been demonstrated to be less effective against solid tumors than lymphoma, as they form an immune-suppressive tumor microenvironment (TME).[10, 11] Another significant unmet need with current mAbs is the induction of unexpected systemic toxic effects caused by off-target delivery of therapeutics, along with autoimmune diseases such as dermatitis, enterocolitis, hepatitis, and hypophysitis.[12, 13]

To overcome the limitations of currently available cancer immunotherapies, nanoparticles (NPs) have emerged as promising carriers for delivering therapeutic payloads to specific target tissues.[14] NPs enhance the stability and solubility of encapsulated cargos, which help to overcome challenges in transferring across biological barriers, such as the intestinal tract or blood-brain barrier.[15-17] In addition, the utilization of NPs prolongs the circulation half-life of drugs and can

improve safety and tolerability compared to conventional immunotherapeutic treatments. Among the various NP-based platforms, polymeric NPs are a promising candidate because of their unique aspects, such as modularity and biocompatibility.[18, 19] Depending on how they are synthesized, polymeric NPs can encapsulate drugs within the core, entrap them in the polymer matrix, or chemically conjugate them to the surface.[20-22] The loading efficiencies and release kinetics of small molecules can also be easily controlled using polymeric NPs by modulating their compositions, molecular weights, surface charges, and stimuli responsiveness.[23, 24] Furthermore, polymeric NPs are generally considered non-toxic and non-immunogenic, providing a relatively safe option for drug delivery.[25] For these reasons, polymeric NPs have great potential to achieve highly efficient yet safe cancer immunotherapies.

Among those polymeric NPs, dendrimers and dendron-based NPs have gained much attention as potential nanoplatforms due to their unique physicochemical properties, including chemically well-defined hyperbranched structure, structural versatility, and importantly, ability to mediate multivalent binding effects effectively.[26] Dendrimers, typically 1 to 10 nm in diameter, present globular structures with 3D branches extending from the central core. Such molecular shape and active functional groups on the surface allow them to be conjugated with various biologically active molecules. Moreover, their flexible and interchangeable branches enable multiple dendronized ligands to bind strongly with cell receptors via multivalent binding or avidity. Previous studies from our group have reported significantly enhanced binding of dendrimers via avidity, which can be proven by a drastic reduction in dissociation rate constants along with improvement in surface targeting *in vitro* and *in vivo*.[27-31]

In this review, we will focus on recent advances in dendritic NPs as effective nanocarriers for cancer immunotherapy by modulating immune responses. **Fig. 1** summarizes three different types

of dendritic NPs discussed in this paper: 1) dendrimer conjugates, 2) dendrimer hybrids, and 3) dendron-based copolymers. To our knowledge, this would be the first review highlighting the latest advances in applying various types of dendritic NPs specifically for targeted cancer immunotherapy. With this overview, we aim to provide insights into developing dendritic polymers for next-generation nanomaterials-based therapeutic strategies.

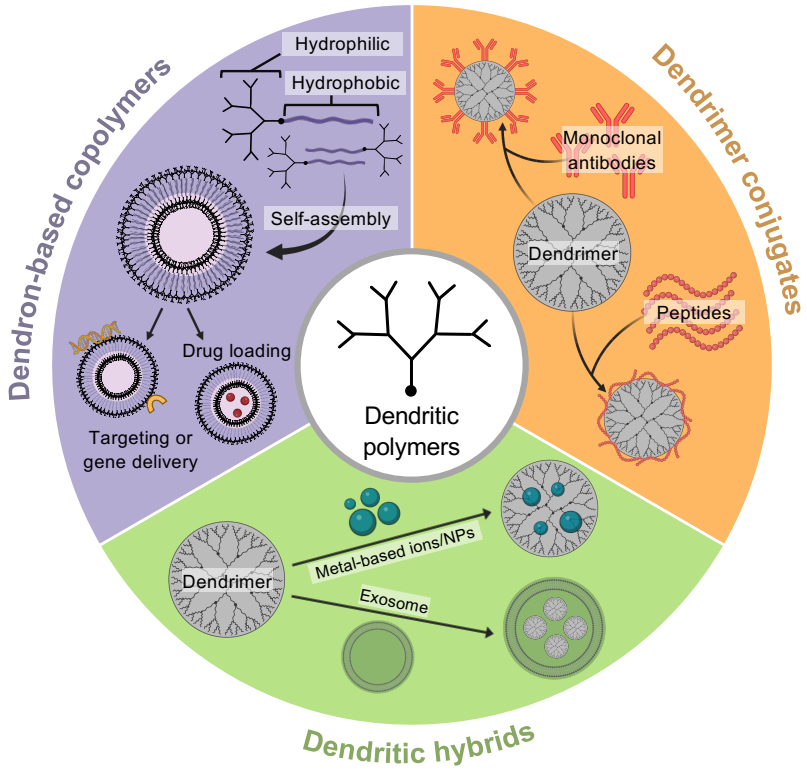

Fig. 1: Schematic diagram of dendritic NPs used for immune-modulating activity.

2. Dendrimer Conjugates

2.1. Dendrimer-antibody conjugates

Monoclonal antibodies (mAbs) have been widely utilized as immune checkpoint inhibitors (ICIs) that target negative immunologic regulators to restore immune responses against cancer.[32] These mAb-based therapies are directed against proteins such as programmed cell

death protein-1 (PD-1), its ligand programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), among others.[33] To date, FDA-approved mAbs that block these proteins include pembrolizumab (Keytruda),[34] nivolumab (Opdivo),[35] cemiplimab (Libtayo),[36] atezolizumab (Tecentriq),[37] avelumab (Bavencio),[38] durvalumab (Imfinzi),[39] tremelimumab (Imjudo),[40] and ipilimumab (Yervoy).[41] Their efficacy has proven beneficial to patients with various cancer types, such as melanoma,[42] renal cell carcinoma (RCC),[43] non-small-cell lung cancer (NSCLC),[44] bladder cancer,[45] gastric carcinoma,[46] head and neck cancer,[47] B-cell lymphoma,[48] and Hodgkin's disease.[49] Despite their significant success in the clinic, tumor heterogeneity, the off-target effect of mAbs, and alternative immune evasion pathways of tumors have hindered the universal success of mAbs for large patient populations.[22, 50]

