

Emerging role of nanotechnology in cancer immunotherapy

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Abstract. Cancer, a genetic and metabolic disease, caused by mutations of gene, dysregulated metabolism or environment factors. Since the cancer rate is among the highest in the 20th century and the incidence keeps elevating in the 21st century, it has been expected that 25% of the population will suffer from cancer during their lifetime. Thus, the study of new targeting vaccines and corresponding specific drugs as well as immunotherapeutic strategies are on in urgent need today. With the rapid development and wide application of nano-sized particles (NPs) and materials in recent years, nanotechnology plays an increasingly significant role in immunotherapy for cancer, such as nano-vaccines, nano-ACT and so on. Despite nanoparticles can be designed in various forms and functions to satisfy personalized demands, this technology still faces many challenges of safety and efficacy. In this review, the latest researches and prospects of for cancer immunotherapies based on nanotechnology are summarized. Additionally, withdraws and focus points of nanotechnology-immunotherapies are also discussed.

Keywords: Nanotechnology, Cancer, Immunotherapy.

1. Introduction

Cancer, as a genetic and metabolic disease [1], is defined by World Health Organization (WHO) as the uncontrollable clone of improper cells that break their normal limits of normal tissues, and which can invade adjacent tissues and migrate to other organs [2]. The subsequent process is called metastasis, and widespread metastasis leads to death in cancer patients as the principal reason [2].

It has been reckoned that there will be approximately 4,820,000 and 2,370,000 new cases of cancer, 3,210,000 and 640,000 cancer deaths in China and the USA respectively in 2022 [3]. Among these new cancer cases, Chinese people are more likely to be bothered by lung cancer, and American people more intend to have breast cancer accordingly. However, lung cancer is the first culprit resulting in death in these two countries [3]. Although some cancer profiles made by government and health care system have reported decreasing burden in some specific cancers, however, the increasing mortality of cancer is a long-term trend, which is more difficult to control effectively in such a short period than the COVID-19 pandemic. Nevertheless, cancer has become a major health issue both in the developed and developing countries.

The progress of malignant tumor is a multi-step process form an in-situ state to invasion and metastasis to nearby or distant organs, resulting morbidity and mortality [1]. As for the treatments of cancer, in addition to the traditional surgical therapy and chemotherapy and radiotherapy, Immunization therapy has become a new generation treatment of malignancy because of the proposal of personalized cancer therapy. Novel immunotherapies include immune checkpoint inhibitors (ICIs) therapy, adoptive cellular immunotherapy (ACT), and tumor therapeutic vaccines [4] mainly.

To treat solid tumors, we can use ICIs by targeting immune checkpoint proteins (ICPs) [5]. Simultaneously we can opt to use CAR-T therapy (a kind of ACT) to treat liquid tumor, like leukemia and lymphoma. What is more, we can also investigate tumor vaccines to induce patients' anti-tumor response. However, disappointing efficacy of ICIs in immune rejection or immunocompromised patients, high cost of ACT. Moreover, tumor vaccines' poor stability, weak immunogenicity and strong toxicity [8]. All of these limitations have hampered researchers for a long time. Fortunately, with the development of nanotechnology, a combinative therapy that unites nanoparticles and immunotherapies provides a solution to break through the bottleneck. For example, the combination of ICI with nano-carrier mediated therapy aiming to treat mammary cancer and non-small-cell lung

carcinoma has entered to the stage of clinical trial, which can make sure activate anti-tumor response and delivery drugs simultaneously that may enhance the therapeutic effects [5]. What is more, the combination of nanotechnology with CAR-T enables more activation of immune cells into tumor microenvironment to deliver personalized therapy [6] and make CAR-T can be applied in the treatment of solid tumor [22-23]. Last but not the least, nano-vaccines with the ability of bio-imitation have been investigated to solve problems of biocompatibility and anti- tumor-associated-antigens (TAAs) [7].

