



REVIEW

# Nanotechnology in the arena of cancer immunotherapy

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**Abstract** Cancer immunotherapy orchestrates the immune system of the human body to fight against cancer cells. By doing this, it has revolutionized cancer treatment. Toxicities arising from dose-limit and low rates of patient response continue to be the major bottlenecks in clinical outcomes. The immune system has a close relationship with tumor. This leads to the combination of nanotechnology and immunotherapy. Nanotechnology can potentiate the efficacy of immunotherapy by enhancing the delivery and retention, and narrowing the toxicity of immunomodulation. In this regard, immunotherapy can combine with nanomedicine to give strategies that could lessen the side effects and improve clinical outcomes in patient populations. In this review, we explore and recapitulate recent advances in nanoparticle-based cancer immunotherapy.

**Keywords** Immunotherapy · Peptides · Nucleic acids · Antibodies · Nanotechnology · Nanomedicine

## Introduction

Cancer, one of the prominent causes of mortality, has gained a lot of scientific devotion. Expansion of cancer knowledge at various levels, including genetic, cellular and physiological, has fostered tremendous progress in cancer treatment over the last decade (Phung et al. 2019). Humans have made much effort towards overcoming this difficult health-related problem using all the methods that can be considered. Conventionally, cancer is treated with chemotherapy upon surgical removal of tumors from patients. However, patients frequently suffer from a variety of off-target effects, which even cause damage to normal organs due to anticancer drugs that are used in the chemotherapy process. This has led to the urgency to develop novel strategies to overcome this undesirable side effect. Immunotherapy is a treatment method that exploits the patients' own components of the immune system to fight diseases. In cancer immunotherapy, the immune system is activated or boosted by agents to attack cancer cells via natural mechanisms, many of which are evaded during disease progression (Rosenberg 2014). In this way, immunotherapy is acknowledged as a promising strategy not only to treat, but also to cure, certain forms of cancer. Aiming to ameliorate antitumor immune response by eschewing or fewer side effects, mainly off target effects, this immunotherapy has shifted the paradigm of cancer treatment (Rosenberg 2014).

Immunotherapy is tremendously remodeling the landscape of clinical cancer treatment. Even though the first immunomodulatory therapeutics, Coley's toxins, were introduced more than a century ago, immunotherapy was accepted as a "stand-alone" modality in cancer treatment only very recently (Hoos 2016). It has achieved unprecedented results on a number of times, not only in complete regression of metastasized tumors but also in long-term

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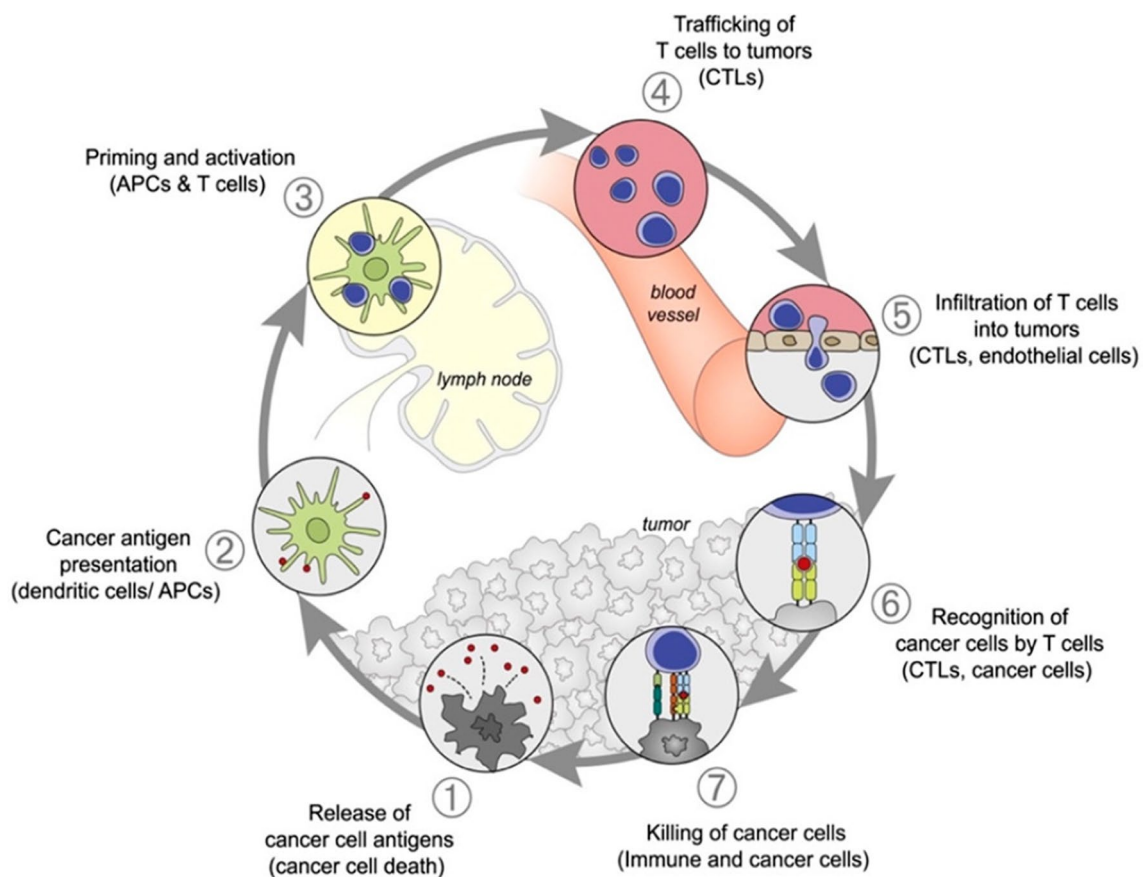
disease-free survival. It outperformed standard-of-care treatment in various cancer types, particularly lung cancer and malignant melanoma (Drake et al. 2014).

From a schematic representation of the cancer immunity cycle, we can understand the principle(s) of cancer immunotherapy (Fig. 1) (Chen and Mellman 2013). Starting with the release of tumor antigens, then passing through a couple of stages and finally promoting the immune reaction cascade again to end the cycle. Tumors can disrupt essential elements of the cancer-immunity cycle through a wide variety of immune regulatory pathways of negative feedback origin. In cancer immunotherapy, these pathways are increasingly becoming the precious targets for successful cancer treatment.

Recent strategies for cancer immunotherapy have mainly focused on tumor-associated antigens (TAAs), known as tumor vaccine, and the induction of antigen-specific T cell-mediated immune responses (Cheever and Higano 2011; Tefit and Serra 2011). With the thriving progress of genomics and proteomics, various potential target antigens, such

as recombinant proteins, synthetic peptides, and DNA, have been studied (De Gregorio and Rappuoli 2014).

Even though the progress made until now in cancer treatment is promising, there are still some limitations in cancer immunotherapy. The effectiveness and success rate of immunotherapy are being thwarted by the immunosuppression of the tumor microenvironment (TME). Several off-target effects can also be aroused concomitantly with these therapies (Kantoff et al. 2010; Maude et al. 2014; van der Burg et al. 2016). To overcome these delicate problems, new breakthroughs are necessitated (Wang et al. 2017). Being an interdisciplinary field, nanotechnology has entered many subject areas including drug development and delivery. Nanotechnology, especially nanoparticle-based drug development has several advantages over conventional drug development approaches. It has demonstrated essential characteristics such as long-term flow and blood concentration, improved binding ability to biomolecules (e.g., endogenous compounds like proteins), and aggregation in target tissues, as well as reduced immune



**Fig. 1** Schematic illustration of the cancer-immunity cycle. The anticancer immune reaction starts with the release of cancer cell antigens (1), which are taken up, processed, and presented by antigen-presenting cells (APCs) to naive T cells in secondary lymphoid organs, such as lymph nodes and spleen (2+3). Subsequently, cytotoxic T lymphocytes (CTLs) are generated, which migrate to and infiltrate tumors and metastases (4+5). In tumors and metastases, CTLs can then recognize (6) and kill (7) cancer cells. (Reprinted with permission from Chen and Mellman 2013; Copyright © 2013 Elsevier Inc.)

responses and tissue oxidative stress (Choi and Han 2018). As nanoparticles (NPs) have a good biocompatibility, it has made notable offerings ranging from targeted drug delivery to biodistribution. Not surprisingly, drug-loaded NPs have an improved bioavailability and stability and prolonged half-life. They are also safeguarded from degradation (Smith et al. 2014; Kapadia et al. 2015; Manjili et al. 2018). Moreover, the different physicochemical properties of each NP are matched to the delivery of adjuvants, antibodies, antigens, cytokines, and vaccines, and they are preferentially allowed to accumulate in vital antigen presenting cells (APCs) such as dendritic cells (DCs) in the draining lymph nodes (Moon et al. 2012; Koshy and Mooney 2016; Song et al. 2017; Hwang et al. 2018). Consequently, this accumulation triggers the downstream effector CD8+ cytotoxic T lymphocytes (CTLs). With the help of T cell receptors and MHC interactions, this CTLs recognize and kill tumor cells, which results in the modification of the tumor microenvironment (TME) and awakening of the immune system (Chen and Mellman 2013). Currently, cancer immunotherapeutic strategies have improved exceedingly with the help of nanotechnology (Le et al. 2018). In this review, we will discuss the current application of several kinds of NPs in cancer immunotherapy as well as consider some improvements in the current methods for future application in cancer immunotherapy.

### Nanotechnology-based delivery systems for cancer immunotherapy

In targeted delivery of immunomodulators as well as other therapeutic agents, ascending numbers of nanoengineered materials have been utilized (Fan and Moon 2015; Fang et al. 2015; Wang et al. 2017; Yong et al. 2018). Versatile composition, changeable size and morphology, and surface modification influence the NPs for this type of utilization (Bracho-Sanchez et al. 2016). Immunotherapy has been of benefit with the help of these properties in several ways. First, there are several ways of cargo loading including encapsulation, affinity binding, electrostatic adsorption, hybridization, cholesterol-mediated linker, and covalent conjugation. High amount loading is achievable with flexible synthetic methods. This method also helps to formulate multiple cargos, and to obtain temporal and spatial control of releasing the cargo, more importantly. Secondly, it can be designed and manufactured with expected size, shape, and surface. Another thing is that their multifunctional properties make them suitable for photothermal therapeutics, imaging probes, or adjuvants in immune stimulation (Koshy and Mooney 2016).