In this context, the approach of conjugating ICI antibodies with dendrimers has been observed to enhance the binding avidity of the antibodies, thus increasing targeting efficacy.[22] In particular, our group has previously reported G7-aPD-L1 conjugates where generation 7 (G7) PAMAM dendrimers were integrated with multiple PD-L1 antibodies (aPD-L1) (**Fig. 2A**). The binding kinetic analysis of the G7-aPD-L1 conjugates using biolayer interferometry (BLI), surface plasmon resonance spectroscopy (SPR), and atomic force microscopy (AFM) all indicated that the dendrimer-ICI antibody conjugates bind to PD-L1 more strongly than free antibody. Furthermore, the improved binding kinetics of the G7-aPD-L1 conjugates were translated into enhanced *in vitro* binding efficiency (**Fig. 2B**) and *in vivo* tumor accumulation (**Fig. 2C** and **D**). When G7-aPD-L1 conjugates were injected in mouse models bearing MOC1 tumors that highly express PD-L1, its accumulation to the tumor site was significantly higher than aPD-L1, suggesting successful *in vivo* selectivity of dendrimer-ICI conjugates. In addition,

we have confirmed that the conjugates can not only enhance the binding efficiency but also improve the blockade of PD-1/PD-L1 interactions by activating T-cell functions. Compared to the aPD-L1 treatment alone, G7-aPD-L1 treatment significantly increased T cell interleukin-2 (IL-2) production by ~35% and cytotoxicity to doxorubicin by ~20% (**Fig. 2E** and **F**).

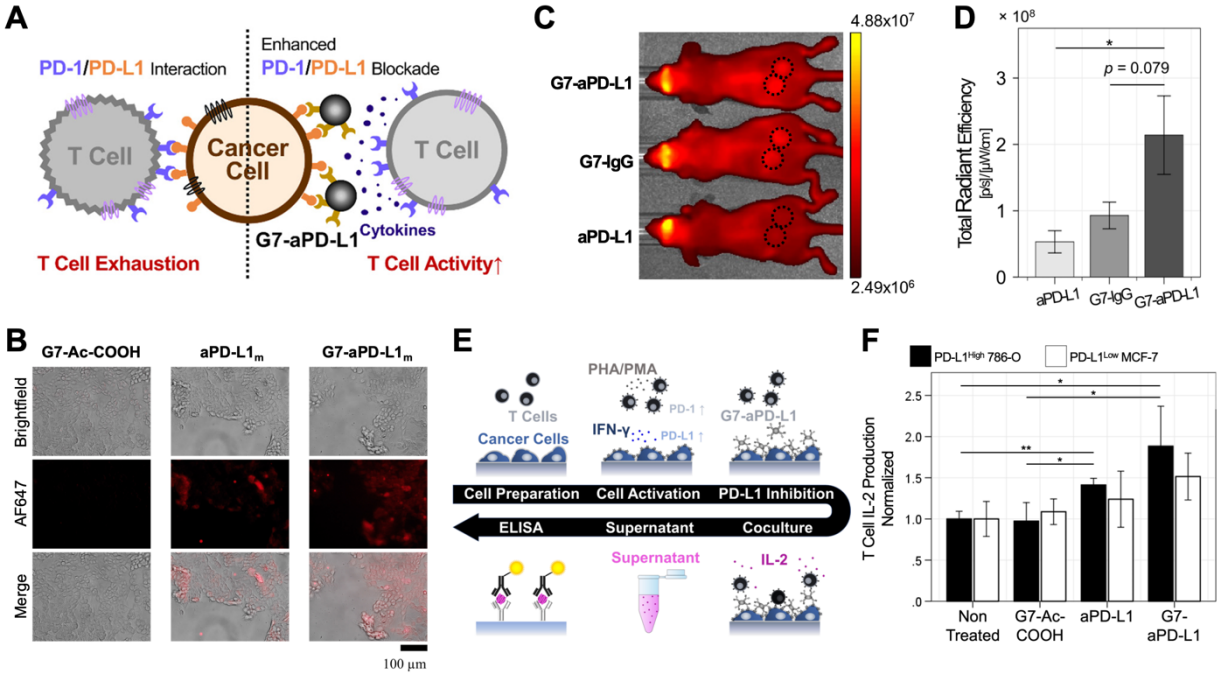

Fig. 2: Dendrimer-ICI antibody conjugates targeting PD-L1 for enhanced immunotherapy. (A) Schematic diagram of dendrimer-ICI antibody conjugates composed of G7 PAMAM dendrimers and PD-L1 antibodies (G7-aPD-L1). (B) *in vitro* cell binding assay of fully acetylated G7 PAMAM dendrimers (G7-Ac-COOH), aPD-L1 alone, and G7-aPD-L1 to PD-L1-expressing MOC1 cells. Note that interactions of G7-aPD-L1 with MOC1 cells observed by the red fluorescence were higher than that of aPD-L1. (C-D) *in vivo* imaging system (IVIS) analysis showing drug accumulation and biodistribution of G7-aPD-L1, free G7 PAMAM dendrimer (G7-IgG), and aPD-L1 in MOC1-tumor bearing mice. Note that the targeting of G7-aPD-L1 to tumors was 2.5-fold higher than that of aPD-L1. (E-F) ELISA assay assessing T cell IL-2 production following the coculture of T cells and cancer cells. Note that the IL-2 section from the T cells was the highest when G7-aPD-L1 was treated on PD-L1 highly expressing 786-O cells. Reprinted with permission.[22] Copyright 2020, American Chemical Society.