2. Nano-vaccine

Nano-vaccine, which is a new generation of vaccine, consists of nanoparticle carriers and/or adjuvants. After injecting into the body, because of size, shape, and surface charge properties, most of nano-vaccines can drain to the lymph nodes (LNs) or spleen directly through lymphatic vessels or blood vessels respectively. The rest of injected nano-vaccines can stay at the injection situ as an antigen depot [8]. Tumor-derived peptides on the surface of nano-vaccines are linked with the major histocompatibility complex class I molecules (MHC- I) on antigen presenting cells (APCs) to T cells in lymphoid organs to initiate cancer-specific adaptive immune reactions [9]. Subsequently, the excitation, proliferation, and differentiation of cancer antigen-specific lymphocytes, especially CD8+ cytotoxic T lymphocytes (CD8+CTLs) play an indispensable role in immunoreaction (Figure 1).

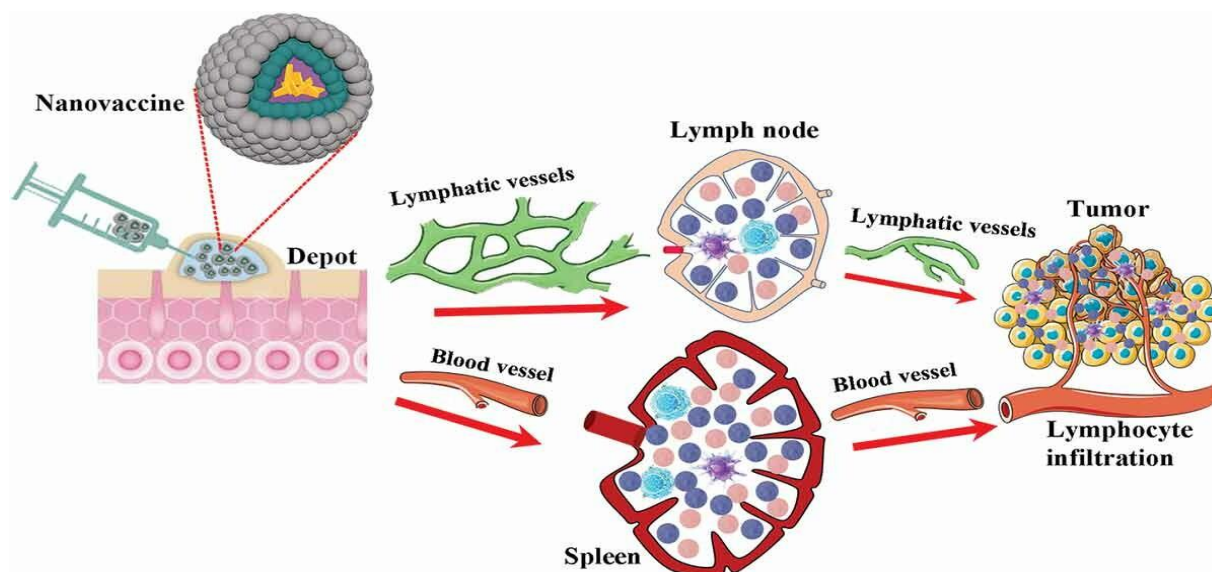


Figure 1. The mechanism of nano-vaccines [8]

Compared with traditional tumor vaccines, nano-vaccines have greater potential in diseases treatment due to their advantages [8]:

- (1) They can induce the situ immunological reaction by delivering tumor antigens to specific organs, tissues, and cells.
- (2) They can enhance the uptake of tumor antigens and the internalization of APCs to facilitate the maturity of immature dendritic cells (DCs) and cross-presentation procedure.
- (3) They can recruit time-dependent APCs by antigen depot effect.
- (4) They can eliminate the degradation from intracellular proteases and release the tumor antigen to improve vaccines' stability.