### Antigenic peptide delivery systems

An antigen is a molecule that binds to antigen-specific receptors, but cannot induce an immune response necessarily in the body by itself. Antigens are usually peptides, proteins and polysaccharides. In cancer diagnosis and therapy, nano-drug delivery systems have been extensively studied. Therapeutic compounds (such as tyrosine related protein 2 (TRP2) or Ovalbumin (OVA)) can be delivered to specific cells (either immune cells or tumor cells) with the help of nanoparticles, therefore, their therapeutic efficacy is improved and toxicity is reduced (Flanary et al. 2009; Wilson et al. 2013). Nanoparticles can deliver multiple immunotherapeutic compounds such as programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) simultaneously to bolster anticancer immune response (Toy and Roy 2016). In fact, nano-drug delivery systems can deliver an improvement in immunotherapy compared to free immunomodulators and antigens. Co-delivery of an immunostimulating agent and a tumor antigen to DCs has been the most expansively studied nanomedicine-based cancer immunotherapy strategy. Various delivery systems, including polymeric systems, lipid-based systems and inorganic nano-structures, have been developed for this purpose (Bahrami et al. 2017a, b).

### Polymeric systems

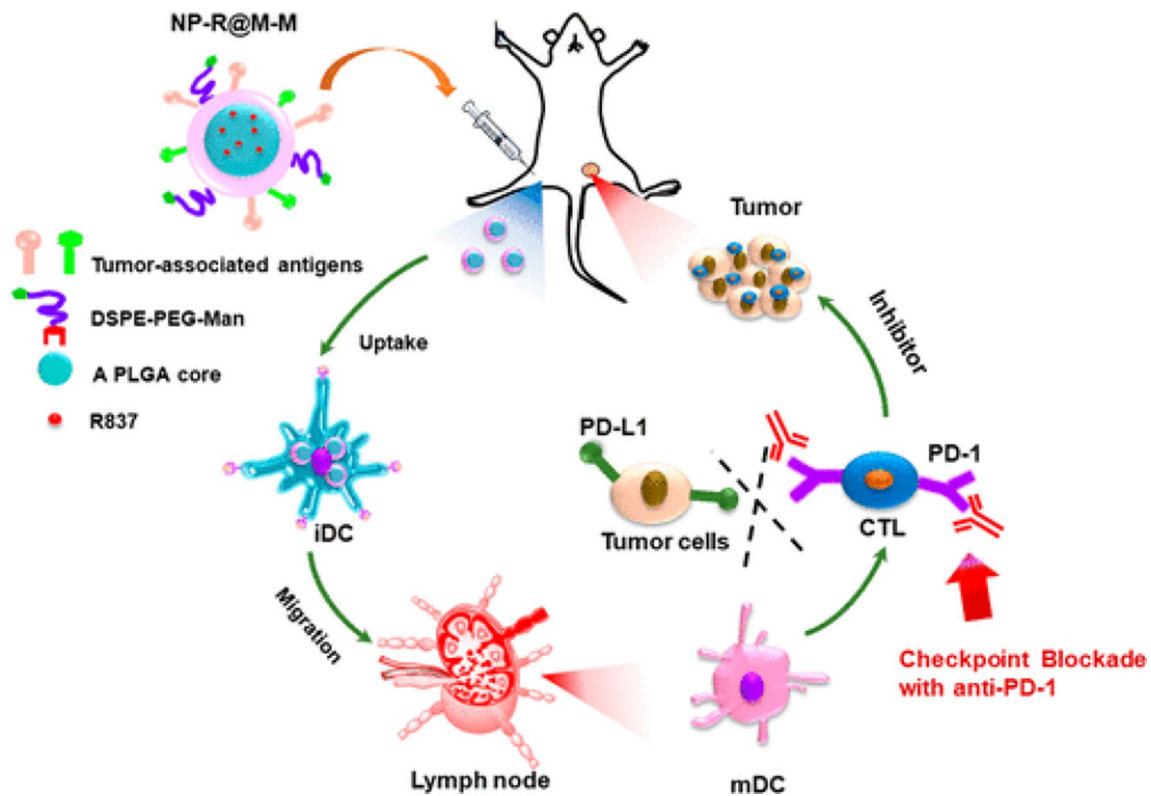
There are many advantages of using polymeric nanoparticles in drug delivery such as biocompatibility and biodegradability, increasing the stability of any volatile pharmaceutical agents, less toxic, targeted drug delivery, nonimmunogenicity, and nontoxicity. Polymeric NPs can be natural polymers like chitosan, gelatin, alginate, and albumen (Zhang et al. 2013a, b). Among the synthetic polymers, polylactic acid (PLA), polycaprolactone (PCL), poly(d,l-lactide-co-glycolic) acid (PLGA), and cyclodextrins (CD), are the most commonly used (Karlsson et al. 2018).

Polymeric carriers, such as NPs, micelles, and hydrogels, have been extensively used in various drug delivery and targeting vehicles. PLGA [poly (lactic-co-glycolic acid)], the FDA approved co-polymer can encapsulate a wide range of biologically active components. As delivery tools, PLGA microspheres can increase the maturation of DCs upon reaching the processing pathways for MHC class I and II molecules (Waeckerle-Men 2005). Over the last couple of years, researchers have combined PLGA nanoparticles (NPs) with cytokine agonists or CpG-coated tumor antigens in order to surge the DC uptake of antigens. Moreover, CTL (CD8+) and T helper cells (CD4+) immune responses are both activated with this platform (Heo and Lim 2014; Kokate et al. 2016; Kim et al. 2018). Hydrogels are comprised of polymeric three dimensional networks whereas

in micellar NPs the inner and external parts comprise of amphiphilic polymers and hydrophilic residues respectively (Luo et al. 2015; Kokate et al. 2016; Bahrami et al. 2017a, b). PLGA, is a biocompatible and biodegradable copolymer (Emami et al. 2019; Perinelli et al. 2019). Esterases facilitate its hydrolytic degradation. By controlling the ratio of glycolic acid to lactic acid, the rate of degradation of this co-polymer can be adjusted. In particulate drug delivery systems, this copolymer is used widely (Cruz et al. 2014; Rowdo et al. 2015). Exhibiting similar size to pathogens (i.e., 100–1000 nm), these PLGA solid core NPs naturally target DCs and are internalized by phagocytosis. Compared to free antigens, they exhibit 100 fold greater uptake by DCs (Kaufman and Disis 2004). PLGA nanoparticle encapsulated tumor antigens trigger antigen presentation with both MHC I and II. This activity facilitates the activation of both Th (CD4+) and CTL (CD8+) immune responses (Pashmine et al. 2005; Waeckerle-Men and Groettrup 2005).

Yang and colleagues successfully constructed a PLGA-based nano formulation composed of numerous key components (Fig. 2). They used TLR agonist R837 (imiquimod), melanoma cell membrane and mannose as an adjuvant for the tumor-specific antigen and APC-recognition moiety respectively. (Yang et al. 2018). These fully biocompatible

nano formulations could be employed as therapeutic vaccines to combat melanoma progression effectively in combination with anti-PD-1 checkpoint blockade treatment. Ahmed et al. showed that PLGA NPs loaded PET lipid increases its adjuvant properties inclusively (Ahmed et al. 2016). This scenario was predominantly noticeable with respect to the stimulation of the immune system's specialized APCs, i.e., DCs. Altogether, they showed the prospect for particle loaded PET lipid A to be used as a vaccine adjuvant. Chen and coworkers developed a therapeutic strategy comprising PLGA, TLR-7 agonist R837 (imiquimod), and the photothermal agent indocyanine green (ICG) for multifunctional nanoparticle formulation (Chen et al. 2016). This nanoparticle platform gives antitumor activity through photothermal ablation, generation of tumor antigen, and a vaccine-like action. They also inhibit metastasis better in combination with anti-CTLA4. To capture diverse set of protein antigens, Min and colleagues used several engineered antigen-capturing PLGA NPs (AC-NPs) (Min et al. 2017). AC-NPs could present tumor derived antigens to APCs upon capturing them. The administration of AC-NPs improved the efficacy of  $\alpha$ PD-1 treatment in a B16F10 melanoma model. Zupančič et al. (2014) developed an antigen-loaded PLGA-PEG-based nanoparticle polymeric platform to deliver breast



**Fig. 2** Schematic illustration to show the structure of tumor cell membrane-coated, R837-loaded, and mannose-modified PLGA NPs (NP-R@M-M) and their functions to induce antitumor immunity as a nanovaccine. (Reprinted with permission from Yang et al. 2018; Copyright © 2018 American Chemical Society)

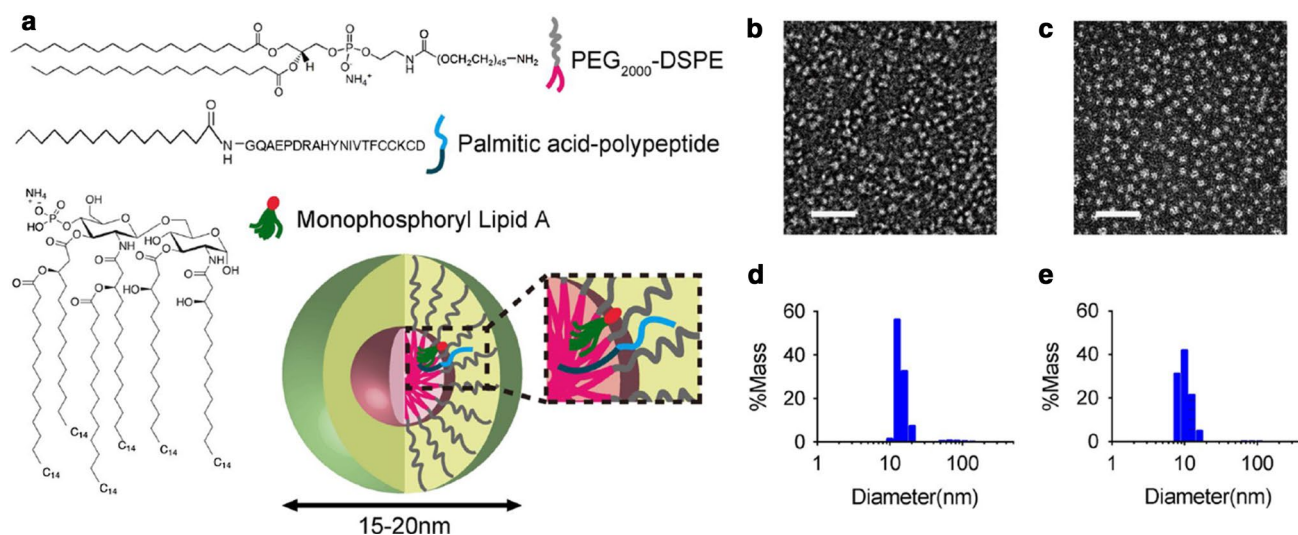


cancer antigens ultimately to DCs. Within the tumor micro-environment, this platform also improved their recognition by T cells. By this, they showed that developing PLGA-PEG-based NPs constitute an auspicious platform for the delivery of tumor-associated antigens to DCs, which are key players in tumor immunology.