2.2. Dendrimer-peptide conjugates

Peptide-based biologics are gaining interest in drug delivery due to advantages like ease of manufacture, tumor penetration, and low immunogenicity.[51, 52] Yet, their inherent limitations, such as low binding strength, short half-life, low tumor retention, and variability in conformational changes, have hindered their widespread use.[28, 52, 53] Incorporating peptides into nanoparticles has become a promising platform to address the issues due to their well-defined molecular structure, chemical modularity for multi-functionalization, biocompatibility, and multivalency.[54-56] Remarkably, our lab has reported a strategy of conjugating PD-L1-binding peptides with dendrimers, resulting in peptide-dendrimer conjugates (PDCs) (**Fig. 3A**).[28] In this approach, engineered PD-L1-binding peptides were isolated from the PD-1 surface and following dendrimer conjugation, stabilized into β-hairpin structures. This is crucial because peptides that do not fold into stable secondary structures risk exhibiting altered binding and physiochemical properties.[28] Furthermore, post-dendrimer conjugation, peptides were displayed in a multivalent fashion, allowing for strong interactions with PD-L1 proteins expressed on tumor cells. Such an approach has been verified by SPR analysis, where PDCs enhanced the binding avidity to PD-L1 molecules by five orders of magnitude compared to the free peptide. The dissociation rate constant (k_d) of PDCs was also ~180 times lower than that of peptide alone, indicating a multivalent effect attributed to the dendrimer. Similar to G7-aPD-L1, the enhanced binding kinetics of PDCs also translated into *in vitro* binding efficiency. PDCs exhibited high PD-L1 selectivity through significant cellular interactions with PD-L1^{high} 786-O cells compared to PD-L1^{low} MCF-7 cells (**Fig. 3B**). Moreover, PDC-treated cancer cells increased IL-2 secretion from T cells by 1.52-fold compared to untreated cells (**Fig. 3C**). Even free peptide-treated cells only showed negligible IL-2 secretion. This result suggested that peptide-based biologics conjugated to dendrimers induced multivalent binding effects that led

to effective inhibition of the PD-1/PD-L1 immune checkpoint pathway. Although this approach has focused on blocking PD-L1 alone, the research can be extended by blocking other immune checkpoint proteins. In a study by Liu et al., they reported a novel lung cancer-targeting peptide, isolated from the utilization of phage display, could specifically target NIC-H460 non-small human lung carcinoma cells and was successfully conjugated to generation 4 (G4) PAMAM dendrimers.[56] Similarly, novel peptide sequences inhibiting other immune checkpoint proteins besides PD-L1 can be investigated with phage display to expand the PDC approach.

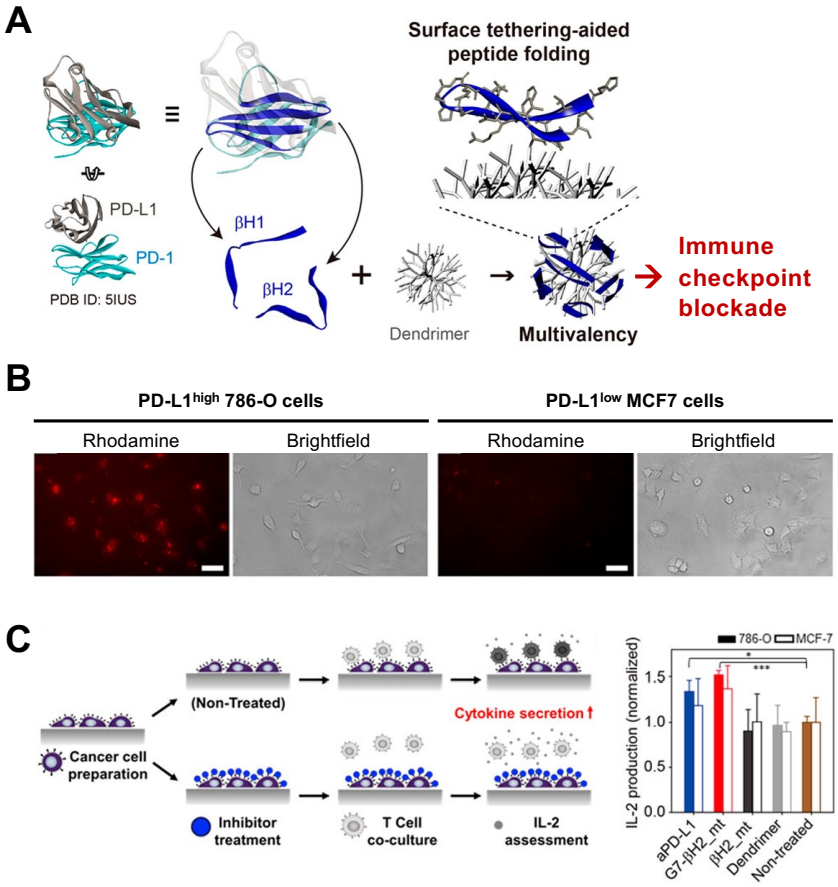

Fig. 3: Dendrimer-peptide conjugates targeting PD-L1. (A) Schematic illustration of peptide-dendrimer conjugates (PDCs) composed of multivalent G7 PAMAM dendrimer and PD-L1-binding peptide, isolated from PD-1 surface. (B) *in vitro* cell binding assays of PDCs to PD-L1^{high} 786-O cells and PD-L1^{low} MCF7 cells (scale bar: 50 μm). Note that stronger cell interactions (red fluorescence from rhodamine) of PD-L1-targeting PDCs are observed in PD-L1^{high} 786-O cells

compared to PD-L1^{low} MCF7 cells. (C) T cell IL-2 production assessments following the coculture of T cells and cancer cells. Cancer cells treated with PD-L1-targeting PDCs (G7-βH2_mt) led to the highest IL-2 secretion from the T cells, suggesting the blockade of PD-1/PD-L1 binding by the conjugates. Reprinted with permission.[28] Copyright 2020, American Chemical Society.