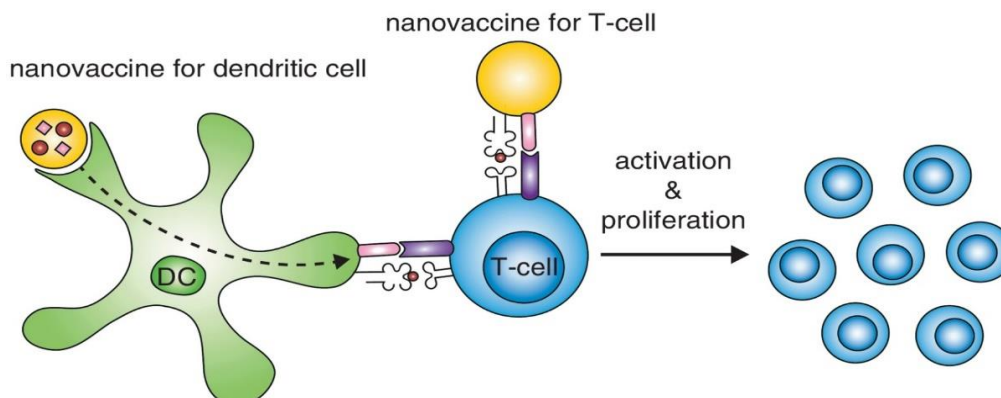


Figure 2. Nano-vaccines targeting T cells and DCs [10]

2.1. Nano-vaccines targeting T-cell (Figure 4)

The most critical point of manufacturing nano-vaccines that activate T cells directly is that we need to imitate the natural procedure of presenting antigens and the capacity of T-cell activation just as natural APCs. Nano-sized artificial antigen presenting cells (a-APCs) aim to stimulate T cells *in vitro* and *in vivo* by animal model and in human body offering a promising strategy for cellular immunotherapies. As for the pathways of nano-sized a-APCs, actually, there are three signals for T-cell activation allowing a-APCs to follow in the treatment of cancer [11]:

(1) Presenting neo-antigen peptide epitopes through their MHC to T cells receptor (TCR) on the surface of T cells (Figure 3A).

(2) Providing co-stimulatory semaphores by enhancing the conjunction between ligands and their receptors on T lymphocytes' surface, such as CD80/CD86, OX40L, and 4-1BBL and their receptors (Figure 3A).

(3) Secreting cytokines such as interleukin-12 (IL-12) or IL-15 to improve the survival, excitation, and proliferation of T-cell (Figure 3B).