Micelles are self-assembling colloidal structures comprising an aqueous solution, a poly (ethylene glycol) (PEG) hydrophilic shell, and amphiphilic block copolymers as a hydrophobic core to act like a drug reservoir. Usually their sizes are below 100 nm with a narrow distribution. Numerous polymers such as polyethylenimine, poly(lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), poly(vinyl alcohol) (PVA), poly(*N*-vinyl-2-pyrrolidone) (PVP), and poly(ethylene oxide) (PEO) are used to manufacture micelles, (Ma and Williams 2018). They have evolved as multifunctional nanotherapeutic platforms for cancer treatment (Cabral and Kataoka 2014; Wang et al. 2018a, b). They are commonly utilized to load poorly soluble compounds into the core, and for cell targeting, the hydrophilic segment can be functionalized as required. Cytosolic antigen delivery to the DCs of the lymph nodes can stimulate processing and cross-presentation of antigens with MHC I, thereby activating cytotoxic T lymphocytes (CTL) immune responses (Morón et al. 2004). Liu and coworkers developed micelles composed of poly (ethylene glycol) (PEG)-phosphatidylethanolamine (PE) incorporating a peptide antigen, either TRP2 or OVA, and a TLR agonist, MPLA, for cytosolic antigen delivery (Fig. 3) (Liu et al. 2017d). This group

also reported that when the micelles were injected subcutaneously to C57BL/6 mice, they showed an approximately 5-time increase in CTL response in vivo in comparison with that for free OVA plus MPLA, which subsequently resulted not only in reduced tumor progression, but also in prolonged survival. In combination with traditional approaches, tumor immunotherapy has also been gradually appreciated in recent times. Liu et al. reported that the co-treatment of micelle vaccine with cisplatin potently suppressed tumor growth in established TC-1 tumor bearing mice (Liu et al. 2017d). This activity continued for not less than 8 weeks from the onset of the treatment. More strikingly, with the co-treatment, more than half the number of mice became tumor free at 3 months. On the contrary, in the case of a single chemotherapy treatment, none of the mice became tumor free.

Basically, hydrogels are three-dimensional structures composed of a cross-linked hydrophilic polymer that can form nano-network or nanofibrous matrix (Leach et al. 2018; Lee et al. 2019). Even though the network is not water-soluble, the physical or chemical bonds formed between the polymer chains drive it towards a high affinity for water. Upon water penetration through this network, the network starts to expand and form hydrogels. A fully expanded hydrogel has similar biocompatible properties of a living tissue, ranging from soft and rubbery viscosity to low interfacial tension with body fluids or water (Hamidi et al. 2008). These properties minimize irritation to surrounding tissues and reduce adverse immune responses after injection.



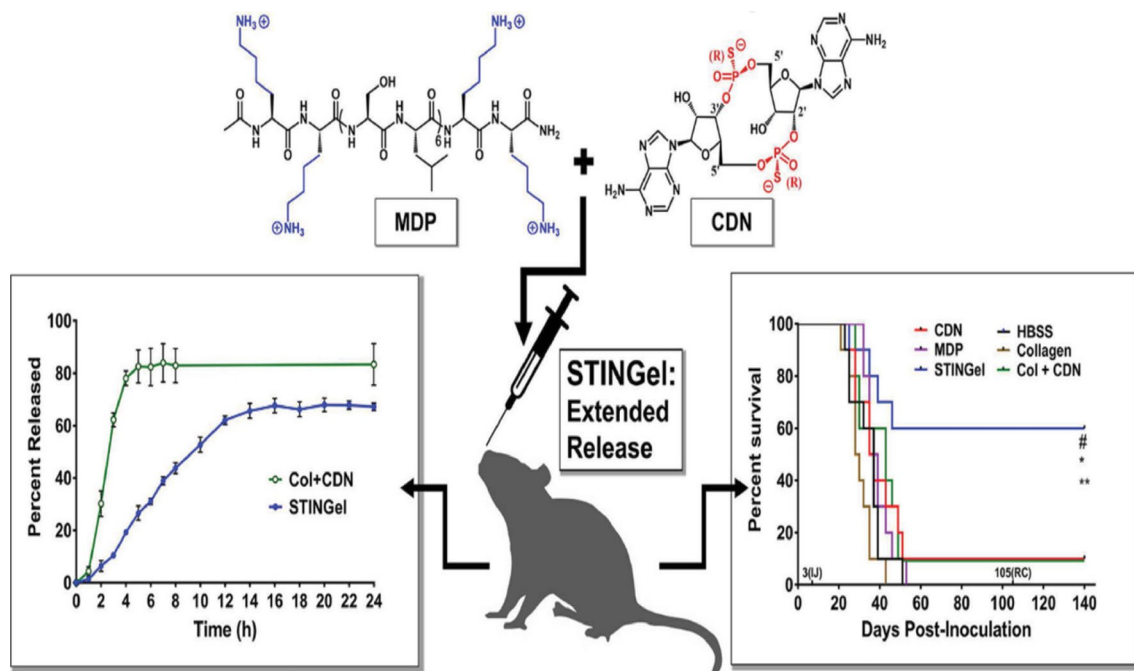
**Fig. 3** Design of cancer vaccine based on PEG-PE micelle. **a** Schematic diagram of self-assembly micelle consist of PEG-PE, palmitoylated polypeptide and MPLA. Upon encapsulation in micelles, the hydrophobic palmitic acid of palmitoylated polypeptide and MPLA can be inserted into the hydrophobic core of the micelles. Transmission electron microscopy (TEM) image of empty PEG-PE micelles **b** and micelle vaccine encapsulating the polypeptide antigen and MPLA **(c)**. Scale bar, 50 nm. Representative size distribution of micelle vaccine **(d)** and empty PEG-PE micelles **(e)** were measured by dynamic light scattering (DLS) analysis. Data represent two or three independent experiments. MPLA, monophosphoryl lipid A; PEG-PE, polyethylene glycol-phosphatidylethanolamine. (Reprinted with permission from Liu et al. 2017d; Copyright © 2017, Springer Nature)

Song et al. developed a polypeptide hydrogel as a sustained delivery platform for immune checkpoint inhibitors and vaccines. This platform can deliver an effective cancer treatment through a cutting-edge combinatorial immunotherapy approach (Song et al. 2019). They used an injectable PEG-*b*-poly(L-alanine) hydrogel for the co-delivery of a tumor vaccine with dual immune checkpoint inhibitors. Tumor immunotherapy efficacy has increased with this platform. The sustained release of tumor antigens and granulocyte-macrophage colony stimulating factor (GM-CSF) has not only tirelessly recruited and activated DCs, but has also triggered a strong T-cell response *in vivo*. This activity was further boosted by immune checkpoint therapy. They also suggested that besides the augmentation of activated effector CD8<sup>+</sup> T cells within the tumors and spleens of the vaccinated mice, this immunotherapy also reduced the ratio of T-regulatory cells (Tregs) significantly.

Injectable hydrogel networks of poly(vinyl alcohol) (PVA) were designed in one study to be responsive to reactive oxygen species. These species are also abundant in the tumor microenvironment (Nathan and Cunningham-Bussell 2013). In a murine model of breast cancer with low immunogenicity, the hydrogel was degraded upon injection, and first released the chemotherapeutic gemcitabine to perform the anticancer activity and generate an immunogenic tumor phenotype, which subsequently released an anti-PD-L1 antibody to kindle antitumor immunity (Wang

et al. 2018a, b). In a murine model of melanoma, post-surgical tumor recurrence can also be inhibited by local injection of hydrogels. It also extends survival compared with free gemcitabine local or systemic injections and an anti-PD-L1 antibody (Wang et al. 2018a, b). Furthermore, Wang et al. suggested that chemotherapies or systemically administered checkpoint inhibitors associated with toxic side effects may be avoided through this system.

Stimulator of interferon genes (STING) agonists and cyclic dinucleotides (CDNs) are a new class of potential immunotherapy drugs currently in clinical trials. Leach and co-workers developed an injectable peptide hydrogel, STINGel to control the delivery of CDN (Fig. 4) (Leach et al. 2018). This group used the self-assembling multidomain peptide (MDP) hydrogels. These mimic the body's extracellular matrix, and encourage cell as well as vascular system growth for tissue repair. The hydrogel turns semisolid inside the body upon administration as a liquid injection and degrades slowly over time. Compared to a collagen containing standard hydrogel, it showed an eight times slower release rate. Through highly localized delivery of CDN, they demonstrated the feasibility of using STINGel as a hydrogel-based immunotherapy platform to increase the efficacy of CDN immunotherapies. This approach has the tremendous potentiality to broaden the horizon of this omnipotent immunotherapy drug to various resistant cancers.