3. Dendritic hybrid NPs

3.1. Dendrimer-exosome hybrids

The development of dendrimer hybrids involves incorporating dendrimers with secondary nanoscale components to utilize the strengths of each element while limiting drawbacks.[57-60] Encapsulation of dendrimers inside larger vesicular compartments is an approach for hybridization that is simpler than direct conjugation involving complicated synthetic routes.[57, 61, 62] The encapsulation process is effectively supported by extracellular vesicles with aqueous cores consisting of synthetic or natural lipids. Although these vesicles lack active targeting to tumor compartments, they leverage their size and extended circulation properties to utilize passive targeting via the enhanced permeability and retention (EPR) effect.[63] Active targeting can also be implemented in these systems by adding targeting ligands, although it would increase the structural complexity of the hybrid system. Notably, a lipid-based NP system with innate tumor-targeting capabilities has been observed through the utilization of cell membranes, including exosomes.[64, 65] Nair et al. found that encapsulation efficiency was dependent upon dendrimer size and surface end-groups when encapsulating dendrimers with cancer-derived exosomes (**Fig. 4A**).[66] Specifically, amine-terminated G7 PAMAM dendrimers demonstrated a loading efficiency of 6.33% when encapsulated within tumor exosomes, significantly higher than carboxyl-terminated dendrimers with only 0.2% efficiency (**Fig. 4B**). In parallel, the loading efficiency of amine-terminated G7 PAMAM dendrimers was higher than those of G4 and G2 PAMAM dendrimers, which was attributable to the higher electrostatic interactions

with increased generation (size) of dendrimers. These surface charge interactions were also required for successful encapsulation using anionic and cationic liposomes. The hybridization of dendrimers within exosomes also diminished the inherent cytotoxic nature of cationic dendrimers, removing a significant disadvantage found with dendrimer-mediated gene delivery (**Fig. 4C**). A major benefit to a dendrimer-exosome hybrid system includes potential homing properties of cancer exosomes, as seen by others.[64, 65, 67, 68] Although the dendrimer-exosome hybrid NP system did not display any homotypic targeting *in vitro*, its potential immune-modulating properties were observed by delivering PD-L1 small interfering RNA (siRNA). The cationic dendrimers were able to condense the PD-L1 siRNA. Through encapsulation with exosomes, they could deliver the gene payload to human breast cancer cells more efficiently than free dendrimers, decreasing PD-L1 expression by more than 3.8-fold (**Fig. 4D**).[66] Downregulation of PD-L1 expression via siRNA delivery can potentially disrupt the ability of cancer cells to evade immune cell checkpoints and has previously been found to be as equally effective as mAb blockade.[69]

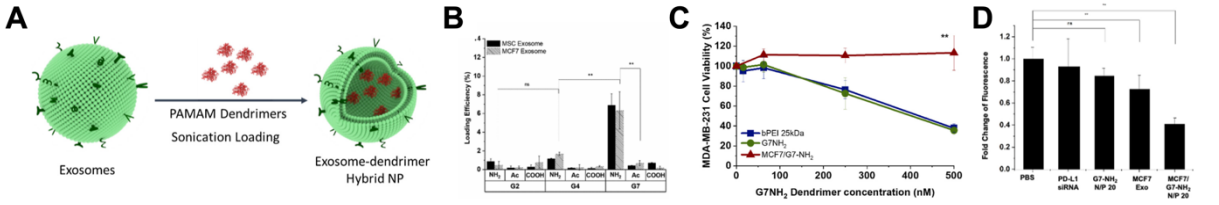

Fig. 4: Dendritic hybrid NPs integrating tumor-derived exosomes and PAMAM dendrimers. (A) Schematic illustration of exosome hybridization with PAMAM dendrimers. (B) Loading efficiencies of various dendrimers into exosomes. Note that the G7 PAMAM dendrimer with positively charged surface termini (G7-NH_2) showed the highest loading efficiency of 6.33% with negatively charged exosomes. (C) Cell cytotoxicity assay of dendritic hybrid NPs compared to G7 only. Note that hybridization of G7-NH_2 with tumor-derived exosomes (MCF7/G7-NH_2) improved the cell viability of MDA-MB-231 cells compared to dendrimer alone. (D) PD-L1 protein expressions after the delivery of PD-L1 siRNA with MCF7/G7-NH_2 NPs. PD-L1 expression was downregulated by 3.8-fold after the dendritic hybrid NP treatment compared to dendrimer alone. Reprinted with permission.[66] Copyright 2023 American Chemical Society.

3.2. Metal-incorporated dendritic hybrids

Another class of dendrimer hybrids used for immune modulation includes dendrimers combined with metal-based ions or NPs. 'Metalloimmunotherapy' is an emerging approach in cancer immunotherapy that utilizes the innate characteristics of metals to modify the TME, either through intrinsic properties or with the help of external stimuli, such as photothermal therapy (PTT).[70-74] For example, Zhang et al. developed a drug delivery system derived from copper sulfide NPs (CuS NPs) and G5 PAMAM dendrimers, named G5-PEG-LyP-1-CuS-DMXAA (GLCD) NPs (**Fig. 5A**).[75] The dendrimer core contained the CuS NPs and an anti-vascular drug, DMXAA. Additionally, the external surface was functionalized with a cancer-targeting peptide, LyP-1. Such NPs exhibited significant *in vitro* tumor cell targeting and cytotoxicity through CuS-mediated PTT and Lyp-1-induced proapoptotic effects. The subsequent *in vivo* study also revealed that the targeted PTT inhibited tumor growth and disrupted tumor blood and lymphatic vessels, preventing lung metastasis without inducing any toxicity (**Fig. 5B**). Moreover, GLCD NPs demonstrated the ability to reverse the immunosuppressive TME by inducing immunogenic cell death via PTT and modulating immune responses through DMXAA, leading to M1 macrophage repolarization. This bimodal approach exemplifies the versatility of dendrimers in delivering multiple anti-cancer agents while efficiently targeting tumor tissue.