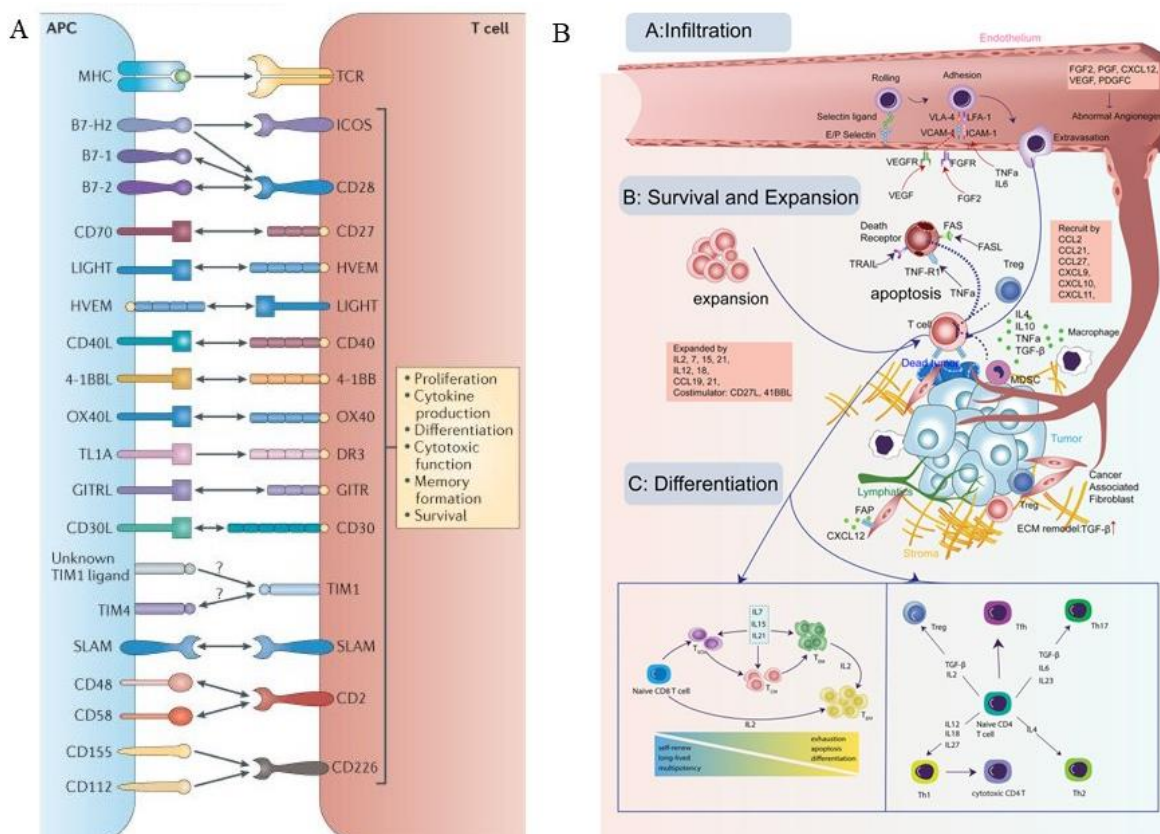


Figure 3. T-cell activation signals in cancer for a-APCs to follow [11-12]

2.1.1. Cellular a-APCs

Cellular a-APCs originate from original or artificially injected human-being cells or cells of the other living organisms, such as murine fibroblasts or insects. They are modified by retrovirus or lentivirus transduction to induce MHC molecules interacting with TCR and co-stimulatory signals [10]. Nano-sized cellular a-APCs are designed by Xiao P et al, with a coat of azido-labeled DC membrane, imiquimod-loaded polymeric NPs, and modify CD3 ϵ antibody through click chemistry [13]. The consequences of this a-APCs vaccine's evaluation shows that it can inhibit tumor inoculation and growth. Furthermore, Sun L et al develop a lymphocyte-based a-APC (LC-a-APC) with DNA engineering. This vaccine can activate tumor antigen-specific T- cell by migrating to peripheral lymph organs forwardly. As the result, it can inhibit the growth of tumor in different models, which may provide a promising future to potentiate cancer cellular immune therapeutics with DNA-Lipid-mediated non-invasive live cell surface engineering.

2.1.2. Acellular a-APCs

The Nano-sized acellular a-APCs are made by using biomimetic biomaterials, which can be modified to exhibit defined features of tumor antigen accurately. Rhodes KR et al make an acellular a-APCs, combining poly-lactic-co-glycolic-acid (PLGA) with cationic poly-beta-amino-ester (PBAE) to form a biodegradable fusion to use acellular a-APC technology *in vivo*. As the result, PLGA/PBAE a-APCs with immuno checkpoint therapy have been efficient in B16-F10 melanoma mouse model. Moreover, Song, S et al. developed a Nano-ellipsoidal a-APCs vaccine called EaAPCPEG/CD47containing PEGylation and CD47-Fc conjugation [13]. EaAPCPEG/CD47 can minimize the cellular assimilation *in vitro* and *in vivo* [13]. At the same time, it also shows a stronger effect to facilitate TRP2180-188-specific CD8+CTL proliferation, local infiltration, and tumor necrosis among the melanoma-bearing murine model and inhibiting tumor growth than other corresponding acellular a-APCs they used in their research [13].

2.2. Nano-vaccines targeting T-cell

After recognizing tumor antigens on the surface of DCs-targeting-nano-vaccines, activated DCs will transfer to secondary lymphoid organs and uptake, dispose and display antigen to T cells. The CD8+CTLs are activated to magnify adaptive anti-tumor immune response. At the same time, $\gamma\delta$ -T cells are elicited by nano-vaccines to induce innate anti-tumor immunity [8].