**Fig. 4** Graphical abstract of STING (Stimulator of Interferon Genes) agonists Cyclic dinucleotides (CDNs) as a powerful new class of immunotherapy drugs. (Reprinted with permission from Leach et al. 2018; Copyright © 2018 Elsevier Ltd.)

## Liposomes

Macrophages and dendritic cells are categorized as APCs. As these cells initiate and activate antigen-specific immune responses, they are regarded as targets for immunotherapy (Banchereau and Steinman 1998; Mellman and Steinman 2001). To deliver antigens to APCs, various functions are required. Several antigen carriers like lipid-based particles, polymeric particles, nanogels, micelles, carbon nanomaterials, and organic–inorganic hybrid materials have been considered in overcoming immune induction barriers. The safety issue, controllability of size, and easy functionalization capability make liposomes a good candidate (Schwendener 2014).

The tumor microenvironment is extremely immunosuppressive. In countering this, the activities of a couple of immunomodulatory and immunostimulatory molecules could be explored. Cytokines, chemokines, and targeted antibodies are the type of molecules that show important roles in this regard. However, there are some shortcomings of these therapies regardless of their immunomodulatory effects in overturning the suppressive tumor microenvironment. To overcome the adverse effects on most organs, and to ensure a safe clinical pathway for the use of immunomodulatory cytokines/chemokines, approaches like nanotechnology hold a great promise for the future. Immunomodulatory molecules containing nanoformulations have prolonged circulation times and a good *in vivo* stability against enzyme degradation and serum inactivation that give them improved bioavailability (Petros and DeSimone 2010; Christian and Hunter 2012). For instance, liposomal intravenous administration of cytokines (IFN- $\gamma$ , IFN- $\alpha$ , IL-2, or TNF- $\alpha$ ) enhances the plasma residence time (Kedar et al. 2000; ten Hagen et al. 2002; Christian and Hunter 2012). Additionally, intramuscular, subcutaneous, intraperitoneal, or intranasal administration of cytokine-carrying liposomes can create local depots, which subsequently gives the immunostimulatory payloads a longer residence time at the site (Eppstein 1982; Anderson et al. 1991). The targeted release of immunostimulatory cargo facilitated by external or physiological stimuli further leads to the improvement of its bioavailability and safety.

Liposomes can conjugate with IL-2 and anti-CD137 antibodies as well as target activated T cells. The direct delivery of immunostimulatory liposomes via intratumoral injections led to increased IL-2 dosing within the tumor as compared to that in systemic injections. A higher ratio of tumor infiltrating CD8+ T cells as compared to regulatory T cells are found in established melanomas with intratumoral treatment (Kwong et al. 2013). Similarly, intratumoral administration of PEGylated liposome formulation have been carried out to deliver TLR agonist CpG molecules and agonistic anti-CD40 antibodies, which resulted in a significant inhibition

of tumor progression. It reduces its systemic leakage during immunostimulatory payload sequestering in targeted tissues, thus minimizes off-target inflammatory effects (Kwong et al. 2011).

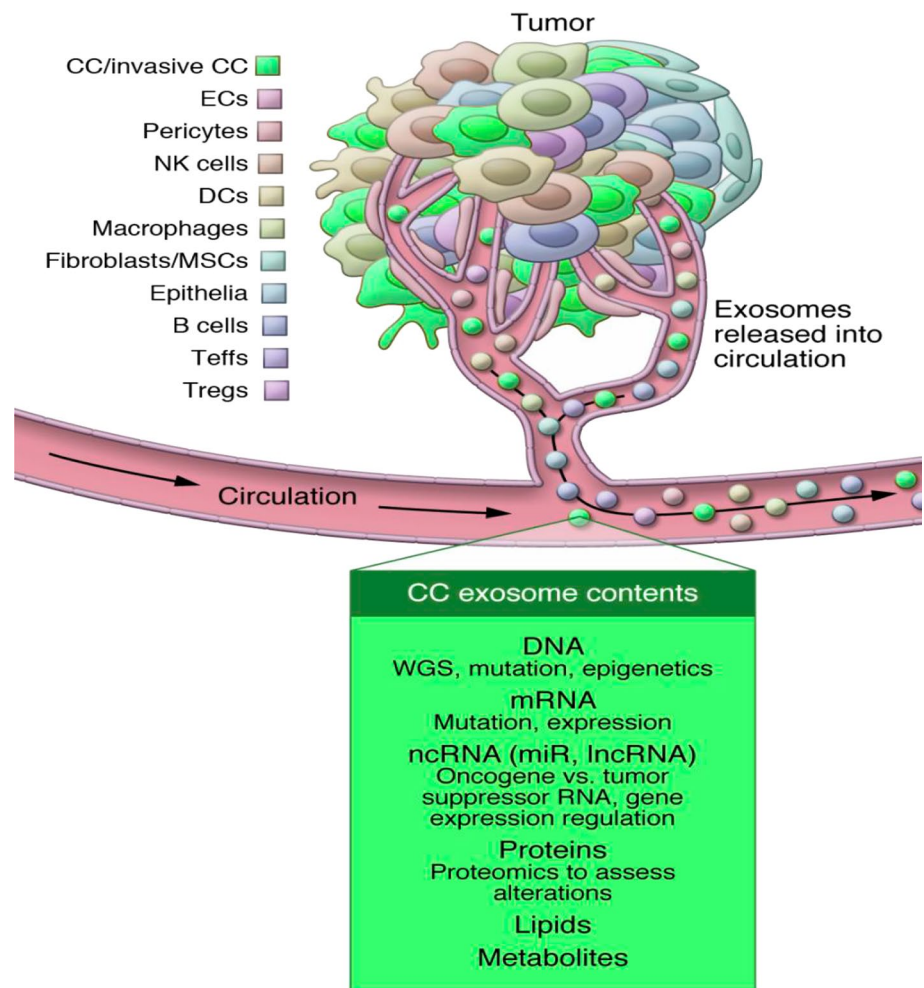
In cancer treatment, adaptive T cell immunotherapy plays a crucial role. Nanotechnology unlocks the door for the *in vivo* targeting, priming, as well as expansion of T cells. Actually, it is possible to expand cancer antigen-specific T cells *in vivo* using vaccines. This activity can also be achieved through ACT. For instance, loading (*in vivo*) of T cells in association with lipid nanoparticle “backpacks” can transport stimulatory cytokines. *In vivo* priming mediated through NPs resulted in 80 times more T cell expansion as well as significant enhancement of ACT efficacy barring systemic toxicity (Stephan et al. 2010). Likewise, upon *in vivo* targeting of circulating adaptive T cells by IL-2-containing liposome resulted in more enhancement of T cell proliferation as compared to that for soluble cytokine administration (Zheng et al. 2013). Following infusion, these tactics stopped the transplanted T cells from decline, precisely in the setting of highly immunosuppressive microenvironment containing solid cancers.

## Exosomes

Exosomes are cup-shaped vesicles, 30–150 nm in size. Releasing from the cells of origin, these nanosized vesicles contain cell-derived lipid membranes, nucleic acids, and proteins (Manandhar et al. 2018). Originating from the fusion between multivesicular body (MVB) and the plasma membrane, exosomes released in the extracellular space (Keller et al. 2006; György et al. 2011). Owing to their immunomodulatory potential, exosomes may also be deployed in innovative immunological approaches to activate adaptive and innate effector cell-mediated anticancer immunosurveillance. The field of exosome-based cancer therapeutics was launched two decades ago, with two seminal publications highlighting the potential of dendritic cell- and tumor-derived exosomes (Dex and Tex, respectively) in cancer immunotherapy (Zitvogel et al. 1998; Wolfers et al. 2001). In the former study, dendritic cells (DCs) pulsed with tumor peptides secreted antigen-presenting Dex capable of eradicating established murine tumors in a T cell-dependent manner (Zitvogel et al. 1998). The other breakthrough was the discovery that Tex are a source of neoantigens and that their internalization by DCs could cross-prime CD8+ T cells and lead to the rejection of syngeneic and allogeneic mouse tumors (Wolfers et al. 2001). Exosomes by transporting bioactive molecules influence the extracellular environment as well as the immune system (Fig. 5) (Kalluri 2016).

Heterogeneity of the tumor includes genomic heterogeneity of tumor neoplastic cells, as well as heterogeneity of non-cancer cell tumor microenvironment (Kalluri 2016).

**Fig. 5** Tumor-associated and circulating cancer-derived exosomes are a heterogeneous population that generates a unique tumor nanoenvironment (TNE) (Reprinted with permission from Kalluri 2016; Copyright © 2019 American Society for Clinical Investigation)



Functional heterogeneity of tumors resulted in varied composition of mesenchymal cells, immune cells, and acellular constituents. Heterogeneity in the tumor nanoenvironment (TNE) is evolving as another stratum of complexity in tumors. Various sizes, EVs, and apoptotic bodies comprise the TNE. Exosomes derived from cancer cells can influence host stromal responses to create a protumorigenic or antitumorigenic environment. However, stromal cell-derived exosomes may promote or halt the progression of cancer in a context-dependent manner.

Early exploration to elucidate the biological functions of exosomes revealed their activities in the regulation of adaptive immunity (Théry et al. 2002; Gould and Raposo 2013). Tumor originated exosomes possess complex and dynamic immunological activities. For instance, they range from antigen presentation modulation of tumor-to-tumor immunity polarization (Clayton and Mason 2009; Greening et al. 2015). Although exosomes have emerged as essential mediators of immune/cancer cell interaction, their anti-tumor and pro-tumor functions remain uncertain, probably indicating the functional heterogeneity of exosomes in the tumor

microenvironment. (Fig. 5). DC-derived exosomes have the ability to activate T and B cells. Exosomes from cancer cells may act as a tumor antigenic source that can be offered to activate T cells (Zitvogel et al. 1998; Wolfers et al. 2001; André et al. 2002; Hwang et al. 2003). Moreover, exosomes from cancer cells may activate NK cells directly through the stress protein, HSP70, presentation (Lancaster and Febbraio 2005). On the contrary, exosomes that originated from mast cells may activate T and B cells indirectly through DC differentiation (Skokos et al. 2003). The role of exosomes in facilitating antitumor immune activities is supported by their reported immune functions. Moreover, by impairing DC maturation through induction of IL-6 expression in BM dendritic precursor cells, they may assist in immune evasion (Yu et al. 2007).