In addition to metal NPs, metal ions have shown potential in enacting anti-tumor effects.[76] Gao et al. reported a dendrimer hybrid derived from manganese ions (Mn^{2+}) and benzoic-acid-modified G5 PAMAM dendrimers, which effectively assisted cancer immunotherapy by serving as a cancer vaccine (**Fig. 5C**). Manganese ions, among other metal ions, have been discovered to act as immunostimulatory adjuvants capable of activating the stimulator

interferon gene (STING) pathway. STING agonists play a critical role in stimulating anti-tumor immunity by inducing natural killer (NK) cells to clear tumor cells that are resistant to T-cell-mediated tumor cell death.[77] The Gao group's nanoformulation, named G5-pBA/OVA@Mn, used dendrimers as a delivery scaffold to co-deliver the immune-modulating ion Mn^{2+} and the tumor antigen ovalbumin (OVA) to antigen-presenting cells.[76] *In vitro* results with this nanovaccine demonstrated that only the dendrimer-assisted codelivery of Mn^{2+} and OVA elicited activation of bone marrow-derived dendritic cells, $CD8^+$ T cell proliferation, and production of interferon-gamma (IFN-γ) (**Fig. 5D**). Then, *in vivo* studies using a B16-OVA melanoma tumor model confirmed that G5-pBA/OVA@Mn worked both as a prophylactic and a therapeutic agent, whereas either Mn^{2+} or OVA alone had minimal effects (**Fig. 5E-F**). Collectively, the ongoing studies of dendrimer-based metalloimmunotherapies represent a developing field that requires additional research to understand the benefits and costs of utilizing metal ions versus metal-based NPs in developing efficacious dendrimer hybrids for cancer immunotherapy.