2.2.1. Targeting to DCs passively

DCs exist in lymphoid organs and can also be found in peripheral tissues, including skin. That is the reason why DCs have the natural ability to phagocytose invasive antigens. Direct nano-vaccines to sites that full of DCs, which is called passive targeting, has been explored [10]. Delivering nano-vaccines to LNs with many DCs has become a noticeable strategy for boosting the outcomes of cancer immunotherapy. For example, Chu, Y et al developed a novel amphiphilic neo-antigen Nano-vaccine, which includes neo-antigens and a polyethylene glycol phospholipid derivative, mixed with Montanide™ ISA 51 adjuvant [14]. They also verified the lymph node-targeted characteristic of their vaccine to activate DCs rapidly, and evaluated the anti-cancer immune effect and security of the neo-antigen nano-vaccine in murine model, to prevent recurrence and metastasis after subcutaneous tumor resection of melanoma [14]. That result can be used in patients with melanoma that are unsatisfied with immune checkpoint inhibitors (ICIs) therapy [14].

2.2.2. Targeting to DCs actively

Nano-vaccines can be installed with ligands that distinctively combine with DCs' surface receptors, such as C-type lectin receptors (CLRs) and some mannose receptors (MRs), so we called active targeting [10]. For instance, Zhang BD et al found that an incorporating of CDG5F (the STING agonist) and 522, as an agonist of the Toll-like receptor 7/8 (TLR7/8), can produced a broader cytokine response, which can used to activate anti-tumor response. Based on this idea, they developed multicomponent nano-vaccines (MCNVs), including a nano-carrier called PC7A polymer to

encapsulate the antigen ovalbumin and adjuvant molecules [15]. Their vaccines have been verified that they could activate bone marrow-derived dendritic cells (BMDCs) to produce multiple pro-inflammatory divisors by activate TLR7/8 actively [15].

3. Novel immunotherapies based on nanotechnology

Numerous innovative technologies have been investigated recently for the confirmed, avoidance, and remedy of cancer. The capacity of nanotechnology to effectively treat malignancy, overcoming the drawbacks of conventional anti-tumor medications, has made it one of the most promising technologies. Wang et al. summarized lasted nano-vaccines in clinical trials to treat malignant tumor (Figure 4).

Name	Nano-formulation	Active components	Cancer type	Clinical stage
FixVac	Lipoplexes	RNA antigens	Stage III B, C and stage IV melanoma	Phase I (NCT02410733)
Combination ONT-10	Liposome	MUC1 antigens and TLR-4 agonists and Varilumab	Advanced ovarian or breast cancer	Phase I (NCT02270372)
mRNA NP vaccine	Liposome (DOTAP)	Autologous total tumor mRNA	Early melanoma recurrence	Phase I (NCT05264974)
DPX-Survivac	Liposome	Survivac, cyclophosphamide and pembrolizumab	Recurrent/refractory diffuse large B-cell lymphoma	Phase II (NCT03349450)
		Survivac, aromatase inhibition, and with/without radiotherapy or cyclophosphamide	HR+HER2-breast cancer	Phase I (NCT04895761)
		Survivac, cyclophosphamide and epacadostat	Recurrent ovarian cancer	Phase II (NCT02785250)
DPX-0907	Liposome complex	7 tumor-specific HLA-A2-restricted peptides, a universal T Helper peptide, a polynucleotide adjuvant	HLA-A2 positive advanced stage ovarian, breast and prostate cancer	Phase I (NCT01095848)
WDVAX	Polymeric NPs (Poly(lactide- co-glycolide) scaffold)	Tumor lysates, GM-CSF and CpG	Melanoma	Phase I (NCT01753089)
IMF-001	Nano-complex	Recombinant NY-ESO-1 protein	Malignancies expressing NY-ESO- 1	Phase I (NCT01234012)
ZYC300	Microencapsulated particles	Plasmid DNA	Advanced stage malignancies	Phase I (NCT00381173)
DC vaccine	DCs	Autologous tumor RNA	Uveal melanoma	Phase III (NCT01983748)
Dex	Dendritic Cell-derived Exosomes	Tumor antigens (MAGE peptides)	Non small cell lung cancer	Phase II (NCT01159288)