Cancer cell derived exosomes can also inhibit NK cell proliferation and cytotoxic activity by lowering of NK group 2, member D (NKG2D) (Clayton et al. 2008). Furthermore, exosomes induce T cell apoptosis, and impact the biology of T cells. Apoptosis of Fas<sup>+</sup> T cells is induced via Fas ligand (FasL) on exosomes derived from cancer



cells (Andreola et al. 2002). Moreover, these exosomes may suppress the activity of the T cell receptor (TCR) (Taylor and Gerchel-Taylor 2005; Söderberg et al. 2007). Muller et al. reported that these exosomes regulate the transcriptome of regulatory as well as effector T cells (Tregs and Teffs) (Muller et al. 2016). Clayton et al. suggested that TGF $\beta$ 1 in exosomes derived from cancer cells induce Tregs cells (Clayton et al. 2007). Altogether, these antitumor immune responses resulted from the effects of exosomes. Morse et al. used dexosomes (DC-derived exosomes) to treat cancer patients suffering from non-small-cell lung cancer. They reported the activation of antitumor immune responses in their clinical efforts (Morse et al. 2005).

All cells, of different origins such as DCs, tumor cells, T cells, and B cells, release exosomes. These released exosomes are intrinsically modified on their surface with specific transmembrane markers including CD9, CD63, CD81, heat shock proteins (Hsp60, Hsp70, Hsp90), MHC I, MHC II, inter-cellular adhesion molecule-1 (ICAM-1), and some endosome-originated peptides (ALIX and TSG-101) (Liu et al. 2017b; Moore et al. 2017). Exosomes derived from dendritic cells (DEXs) stimulate tumor specific CD8+ and CD4+ immunoresponses. They can transfer MHC-peptide complexes to T cells from original DCs. By doing this they present their antigens. The co-stimulatory molecules' surface expression of CD80 and CD86 play a vital role in the immunogenicity of the DEXs and in T cells' maturation enhancement (Théry et al. 2001, 2009; Utsugi-Kobukai et al. 2003). Furthermore, the surface expression of BAT3, IL-15, TNF, and NKG2D DEXs can trigger natural killer cell (NK cell) oriented immune reaction (Simhadri et al. 2008; Viaud et al. 2009; Munich et al. 2012). Munich et al. reported that DEXs having TNF, FasL, and TRAIL exhibited strong efficacy for killing various tumor cells (Munich et al. 2012). They also demonstrated that in cell culture, this activity is displayed not only in a time-, but also in a dose-dependent manner.

Specific tumor antigens containing tumor derived exosomes (TEXs), can be taken up by DCs to trigger a strong tumor specific CTL activity. Liu et al. reported that exosomes derived from C6 glioma combining with  $\alpha$ -galactosylceramide ( $\alpha$ -GalCer), an activator of invariant natural killer T cells (iNKT), were used for immunotherapy in glioblastoma bearing rats (Liu et al. 2017a). Compared to the co-administration of the  $\alpha$ -GalCer and tumor cell lysate, serum level increased in subcutaneous combination treatment of IFN- $\gamma$  and TNF- $\alpha$ . Enhanced Th1 immunoresponse was observed via this outcome. In another experiment, intraperitoneal therapy of ascites-derived exosomes of murine T-cell lymphoma activated splenocytes. It also induced specific CD4+ and CD8+ lymphocytes. Increased survival of the animals ensued in both cases (Menay et al. 2017).

## Nucleic acid delivery systems

Recently, nucleic acid therapeutics has appeared as a consequential segment of cancer immunotherapy. Although there are tremendous prospects for nucleic acid therapeutics, many delivery challenges for both in vivo and ex vivo applications have hindered their translation into the clinic. Being highly unstable, nucleic acids degrade quickly in the existence of nucleases, and it happens before they reach the desired tissues (Kauffman et al. 2016). Moreover, without the help of physical techniques (such as electroporation) or transfection reagents, nucleic acids are not able to move into cells. However, these required reagents are highly toxic to the cells ex vivo that makes it unsuitable for in vivo use (Stewart et al. 2016; Moffett et al. 2017). Some nucleic acid therapeutics has to face another delivery obstacle of nuclear membrane crossing to be transcribed within the nucleus (McNamara et al. 2015). Therefore, there is tremendous interest in designing and developing novel delivery systems that can encapsulate and guard nucleic acids, as well as facilitate their delivery into the targeted tissues and cells to exploit their prevailing therapeutic potential.

## Polymeric systems

Nucleic acids can be encapsulated into polymeric NPs for targeted delivery to DCs and other APCs. This strategy is one of the most attractive strategies in cancer immunotherapy (Ghaemi et al. 2007). Nucleic acids can function as an immune-booster (e.g., CpG ODN, pDNA and poly I: C), an immunosuppressive silencing agent (e.g., siRNA), or an immunoadjuvant (e.g., pDNA, mRNA), or express a tumor antigen.

### *siRNA polymeric nanoparticles*

Polymeric NPs are used to deliver siRNA to silence an immunoinhibitory molecule that resulted in a boost for the antitumor immune response. For instance, CD73 (ecto-5'-nucleotidase) is over-expressed in immune and tumor cells. Adenosine is catalytically produced from AMP along with it. Binding of adenosine to the A2A adenosine receptors (A2AR) at the surface of T cells triggers decreased T-cell proliferation, decreased co-stimulatory cytokines production such as TNF- $\alpha$  and IFN- $\gamma$ , suppressed activity of NK cell, and impaired secretion of cytolytic molecules such as FasL and perforin. In tumor cells, CD73 is involved in tumor neovascularization, metastasis, as well as in invasion (Zhang 2010). Another group used tripolyphosphate (TPP) in ionic-gelation to load CD73 siRNA into chitosan NPs (~ 100 nm) (Jadidi-Niaragh et al. 2017). Upon intravenous administration to 4T1 mice (breast cancer bearing), NPs accumulated in the tumor, which subsequently downregulated CD73

in tumor associated immune cells, such as DCs, MDSCs, and Treg. Reduction of inhibitory cytokine IL-10 stemmed from that activity, but the secretion of immunostimulatory cytokines (IL-17 and IFN- $\gamma$ ) increased. Consequently, the primary tumor growth decreased with reduced lung metastases. However, saline treated mice and the control siRNA showed no action. Luo et al. prepared a polyplex formulation and mixed it with siRNA against STAT3 and OVA. Polyplex consisted of poly I:C (a TLR 3 agonist immunoadjuvant) and poly(ethyleneglycol)-b-poly(l-lysine)-b-poly(l-lucine) (PEG-PLL-PLLeu). After subcutaneous administration of the polyplex (~ 142 nm) to mice having C57BL/6 J B16 OVA tumor showed significant uptake by DCs in the draining lymph node. STAT3 level in the DCs and the number of immunosuppressive cells in the lymph node reduced. Subsequently, superior antitumor efficacy was found compared to the formulation that did not have siRNA (Luo et al. 2015).

#### *Oligodeoxynucleotides (ODNs) polymeric NPs*

In a study, carboxyl-styrene/acrylamide (PS) copolymer nanospheres were modified by polyethylenimine to develop a delivery system. Unmethylated cytosine-phosphate-guanine (CpG) oligodeoxynucleotides in combination with transforming growth factor-beta (TGF- $\beta$ ) receptor I inhibitors were delivered using that platform to obtain cancer immunotherapy outcomes (Liang et al. 2016). Intra-tumoral (i.t) injection of the formulation to a BALB/c mice bearing H22 liver tumor triggered an increase in splenic CD8+ T cells and a decrease in tumor volume compared to that for each component alone and the control. However, its application is limited to i.t only and the preparation technique is complicated, poorly reproducible, as well as time-consuming. To scale-up the manufacturing, significant obstacles have to be overcome. Heo and co-workers developed a chemoimmunotherapy platform, which is the combination of chemotherapy and immunotherapy. They combined chemotherapy, using the hyaluronic acid and paclitaxel complex (HA/PTX), and immunotherapy, using CpG ODN-encapsulated PLGA NPs, PCNs, and IL-10 small interfering RNA-encapsulated PLGA NPs, PINs. This strategy efficiently inhibited tumor growth, and not surprisingly increased the animal survival rate as well. It gave a superior antitumor activity compared to that of the control groups and PTX only (Heo et al. 2015).

Another group tried to augment the cytosolic delivery of ODNs (Wilson et al. 2013). They developed a pH-sensitive diblock-co-polymeric micelle (~ 30 nm) containing components of a cationic moiety (dimethylaminoethyl methacrylate), disulfide, and propyl acrylic acid for nucleic acid complexation, chemical conjugation with a peptide antigen, and pH-dependent conformation change, respectively. Intradermal injection of the CpG ODN and an OVA peptide co-formulation using polymeric micelle displayed greater DC

uptake via macropinocytosis, clathrin-, and caveolae-mediated endocytosis. This gave an augmented MHC I antigen cross-presentation and superior IFN- $\gamma$  production compared to co-delivery of free CpG and OVA. There are some other approaches by which increased DC delivery can be achieved. For instances, mAb targeting DC-SIGN (Cruz et al. 2011) or surface coating of NPs with mannose (Zu et al. 2013; Silva et al. 2015), DC 205 (Bonifaz et al. 2002), LOX1 (Delneste et al. 2002), Dectin-1 (Carter et al. 2006), MAC1 (Fayolle et al. 1996), CD11c, CD40 (Zhang et al. 2003), and CD18 receptors (van Broekhoven et al. 2004).