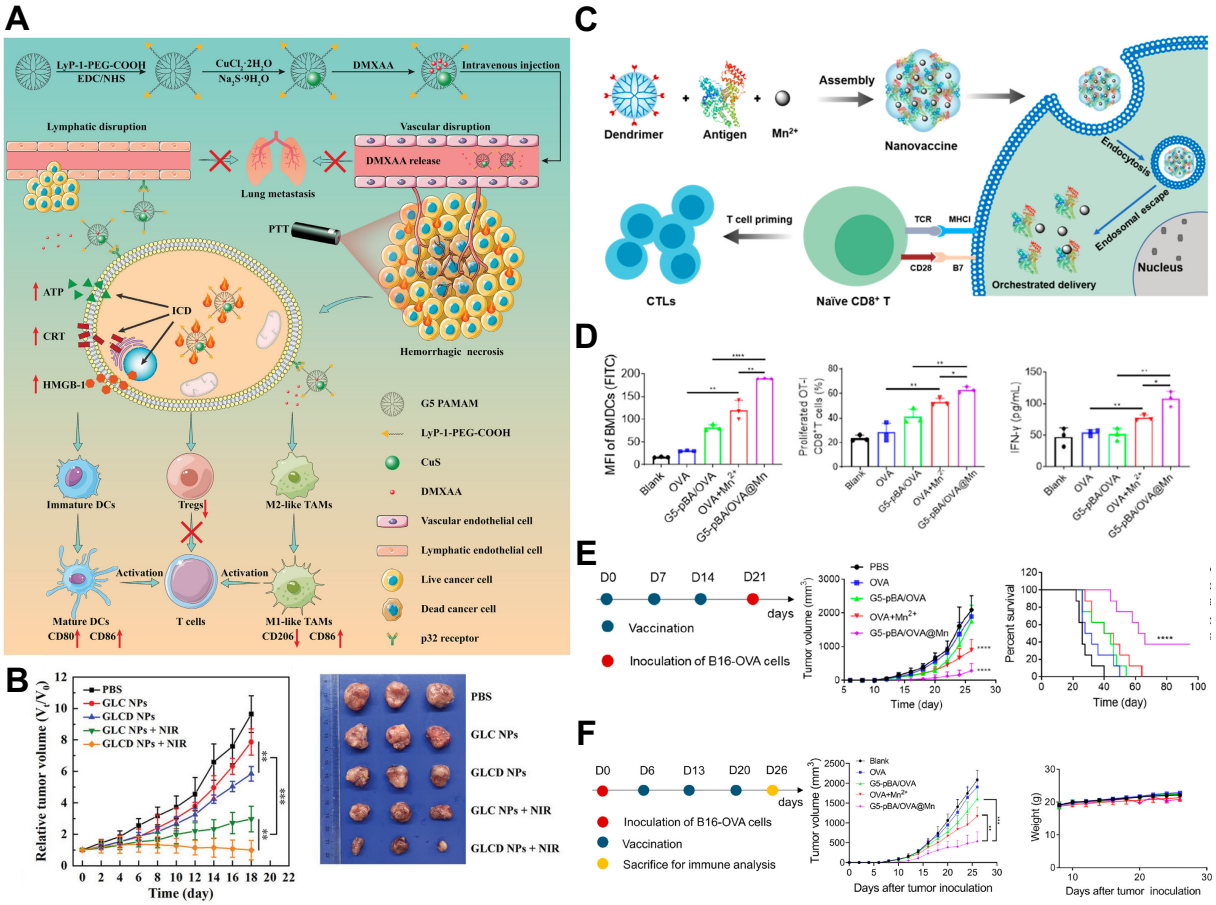

Fig. 5: Metal-incorporated dendritic hybrids for metalloimmunotherapy. (A) Schematic illustration outlining the formation of GLCD NPs for immune modulation-mediated combination tumor therapy as reported by Zhang et al. (B) Relative tumor volume change in 4T1 tumor-bearing mice after different treatments (left), alongside tumor tissues collected at day 18 (right). Note that 4T1 tumor-bearing mice treated with GLCD NPs and near-infrared (GLCD NPs + NIR) showed the most effective tumor inhibition efficacy due to the combination therapy of DMXAA and PTT induced by NIR. (C) Schematic drawing depicting the formation of G5-pBA/OVA@Mn complexes and their mechanisms for improving intracellular delivery of antigen OVA for enhanced cancer immunotherapy as reported by Gao et al. (D) Quantifications of activated bone marrow-derived dendritic cells (BMDCs, left), $CD8^+$ T cell proliferation (middle), and production of interferon-gamma (IFN-γ) measured via flow cytometry or ELISA. The significant increase was observed only in the G5-pBA/OVA@Mn-treated group. (E) Prophylactic effect of G5-pBA/OVA@Mn in the B16-OVA tumor models. Note that G5-pBA/OVA@Mn-immunized mice substantially inhibited the B16-OVA tumor growth. (F) The therapeutic effect of G5-pBA/OVA@Mn in the B16-OVA tumor models. Note that G5-pBA/OVA@Mn treatments significantly delayed tumor growth without any toxicity. Reprinted with permission.[75, 76] Copyright 2023 John Wiley and Sons for A-B, and Copyright 2023 American Chemical Society for C-F.

4. Dendron-based copolymers for immune modulation

Dendrons, wedge-shaped molecules originating from a central branch, provide a unique platform for synthesizing novel block copolymers. Unlike dendrimers that merely serve as templates for outward growth in a spherical direction, dendrons enable the creation of distinctive copolymers.[78] In this innovative approach, a single linear polymer, linked to a dendron, extends to multiple end groups and optionally integrates with a third polymer.[79] In contrast to the conventional hybridization of dendrimers with secondary nanocarriers, researchers are studying new structural designs where dendritic scaffolds are directly conjugated to secondary compounds to create singular nanoplatforms with dual characteristics.

4.1 Dendron micelles

Introducing dendron segments as the hydrophilic component within amphiphiles has shown beneficial in the development of thermodynamically stable micelles with low critical micelle concentrations (CMCs), ranging between 10^{-6} and 10^{-8} M.[80, 81] These lower CMC values are attributed to the conical dendron structure lowering the entropic cost necessary for micelle self-assembly compared to linear counterparts.[82] Further physiochemical differences between dendron-containing micelles and others also translate into *in vitro* efficiency. Hsu et al. observed polyester-containing dendritic micelles with polyethylene glycol (PEG) shells and a polycaprolactone (PCL)-containing core offered a 2-fold higher half-life in 50% FBS compared to the linear copolymer counterparts.[83] Other benefits include the orientation of external ligands such as PEG. Pearson et al. confirmed by molecular dynamic simulations that PEG chains could homogeneously cover the hydrophobic core of micelles more effectively compared to linear copolymer-based micelles.[82, 84] Since many NPs entering clinical trials include some degree of PEGylation to prolong blood circulation via reduction of protein corona

formation, it is crucial to understand the molecular dynamics of PEG on the surface of dendritic copolymers.[85] When PEGylation is included in addition to targeting moieties in the same dendritic NP, understanding the ligand conjugation strategy is important to optimize receptor binding. Pearson et al. determined the spatial flexibility of a targeting ligand, folic acid, in relation to the external PEGylated NP surface is directly related to the degree of receptor binding. This way, PEG linkers conjugated between the dendron and targeting ligand can extend the ligand further away from other shorter PEG chains to allow for efficient receptor engagement.

Functional modifications of dendron micelles include the introduction of various targeting moieties, immune checkpoint inhibitors, and immune-modulating drugs.[86, 87] For example, Li et al. found a dendronized copolymer developed from N-(2-hydroxypropyl)methacrylamide (HPMA) and G2 lysine-based dendrons conjugated to oncolytic peptides formed stable alpha helix structures (**Fig. 6A**).[88] Proton nuclear magnetic resonance spectra and circular dichroism results indicated that only conjugation to the dendronized co-polymer and not a linear counterpart could produce the alpha-helical structures crucial for obtaining oncolytic peptides' membrane lysis potential of oncolytic peptides.[88] With these peptide-stabilized dendron constructs, a significant occurrence of immunogenic cell death was observed. This was substantiated by the dual release of damage-associated molecular patterns (DAMPs), which can recruit immune-modulating cells to the tumor microenvironment and increase $CD8^+$ T cell populations.[88] However, a co-delivery approach with PD-L1 blockade was necessary to eradicate tumors and ultimately produce effector memory T cells. This synergistic response in anti-tumor activity was also observed where a phosphorous comprised of dendritic micelle encapsulating doxorubicin was co-delivered with aPD-L1.

Zhan et al. found phosphorus-based NPs can trigger intrinsic immune-modulating activity by inducing the secretion of IFN-γ, perforin, and granzyme B, allowing the cytotoxic impact of immune cells towards cancer cells (**Fig. 6B**).[89] Further sensitization of cancer cells was obtained with DOX-mediated immunogenic cell death paired with aPD-L1 immune checkpoint blockade. As a result of this trimodal anti-cancer approach, it produced robust populations of central and effector memory T cells. It generated the potential to achieve adaptive immunity towards recurring metastases. Considering this, a careful design of dendronized micelles can be developed to enhance the anti-tumor potential of therapeutic payloads encapsulated or conjugated to the dendron exterior. Multiple studies are underway to discover the structural characteristics of dendrimers that contain innate therapeutic benefits[90].