Figure 4. Cancer nano-vaccines in clinical trials [16]

3.1. Novel adoptive cell transfer therapy (ACT) therapies

Adoptive cell transfer therapy (ACT) includes Tumor-infiltrating lymphocyte (TIL) therapy, chimeric antigen receptor (CAR) T cells therapy, and T- cell receptor therapy (TCR). In this immunotherapy, effective lymphocytes are injected into patients to treat malignancy. Nonetheless, the low concentration of effective lymphocytes in tumor tissues because of traffic and activity is still the main roadblock. Combining ACT with nanotechnology, by implanting or injecting nano-polymer or hydrogen scaffolds to create a modelled, customized tumor microenvironment, co-localized inflammatory molecules, tumor antigens, and immune signals can be generated in tumor situ, which has increased the concentration of effector cells. For example, Sanz-Ortega L et al. have demonstrated the potential for ACT-magnetic nanoparticles-loaded lymphoid effector cells with external magnetic field *in vitro* and *in vivo* [17]. Furthermore, the integration of nanotechnology and CAR-T/TCR therapy makes it possible to treat solid tumors. Application of nanotechnology in CAR-T cell therapy for solid tumors can be divided into three main points (Figure 5).

- (1) Helping effective T-cell target migrate to tumor tissues;
- (2) Remolding tumor microenvironment (TME) to stimulate CAR-T cells;
- (3) Aiding CAR T cells survival and proliferation [18, 19].