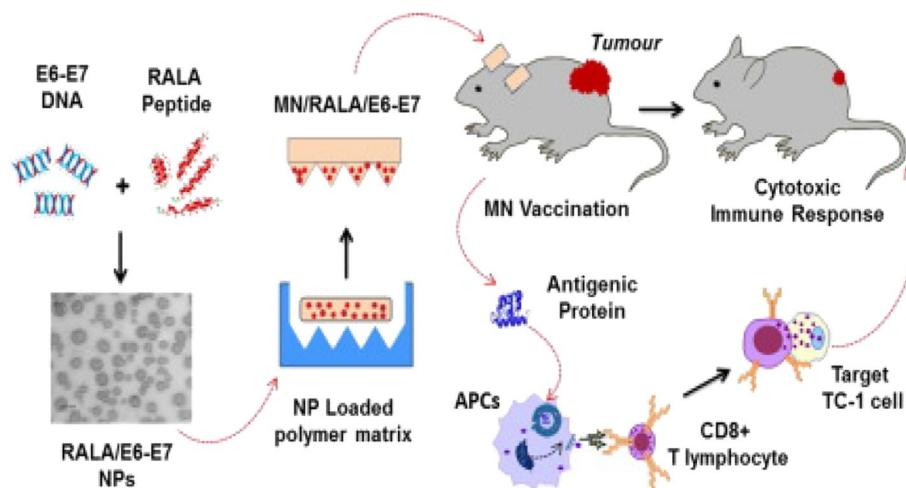
#### *pDNA polymeric nanoparticles*

To be recognizable as an immunoadjuvant by TLR9, plasmid DNA (pDNA) is a more versatile nucleic acid for immunotherapy compared to CpG, ODN, and siRNA. It can be encoded with an immunostimulating molecule or a tumor antigen. In a study, TNF- $\alpha$  encoding by pDNA was condensed with a polyarginine (HCR) peptide. That peptide was a modified cationic histidine- and cysteine. The encapsulation into the PLA-PEG NPs was carried out using a double emulsion method. Cytosolic delivery and nuclear translocation of pDNA was facilitated by HCR peptide. After intra-tumoral delivery, significant expression of TNF- $\alpha$  was measured with increased tumor cell apoptosis (Shukla et al. 2017).

As APCs, namely dermal DCs, keratinocytes, and Langerhans cells, are abundantly located in the dermal region, intradermal pDNA delivery using microneedles triggered elevated immunoresponses in comparison with those in subcutaneous or intramuscular deliveries (Kang et al. 2011; Guan et al. 2010). Upon making a complex with a cationic RALA peptide (repeated arginine-alanine-leucine-alanine units containing 30 amino acid peptide) pDNA encoded with HPV-16 E6 and E7 was delivered into the dermal compartment with the help of a PVP microneedle. In response to raised levels of DCs, secreted dermal IgG and TNF- $\alpha$  sequentially expressed E6/E7 that led to superior antitumor efficacy than in the intramuscular delivery (Fig. 6) (Ali et al. 2017).

Oral delivery, the most favored route for vaccine administration, also presents various barriers, including a tough acidic environment, a mucus layer, endogenous nucleolytic enzymes, and microbial flora (Bhavsar et al. 2007). Hu and colleagues (Hu et al. 2015) coated the salmonella bacteria with polyplex composed of pDNA encoding mAb against VEGFR-II and PEI. The viability of salmonella in the GI system increased with PEI interaction. It was suggested that this organism could be engulfed by the Peyer's patches DCs, resulting in improved pDNA transfection. This oral treatment induced the expression of mAb against VEGFR-II and enhanced TNF- $\alpha$  and IFN- $\gamma$  production. Both CD4+ and

**Fig. 6** Two two-step delivery of DNA vaccine for cervical cancer. (Reprinted with permission from Ali et al. 2017; Copyright © 2016 Elsevier Inc.)



CD8<sup>+</sup> T cells population in spleen and antitumor efficacy were enhanced. There are some other proteins that have also been encoded in pDNA for cancer immunotherapy such as the immunostimulatory cytokines IL-12 (Kim et al. 2006), CD40L (Daftarian et al. 2011) and interferon- $\gamma$ -inducible protein-10 (IP-10) (Lai et al. 2014), as well as tumor antigens TRP-2 (Daftarian et al. 2011) and OVA (Yata et al. 2017).

### Lipid-based nanoparticle systems (LNPs)

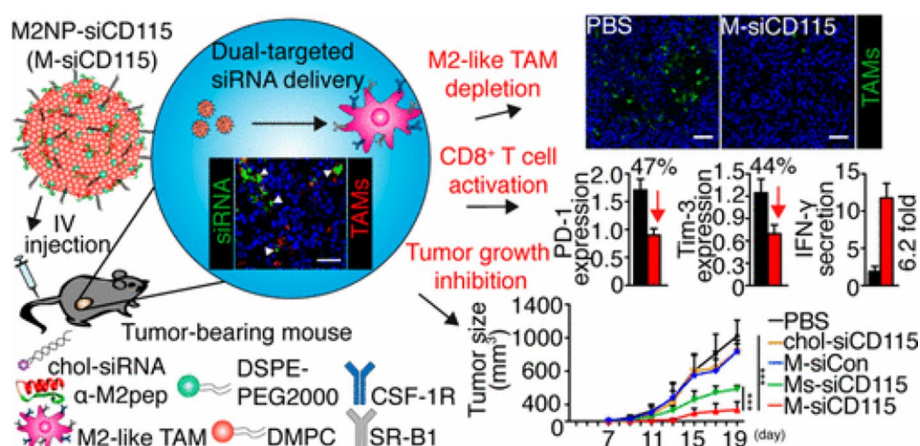
Being tedious to manufacture and construct, viral vectors have limited carrying capacity, and are immunogenic (Thomas et al. 2003). Thus to deliver nucleic acid based drugs, researchers usually opt for non-viral carrier systems. Lipid-based nanoparticle (LNP) systems are one of the most promising means of colloidal carriers for bioactive organic molecules. In nucleic acids, non-viral delivery systems like LNPs are one of the most prominently explored delivery systems. Nuclease-mediated degradation, poor cellular uptake, non-specific tissue distribution, and rapid clearance are the typical delivery barriers. In order to interact

and load negatively charged nucleic acids, lipids of cationic origin or ionizable cationic lipids are regularly used in LNPs to overcome these delivery barriers (Bally et al. 1999; Semple et al. 2000; Maurer et al. 2001).

### siRNA LNPs

LNPs are the most promising delivery systems for empowering the therapeutic potential of small interfering RNA (siRNA). Qian and co-workers developed a siRNA loaded dual-targeted lipoplex for silencing the receptor in TAMs namely colony stimulating factor-1receptor (CSF-1R) (Fig. 7) (Qian et al. 2017). Scavenger receptor B type 1 (SR-B1) that targets  $\alpha$ -peptide and M2 macrophage binding peptide (M2pep), and contains fused targeting peptide, was labeled at the surface of lipoplex for TAM targeting. Lipoplex was administered intravenously to the tumor bearing mice. Dual targeted lipoplex showed 4- to 5-times increased TAM uptake compared to that for either components of the modified lipoplex. This treatment led to improved antitumor efficacy via reduction in inhibitory cytokines such as TGF- $\beta$  and IL-10, as well as downregulation of T cell

**Fig. 7** M2-like TAM dual-targeting nanoparticles (M2NPs) (Reprinted with permission from Qian et al. 2017; Copyright © 2017 American Chemical Society)



immunoglobulin and mucin-domain containing-3 (Tim-3) in the microenvironment of the tumor, and co-inhibitory receptors including programmed cell death protein 1 (PD-1).

For targeting TAMs, some other ligands such as mannose and folic acid were also utilized (Daldrup-Link et al. 2011; Zhu et al. 2013; Chen et al. 2014a, b). One group showed improved endosomal escape and enhanced cytosolic delivery of siRNA against suppressor of cytokine signaling 1 (SOCS1). This inhibited the immunosuppressing STAT pathway in association with a pH-sensitive and cell-penetrating lipoplex preparation, which contained small unilamellar vesicles (R8/GALA-MEND (SUV)) (Akita et al. 2010). Subcutaneous administration of this lipoplex reduced SOCS1 expression in DCs significantly, leading to increased IFN- $\gamma$  and IL-6 secretion and eventually the antitumor effect increased. siRNA against immune check point inhibitor, CTLA-4 (that inhibits T cell-DC interaction), was mixed with BHEM-Chol, a cationic lipid, to form hydrophobic complexes. These systems were loaded within PEG-PLA polymeric micelles (~140 nm) and administered intravenously to mice presenting B-16 melanoma (Li et al. 2016a). The formulation decreased the expression of CTLA-4 in T cells, which subsequently augmented infiltration of CD4+ and CD8+ T cells in the tumor and decreased Treg population compared to that in the control siRNA formulation. Immunostimulatory RNAs (isRNAs, 19-bpdsRNAs) could be united with lipoplex to activate TLR 3/7/8 (Chen et al. 2014a, b; Kabilova et al. 2016). This RNA-lipoplex was injected peritoneally to B16 melanoma bearing mice resulting in increased IFN- $\gamma$  production and increased antitumor, as well as anti-metastatic activities.

#### *Oligonucleotides LNPs*

In an experiment, mannose modified lipid-calcium phosphate (LCP) core was prepared by CpG ODN and phosphorylated Trp2 peptides (p-Trp2) were co-precipitated with the help of  $\text{Ca}^{2+}$ , which was subsequently coated with lipids (Xu et al. 2013). LCP injected subcutaneously to mice bearing B16F10 melanoma gave a high accumulation value of the NPs in the lymph nodes (~35% of the injected dose). This also increased the uptake of DC and enhanced IFN- $\gamma$  secretion, which led to inhibition of tumor growth. Liu and colleagues loaded CpG ODN into PEGylated cationic liposomes to target MDSCs. This formulation was modified in the surface with an IL-4R $\alpha$  RNA aptamer to reverse immunoinhibitory phenotype of MDSCs. Upon subcutaneous injection to BALB/c mice, the formulation reduced tumor angiogenesis significantly (Liu et al. 2017c). As an immunoadjuvant, apart from CpG ODN, cyclic dinucleotides derived from bacteria can also act. Cyclic di-GMP (c-di-GMP) showed not only high affinity binding with RNA helicase DDX41 (cytosolic

ATP-dependent), but also formed stable complexes to trigger an interferon gene protein STING, that resulted in the activation of a TANK binding kinase1-interferon regulatory factor 3 (TBK1-IRF3) pathway, and promoted IFN production (Zhou et al. 1999; Miyabe et al. 2014; Liu et al. 2017c). Miyabe and colleagues found improved cytosolic delivery with the complex of negatively charged c-di-GMP and cationic pH-sensitive liposome. Lipoplex administered subcutaneously to mice bearing OVA-melanoma promoted IFN- $\beta$  secretion from macrophages and boosted the anti-tumor activity.