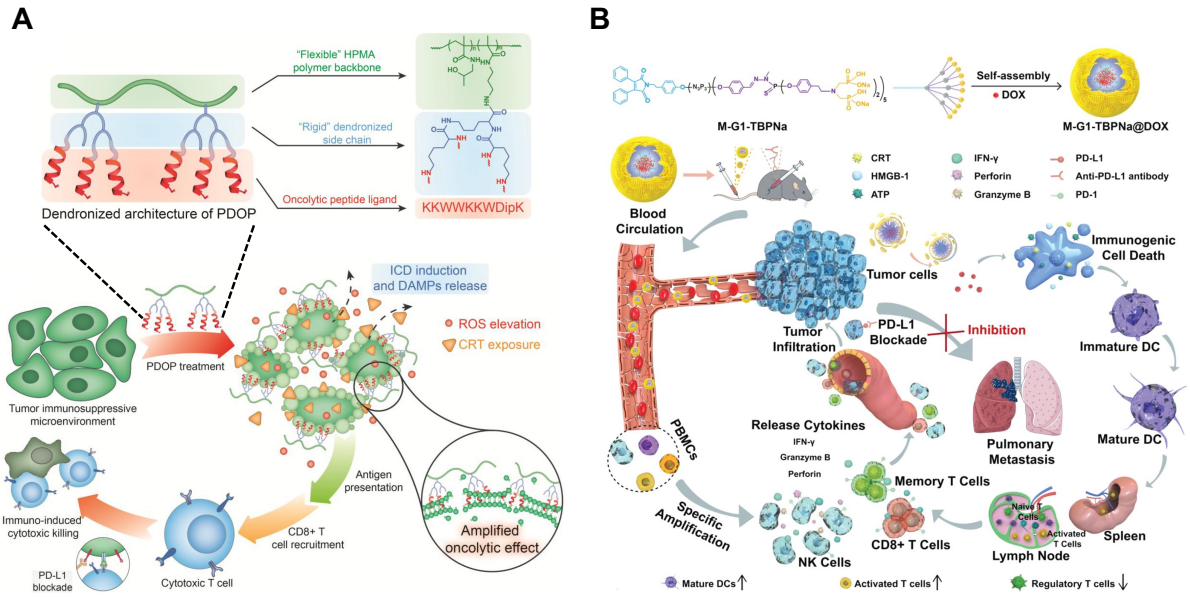

Fig. 6: Functional modifications of dendron micelles with targeting moieties. (A) Schematic illustration of dendronized copolymer developed from N-(2-hydroxypropyl)methacrylamide (HPMA) and G2 lysine-based dendrons conjugated to oncolytic as reported by Li et al. Reprinted with permission.[88] Copyright 2021 Journal of Controlled Release. (B) Schematic illustration of chemotherapeutic drug doxorubicin-loaded phosphorous dendron micelles combined with aPD-L1 for inherent immunomodulatory activity as reported by Zhang et al. Reprinted with permission.[89] Copyright 2022 Advanced Materials.

4.2 Dendritic LNPs (dLNPs)

Recent FDA approval of Onpattro and COVID-19 vaccines have illustrated the clinical potential of lipid nanoparticles (LNPs) as effective drug carriers for gene delivery.[91] As a result, numerous LNP developments are underway in the field of orphan diseases and vaccine development.[92, 93] However, LNP-based carriers utilized towards cancer immunotherapies and other combinatorial cancer therapies are still lacking. Based on the physiochemical enhancements of dendron-based micelles compared to linear analogs, researchers have developed next-generation LNPs through the combination of dendrons with natural phospholipids.

One of these dendritic copolymers includes the direct conjugation of hydrophilic generation 3 (G3) PAMAM dendrons to hydrophobic phospholipids, specifically dioleoylphosphatidylethanolamine (DOPE). DOPE is a neutral lipid that can induce membrane fusion within the endosomal lumen. One of the benefits of using the DOPE for the hydrophobic segment is the inherent biocompatibility, as it is a natural component in human cell membranes and is classified as a helper lipid for gene delivery.[94] The amphiphilic structure has inherent self-assembling properties, forming micelles with hydrophobic cores and hydrophilic shells. Post micelle self-assembly, the dendronized lipid-bearing unimers form dendritic lipid nanoparticles (dLNPs). The synthetic method of dendritic amphiphiles must be carefully considered to denote the structural characteristics required for the high i*n vivo* efficacy of this drug delivery platform. One method to understand the optimal features of efficient dendritic copolymers involves the creation of structure-activity relationships. Nair et al. observed when using dLNPs of G3 versus G2 PAMAM dendrons for drug and gene co-delivery, G3 dLNPs significantly outcompeted G2 dLNPs, resulting in efficient transfection of DNA plasmids and

cytosolic delivery of hydrophobic payloads.[80] The superior capability of G3 dLNPs was owed to its heightened proton buffering capacity, allowing efficient endosomal escape compared to G2 counterparts.[80, 95] Other investigators also confirmed higher generation dendritic NPs offer optimal drug delivery results.[96, 97]

5. Current clinical status of dendrimer-based systems

Although numerous papers and patents have been published over the past few decades, the clinical translation of dendrimers and dendritic NP systems remains limited. This section delves into the clinical status of dendrimer-based systems, focusing on ongoing trials and limitations hindering their clinical translations. Despite the recent expansion of dendrimer-related clinical trials, only a few have advanced to phase 3. Safety and efficacy assessments primarily concentrate on poly-lysine and PAMAM dendrimers, which are being tested for cancers, bacterial vaginosis, and COVID-19 treatments. Understanding *in vivo* interactions with dendrimer-based structures involves critical nanoscale design parameters (CNDPs), which significantly impact complement activation, excretion, protein interactions, cytotoxicity, cellular uptake, and biodistribution.[98, 99] Noteworthy among PAMAM dendrimers is the so-called OP-101, which has improved survival rates in COVID-19 treatment.[100] The Starpharma's dendrimer portfolio includes AZD0466, which has demonstrated efficacy against cancer with reduced side effects.[101] Clinical trials also involve G4 PAMAM dendrimers, denoted as D-4517-2, for eye diseases[102] and KK-46, aiding COVID-19 treatment.[103] Poly-L-lysine dendrimers, such as Gadomer-17 and VivaGel®, have demonstrated potential in MRI and antiviral applications.[104, 105] The literature indicates that VivaGel® impedes bacterial growth associated with bacterial vaginosis (BV) via a novel mechanism of action, in contrast to conventional antibiotics, by obstructing bacteria attachment to cells and hindering

biofilm formation and disruption. **Table 1** presents an overview of various clinical trials conducted with VivaGel® alongside other dendrimer-based systems.