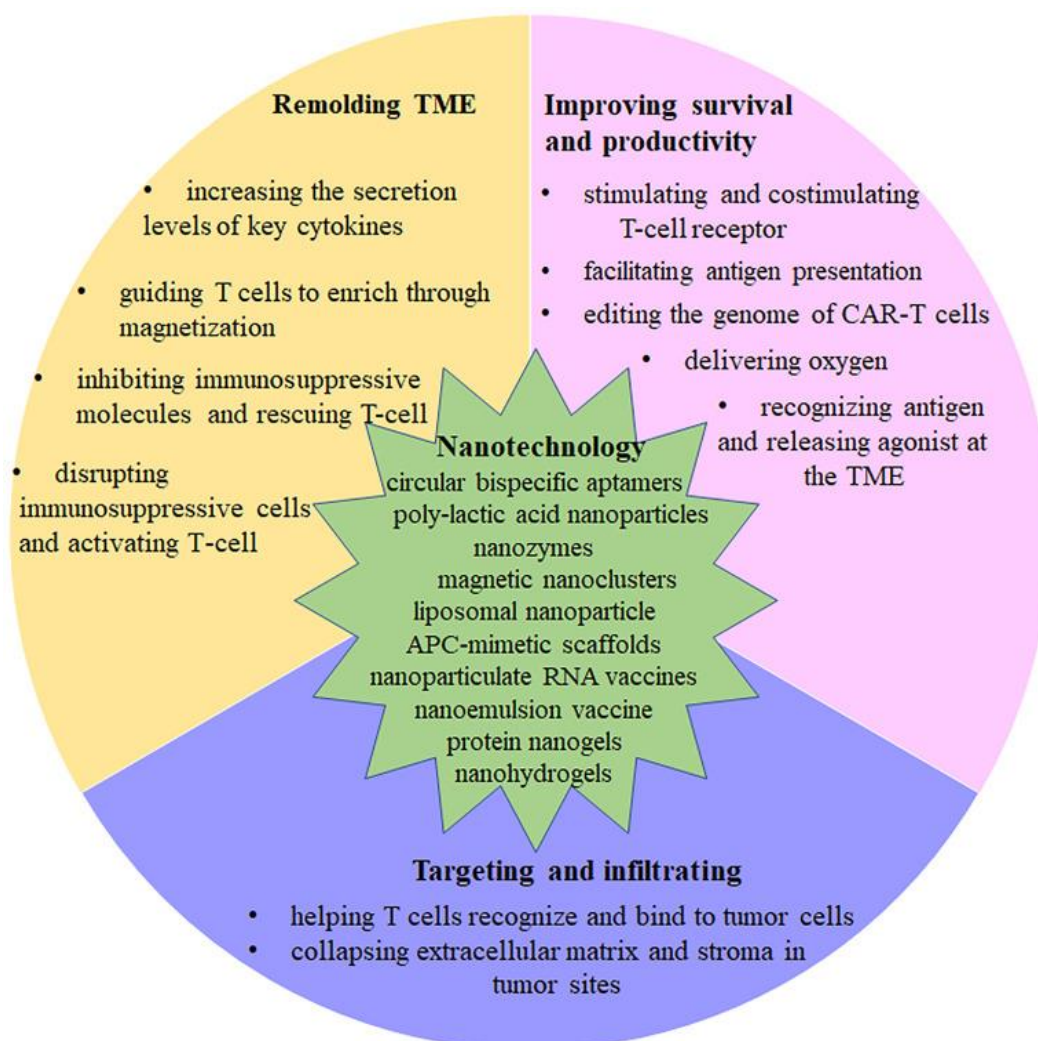


Figure 5. The main point of the fusion of nanotechnology and CAR-T [19]

3.2. Immunotherapy targeting malignant cells and macrophages

Tumor-associated macrophages (TAMs) cluster in the location where malignant cells are located, which is called the tumor microenvironment (TME). TAMs and TME are the symbol of low survival in cancer patients. Therefore, targeting TAMs or TME to evoke cancer cell killing through cellular phagocytosis have become a promising malignancy immunotherapy. Combining nanotechnology with this strategy has become an emerging trend to get rid of the drawbacks of this strategy, such as the lack of efficiency [20]. Zhao C et al. summarized different therapeutic pathways based on nanotechnology to modulate macrophage's function [21]:

- (1) Inhibiting macrophages recruit to tumor;
- (2) Depleting M2 –type-TAMs or reprogramming M2-type-TAMs to M1-type-TAMs;
- (3) Obstructing of the pathway of CD47-signal-regulatory protein alpha (CD47-SIRP α).

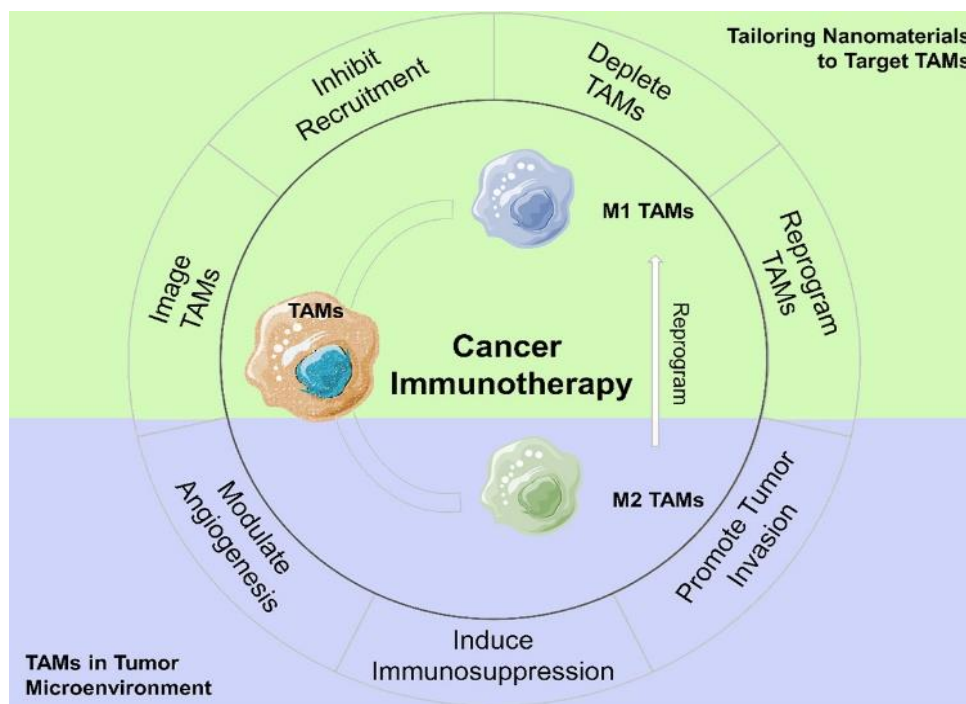


Figure 6. Different ways of modulating the function of TAMs with nanotechnology [21]