#### *pDNA LNPs*

To activate the therapeutic potential of macromolecules such as plasmid DNA (pDNA), sophisticated delivery technologies are required. pDNA are liable to breakdown in biological fluids; therefore, upon systemic administration, they do not accumulate at target sites, and even if they reach the target cells, they cannot enter the intracellular sites of action (Yin et al. 2014; Maclachlan and Cullis 2005). LNP-based delivery systems for pDNA must shield the pDNA from breakdown, enhance uptake into target cells, and inspire cytosolic release of encapsulated pDNA, and finally facilitate pDNA access into the nucleus (Maclachlan and Cullis 2005; Akhtar and Benter 2007).

To induce antitumor immunity, an antigen or immunostimulating cytokine encoded by pDNA can be delivered through lipoplex. Upon peptide antigen-coupled subcutaneous injection, lipoplex with IFN- $\gamma$  pDNA enhanced the antitumor efficacy (Yuba et al. 2015). Garu and co-workers fabricated cationic liposomes, which were similar to mannose in respect of structure (Garu et al. 2016). The cationic liposomes together with a melanoma antigen encoding pDNA (pCMV-MART1) were mixed to prepare lipoplex, which was delivered to mice. The lipoplex was taken up selectively by DCs in the draining lymph node, which led to a surge in the secretion of immunostimulating cytokines (IL-4, IL-6, IL-12, TNF- $\alpha$ , IFN- $\gamma$ ), and reduced tumor growth in comparison with that for the administration of mannose labeled lipoplexes. In another study, a pDNA expressing chemokine ligand 21 (CCL21, an immunoadjuvant that enhances DC maturation) and a pDNA encoding telomerase reverse transcriptase (TERT, a tumor antigen) were incorporated into lipoplex. The formulation was then mixed with UV inactivated hemagglutinin virus of Japan (HVJ) to have superior gene delivery. The HVJ-lipoplex was injected intramuscularly to mice that was bearing TS/A breast cancer, which in turn induced immunostimulating cytokine secretion and slowed tumor growth (Yamano et al. 2007).



### mRNA LNPs

Recently mRNA-based nanovaccine has appeared as a new approach for cancer immunotherapy (Persano et al. 2017; Voshavar et al. 2017). Delivery of mRNA LNPs is non-infectious and non-integrating as well as, and can be used to express several proteins in both dividing and non-dividing cells (Kallen and Theß 2014; McNamara et al. 2015; Pardi et al. 2017). Upon the subcutaneous administration of OVA mRNA containing lipopolyplex, a significant DC uptake via micropinocytosis resulted. Increased production of IL-12, IFN- $\beta/\gamma$ , and TNF- $\alpha$  co-stimulatory cytokines was measured with enhanced antitumor efficacy compared to an OVA peptide containing a lipid-polymer formulation (Persano et al. 2017). Another group prepared an OVA expressing mRNA containing DOTAP cationic lipoplex (~ 100 nm) (Sayour et al. 2017). Lipoplex with mRNA and DOTAP in a ratio of 1:15 were exceedingly effective in transfection of the DCs, they found. After lipoplex intravenous injection to mice (C57BL/6 melanoma-bearing), the numbers of antigen specific CD8+ T cells increased in both splenocytes and lymph nodes. Consequently, a significant decreased in tumor growth was noted.

Systemic intravenous administration of lipoplex having mRNA is recommended to mediate APCs activation in the reticuloendothelial system (RES) for inducing MHC I/II over-expression and systemic immunoresponse enhancement. One group manufactured a lipoplex formulation of cationic liposomes (DOTAP/DOTMA/DOPE) and several antigenic peptides like gp70, OVA, TRP-1, or oncogene E6/E7HPV-16 encoded by mRNA (Kranz et al. 2016). They showed that, following intravenous injection in mice, the lipoplex with a low lipid: mRNA ratio (i.e., 1:5) accumulated in the spleen effectively. However, in the case of high lipid: mRNA ratio (i.e., 5:1), the formulation was mostly taken up by the lungs. Within the spleen, the lipoplex accumulated in the DCs rather than in other APCs via micropinocytosis, and caused enhanced secretion of IFN- $\alpha$  and upregulation of CD40 and CD86. The upregulation led to the maturation of CD4+ and CD8+ cells. CD11c, TLR9, and IFN- $\alpha$  receptor 1 (IFNAR1) have been mediating these immunostimulatory effects. Clinically, an efficient CD8+ T cell-mediated immunity was found after testing lipoplex in three patients that showed individually a reduction in tumor size and metastatic nodules.

### Monoclonal antibody (mAb) delivery systems

Treatments based on antibody have finally set forth as auspicious strategies to combat cancers. By binding to target proteins with a high degree of specificity, off-target side effects are limited. On the contrary, limitations such as scant

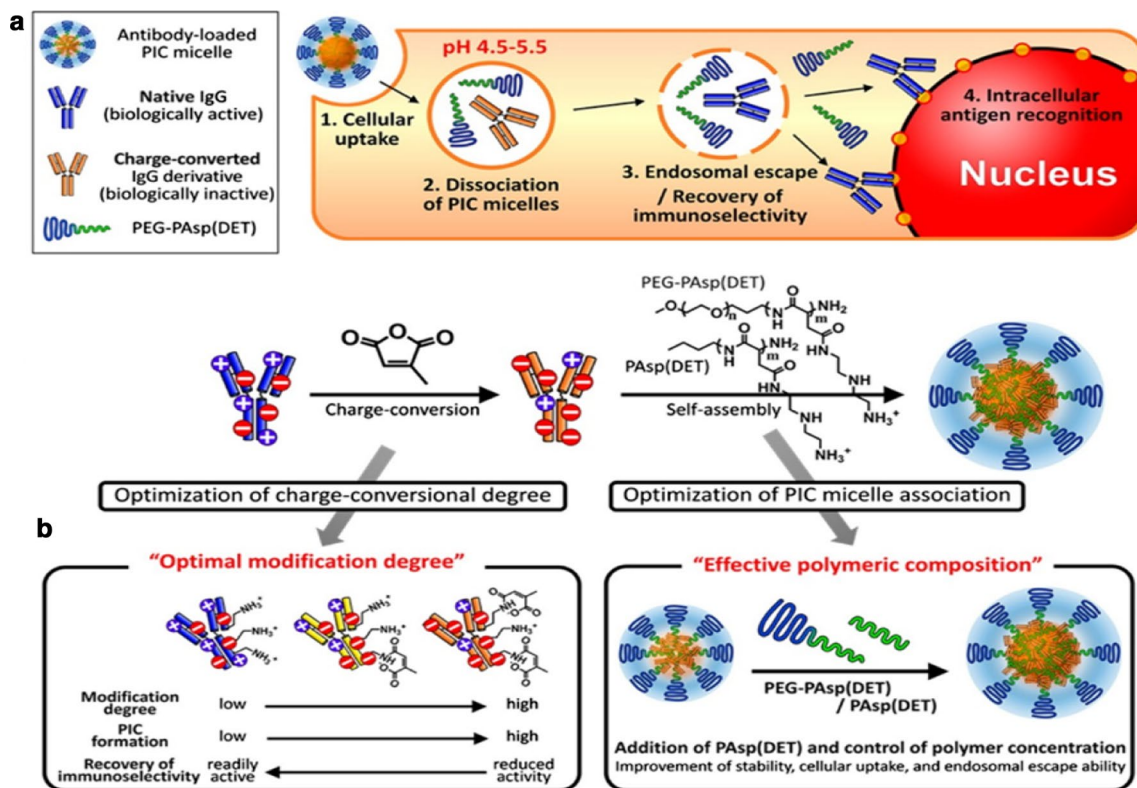
pharmacokinetics, poor tissue penetration, and impaired interactions with the immune system have been identified. NPs as novel delivery systems are being explored as candidates to overcome these deficiencies (Elhissi et al. 2012).

In a study, Kim et al. found that an appropriate ratio of homo- and block-cationomers in antibody-loaded polyion complex micelles (PICs) improved the endosomal escape ability of the loaded antibody. This subsequently enhanced the recognition of intracellular antigens. In order to get this effect, they tailored the structure of PICs to load transiently charged antibody derivatives for enhanced stability, delivery to the cytosol, and antigen recognition inside the cells (Fig. 8) (Kim et al. 2016).

In another study, Chen et al. constructed a biodegradable PLGA-NP carrying anti-OX40 mAb. Being a TNF receptor expressed on T cells, this antibody can transmit an effective activating signal (Chen et al. 2014a, b). Even though, the free anti-OX40 mAb failed to display the desired clinical activity in phase I clinical trials, it showed promise in loading anti-OX40 onto a PLGA-NP. This subsequently induced CTL proliferation, tumor antigen-specific cytotoxicity, and produced cytokine more strongly than in the free anti-OX40 mAb.

In order to increase responses, Lei et al. used functionalized mesoporous silica (FMS) to load CTL-associated protein-4 (CTLA-4) at super high densities through non-covalent interactions. This system provided long-lasting and localized release of CTLA-4 (Lei et al. 2010). Moreover, it prompted a much superior and extended therapeutic effect as compared to the systemic administration of same amount. Not surprisingly, a better tuning in the rate and durability of mAb release could be obtained with functional modification of FMS NPs.