The clinical applications of dendrimers are constrained by certain limitations despite the numerous potential advantages. PAMAM dendrimers are cationic polymers that can induce cytotoxicity by disrupting cell membranes due to their high binding affinity.[106] However, this concern may be mitigated by incorporating surface chemistries that covert surface amine groups to carboxyl, hydroxyl, or acetyl functional groups to diminish the surface positive charge.[98] Another challenge includes that higher-generation dendrimers (G5 or above) can reach sizes comparable to biomacromolecules, including DNA and proteins, complicating renal elimination and hepatic metabolism.[107] Targeted dendrimers containing drug molecules may also potentially exhibit lower efficacy than antibody-drug conjugate counterparts due to steric hindrance and limited selectivity issues.[108] For dendritic NPs to be used as vaccines, their mechanisms in clinical situations must be further studied and optimized.[109] Lastly, scaling up multifunctional dendrimers presents challenges since each addition of a moiety follows a distribution curve, resulting in an undefined mixture instead of reproducible products.[110] Emphasizing the necessity rather than the quantity of surface functional groups may be critical. Overcoming the limitations mentioned above could unlock the potential of dendritic NPs as a next-generation drug delivery method, specifically in cancer immunotherapy.

Study Number	Phase	Aims	Date Posted
NCT04865419	1&2	Tolerability, and pharmacokinetics of AZD0466 in advanced hematological malignancies	09/11/2023
NCT04458298	2	Efficacy of OP-101 in severe COVID-19.	02/13/2023
NCT05208996	1	Dose efficacy of KK-46 in severe COVID-19SARS-CoV-2 inhibition	26/01/2022
NCT05105607	1	Tolerability, and pharmacokinetics of D4517-2 in eye diseases	21/09/2022
2014-000694-39	3	Prevention of recurrence of BV (female) with VivaGel®	21/11/2014
NCT02237950	3	Prevention of recurrence of BV (female) with VivaGel®	12/09/2014
NCT02236156	3	Prevention of recurrence of BV (female) with VivaGel®	10/09/2014
2012-000752-33	3	Treatment of BV (female) with VivaGel®	22/06/2012
NCT01577537	3	Treatment of BV (female) with VivaGel®	16/04/2012
NCT01577238	3	Treatment of BV (female) with VivaGel®	13/04/2012
NCT01437722	2	Prevention of recurrence of BV (female) with VivaGel®	21/09/2011
NCT01201057	2	Efficacy against BV (female) with VivaGel®	14/09/2010
NCT00740584	1&2	Retention and duration of activity (female) with VivaGel®	25/08/2008
NCT00490152	1	Adherence, acceptability (female) of VivaGel®	22/06/2007
NCT00442910	1	Safety and acceptability (female) of VivaGel®	5/03/2007
NCT00370357	1	Safety (male) of VivaGel®	31/08/2006
NCT00331032	1	Safety and tolerability (female) of VivaGel®	29/05/2006

Table 1. An overview of clinical trials of dendrimer-based systems.

6. Conclusions

Dendrimers represent a class of nanoscale macromolecules characterized by a highly-branched spherical structure, excellent biocompatibility, and customizable surface properties. Various modified dendrimers and dendrimer-based hybrid NPs have been investigated for their

applications in cancer treatment and diagnosis. Recently, these dendrimers have shown promise in cancer immunotherapy. Our review provides a comprehensive summary of the immunomodulatory effects of dendrimers and dendritic polymers, focusing on recent developments in dendrimer-assisted cancer immunotherapy and visualization of cancer immunity.

Current studies on dendrimer or dendron-mediated delivery systems have focused on mitigating the shortcomings of cancer immunotherapies by synthesizing dendrimer-based drugs with innate immune properties or utilizing co-delivery approaches. However, these cancer treatment methods do not fully exploit the functionalization opportunities dendron scaffolds offer. Based on current preclinical results, we are growing to understand the multivalent effect owed to dendritic scaffolds is robust enough to produce effective results *in vitro* and *in vivo* and can substitute or, in some cases, outcompete current FDA-approved drugs.[22, 28] Furthermore, dendrimers and dendron-hybrids have been observed to have more efficient tumor targeting compared to alternative nanocarriers.[22, 66, 82, 111] For maximal efficacy, researchers must carefully design next-generation dendron-based delivery systems, which comprise innate immune stimulating properties, targeting or therapeutic ligands, and dual drug loading, all within the same NP construct. In doing so, we can eliminate the need to co-administer multiple monotherapies and co-create a single nanoplatform with an array of utilities for cancer therapy. This concept is further highlighted when limited success has been observed in delivering dendronized copolymers conjugated to therapeutic peptides, as tumor regression was not observed until co-administered with the immune checkpoint inhibitor, aPD-L1.[88] An important benefit of creating a multifunctional dendrimer or a dendron-based drug delivery platform includes the ability to direct multiple therapeutic components to the same site in the body. This method alleviates challenges clinicians face when considering the pharmacokinetic profiles of various drugs, ultimately

reducing significant side effects. As ongoing research delves into understanding the mechanistic roles of various substrates once conjugated or encapsulated by dendron-based structures, valuable insights will emerge, enlightening the field of NP-mediated cancer immunotherapy. Clinical data have indicated the safety and efficacy of specific dendrimer-based nanosystems, particularly in treating conditions such as COVID-19, eye diseases, and sexually transmitted infections. However, the application of dendritic scaffolds in the context of cancer immunotherapy remains largely unexplored, requiring further development in the synthesis, physiochemical, and biological characterization of these nanocarriers. In summary, utilization of the entire therapeutic capabilities of dendron- and dendrimer-based structures in cancer immunotherapy requires a collaborative effort to bridge the current gaps and expand the field toward the new era of cancer treatments.

Conflicts of Interest

There are no conflicts to declare.

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