3.3. Immunotherapy using nano-system to deliver chemotherapeutic drugs

Chemotherapy is still the first choice for some types of tumors because of the economic consideration and clinical practicality. However, the decreased therapeutic efficacy and side effects have become the main roadblocks. The combination of chemotherapeutic drugs and nanotechnology to improve their concentration into the location of tumor shows great potential for anti-tumor treatment. For instance, Cao L et al. have designed a ROS-responsive nano-particles delivering system to activate cGAS-STING pathway, which is an essential pathway for intracellular immune response [22]. Furthermore, a mannose nano-gel (DM NGs) was developed by Ma X et al, which has shown excellent micellar stability, selective releasing chemotherapeutic drug and prolonged life span in breast-cancer mouse model [23]. Moreover, a novel mitochondria-targeted carbon-nanodots-based drug delivery system (TPP-CNDs-S-CPT) developed by Gong X et al. showed marvelous water dispersibility, superior fluorescence properties, satisfactory cell permeability, and favorable biocompatibility both *in vitro* and *in vivo* [24]. TPP-CNDs-S-CPT provides a promising future for the precise chemotherapeutic drug release [24].

3.4. Immunotherapy that regulates immune cell differentiation

Wnt/ β -catenin signaling is a highly invariable pathway to regulate cell proliferation, differentiation, apoptosis, stem cell-renewal, tissue homeostasis, and would coalescence [25]. Zhou Y et al. have summarized that abnormal activation of this signaling pathway benefits immune rejection and hinders T- cell mediated anti-cancer responses. Accordingly, the excitation of Wnt-pathway results in increased resistance to immunotherapies [25]. Therefore, Wnt/ β -catenin signaling pathway combined with nanotechnology is a possible strategy to treat cancer in the future. For instance, Mohapatra P et al. have repurposed a nanotechnology-based drug that target Wnt/ β -catenin signaling to treat liver carcinoma [26].

4. Conclusions

The emergence of nanotechnology in malignant tumors' immunotherapies has brought a reformation to personalized cancer treatments, providing clinicians and researchers an efficient and effective tool to elicit and strengthen patients' anti-tumor immune response. Nanoparticles are used to

make cancer therapeutic vaccines targeting T-cell, DCs, aiming to activate tumor antigen-specific lymphocytes. There are also many immunotherapies combined with nanotechnology, such as nano-based ACT, nano-based TAMs-targeting therapy. Furthermore, researches also try to use it as a chemotherapeutic drugs carrier, which can enhance the therapeutic effects.

The merits of nanotechnology using in immunotherapies includes their ability to be modified and functionalized [27]. Targeting transportation of nanoparticles to tumor sites straightway or mediate, can use the hyper-permeability of tumor vascular system, and decrease the side effects of anti-tumor drugs at the same time. However, despite the advantages of nanotechnology using in immunotherapies, some drawbacks of this strategy still need further study, including immunogenicity, non-targeted cellular uptake, cytotoxicity, and the accurate interactions from nanoparticles and patients' immune system. Moreover, the lack of evidences on immune system's role in the progress of cancer makes it is hard to transfer nanoparticle to clinical applications [27]. Simultaneously, there are also some key focus points from therapeutic views, such as the extent of vulnerabilities of NPs, reliable and monitored biomarkers, and the mechanisms of resistance [28].

To sum up, the emergence of nanotechnology in cancer immunotherapeutic will appeal more researchers to devote themselves before it can be converted into practical clinical applications maturely, such as the optimization of NPs products in *vitro* and in *vivo* animal model and non-invasive appropriate biomarkers to estimate early anti-tumor immune responses.

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