There are some evidence of successful NP co-delivery of antibodies with cytokines. In a murine B16F10 model, NPs cured many established primary tumors after intratumoral administration. This system also prompted protective antitumor memory as well as avoided the soluble immunotherapy (equivalent dose) associated lethal inflammatory toxicities. Li et al. found improved antitumor activities of celecoxib and PD-1 mAb in combination with alginate hydrogel delivery system. They utilized this system to treat tumor bearing mice upon local delivery of celecoxib and PD-1 mAb (Li et al. 2016b). These activities were related to the ability of drugs to sustain high concentrations in peripheral circulation as well as in the regions of the tumor. A dual system NP platform was developed another research group. They Combined anti-PD-L1 with anti-4-1BB, a T cell co-stimulatory signal to develop that platform (Fig. 9) (Kosmides et al. 2017). To recognize target cells, this system redirected effector T cells. At the same time, this dual system blocked inhibitory checkpoints that resulted in a sixfold increase in IFN- $\gamma$  production in vitro with an exhausted phenotype in the presence



**Fig. 8** **a** Pathways for Successful Intracellular Antibody Delivery with PIC Micelles. **b** Formation of PIC Micelles Incorporating Charge-Converted IgG Antibody Derivatives and Strategies To Engineer the Systems in This Study. (Reprinted with permission from Kim et al. 2016; Copyright © 2015 American Chemical Society)

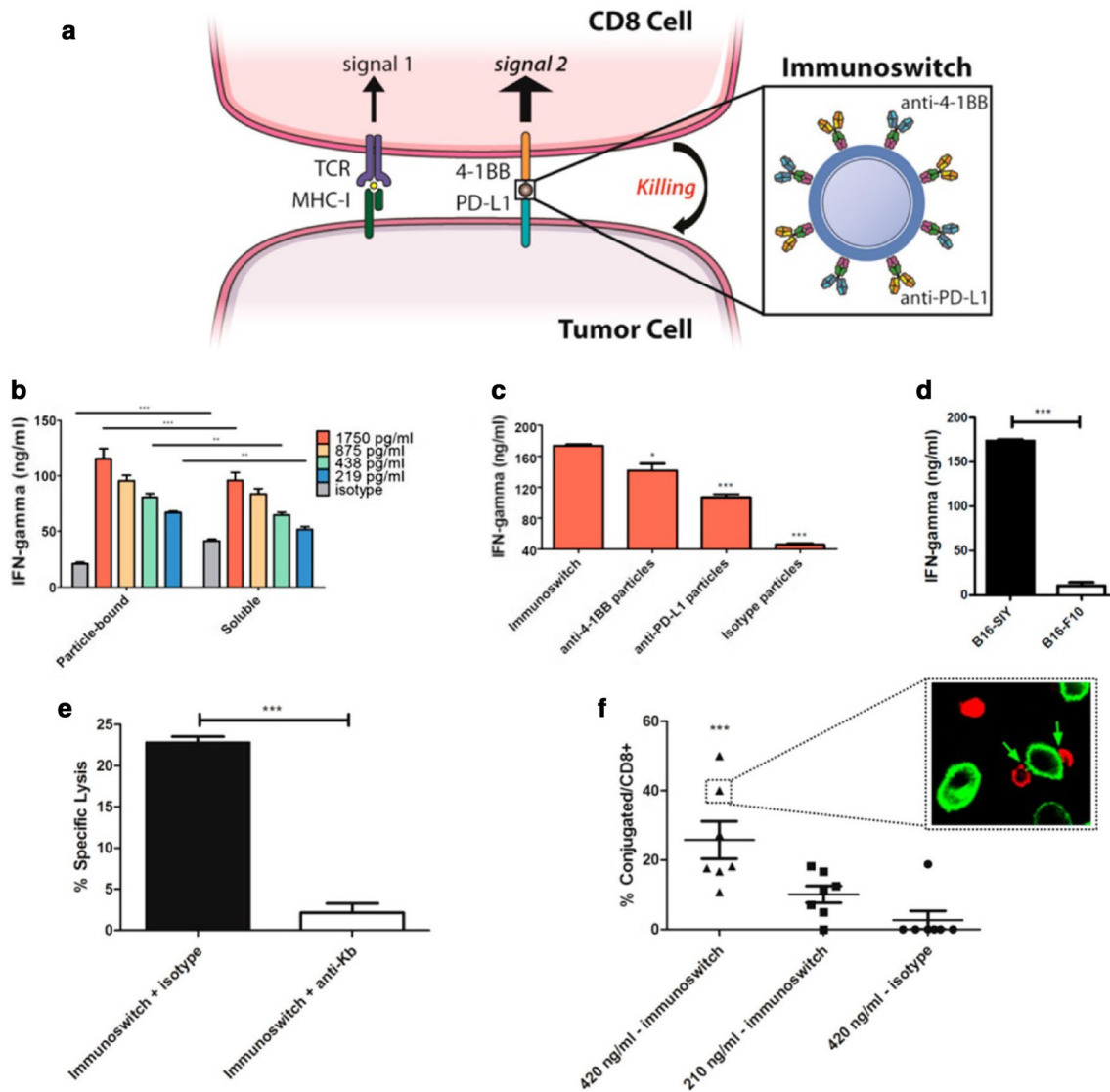
of tumor cells. Furthermore, growth of tumor stalled and in the tumor infiltrating lymphocytes, a significant extent of PD-1 expression plunger.

Some immunotherapeutic antibodies successfully inhibit cancer's immunosuppressive checkpoint pathways (Table 1).

### Future implications and conclusion

In this review, we recapitulated the principles and usage of nanotechnology in the landscape of cancer immunotherapy. The recent achievements in immunotherapy have attracted the development of novel immunotherapeutics. However, the complexity of tumors and the tumor microenvironment (TME), low immunogenicity and off-target side effects are still challenges for anticipated cancer immunotherapy. The activity of tumor cells, tumor-infiltrating immune cells, tumor associated stromal cells, and various chemokines or cytokines regulate the suppression of tumor specific T cells. In this complex system, one of the key and arduous activity

is to rekindle the immune response. However, nanotechnology with its unique advantages can augment cancer immunotherapy considering the current obstacles and technical challenges. Actually, advancement of nanotechnology, more especially NPs, gives a new paradigm for cancer immunotherapy. For instance, we can say some investigators obtained PD-1-expressing cellular NPs using genetically engineered cells to deliver immunological molecules of smaller sizes. Novel ideas for personalized immunotherapy may be attained through these strategies. Even though targeting ability of NPs is limited by the controllability, they play an exclusive role in targeted delivery for cancer immunotherapy. Therefore, development of measurement and characterization techniques drives the application of NPs. Continuous updating of clinical data is another factor that plays a great role in NPs application. Distribution of the delivered drug at tumor sites is restricted by the selectivity and effectiveness of NPs. This restriction directly confines the clinical application of NPs. This leads to a lack of impact on human patients and clinical transformations of NPs. Finally, biosafety of NPs as a drug carrier needs to be taken seriously. A detailed study



**Fig. 9** Immun SWITCH particles link PD-L1 checkpoint blockade with 4-1BB co-stimulation. **a** Schematic showing immun SWITCH particle interaction with CD8 + T cell and cognate target cell. Inset: Immun SWITCH particles are synthesized by conjugating anti-4-1BB and anti-PD-L1 monoclonal antibodies to the surface of 80 nm particles. **b** IFN- $\gamma$  secretion from PD-1<sup>hi</sup> 2C CD8 cells co-incubated with PD-L1<sup>hi</sup> cognate B16-SIY cells and immun SWITCH particles or soluble antibody. **c** IFN- $\gamma$  secretion from PD-1<sup>hi</sup> CD8 cells co-incubated with PD-L1<sup>hi</sup> B16-SIY and 9  $\mu$ g/mL of the indicated particle type. **d** IFN- $\gamma$  secretion from PD-1<sup>hi</sup> 2C CD8 cells co-incubated with PD-L1<sup>hi</sup> cognate B16-SIY cells or noncognate B16-F10 cells and immun SWITCH particles. **e** Percent specific lysis of B16-SIY cells by 2C CD8+T cells when co-incubated for 4 h at a 1:1 effector-target ratio in the presence of immun SWITCH particles and 100  $\mu$ g/mL anti-Kb blocking mAb or isotype control. **f** PD-1<sup>hi</sup> 2C CD8 cells and PD-L1<sup>hi</sup> B16-F10 cells were labeled with a red and green membrane dye, respectively. (Reprinted with permission from Kosmides et al. 2017; Copyright © 2017 American Chemical Society)

of the pharmacokinetic attributes of NPs in animal models is required. Another factor is the assessment of host immune reactions by various components also need to be considered.

Even though nanotechnology is still in the developmental stage, it undoubtedly has an important role to play in immunotherapy to bring relief to cancer patients.

**Table 1** Food and Drug Administration approved anti-cancer immunotherapy (Redrawn with permission; Qiu et al. 2017)

Drug name	Immunotherapy	Cancer type	FDA approval time
Rituximab	Monoclonal antibody: CD20	CD20-positive B-cell Non-Hodgkin's lymphoma	26/11/1997
Trastuzumab	Monoclonal antibody: Erb B2 (HER-2)	Metastatic breast cancer	25/09/1998
Alemtuzumab	Monoclonal antibody: CD52	B-cell Chronic lymphocytic leukemia	07/05/2001
Ibritomomab	Monoclonal antibody: CD20	B-cell non-Hodgkin's lymphoma	19/02/2002
Tositumomab	Monoclonal antibody: CD20	CD20-positive B-cell Non-Hodgkin's lymphoma	27/06/2003
Cetuximab	Monoclonal antibody: EGFR	Metastatic colorectal and head and neck carcinoma	12/02/2004
Bevacizumab	Monoclonal antibody: VEGF-A	Metastatic colorectal and non-small cell lung carcinoma	26/02/2004
Panitumumab	Monoclonal antibody: EGFR	Metastatic colorectal carcinoma	27/09/2006
Sipuleucel-T	Cancer vaccine	Metastatic castration-resistant prostate cancer	29/04/2010
Ipilimumab	Immune check-point inhibitors: anti-CTLA-4	Advanced melanoma	25/03/2011
Pembrolizumab	Immune check-point inhibitors: anti-PD-1	Advanced refractory melanoma and non-small cell lung cancer	04/09/2014
Nivolumab	Immune check-point inhibitors: anti-PD-1	Unresectable or metastatic melanoma Squamous non-small cell lung cancer	22/12/2014
Talimogene laherparepvec	Oncolytic virus therapy: HSV-1	Advanced melanoma	27/10/2015

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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