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Nanotechnology: Logical Approach in Cancer Immunotherapy

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ABSTRACT

Nanotechnology based drug delivery system has several advantages. This can be beneficial in cancer immunotherapy which is one of fatal diseases today. Recently immunotherapy has proven to best effective treatment today. Present review deals with several approaches, which based on nanotechnology and can be beneficial in cancer vaccine for the prevention and treatment of cancer. Immune system can inhibit oncogenesis by actively identifying and eliminating cancerous cells, by immune surveillance. Among different types of tumor antigens, oncoproteins, which are either mutated or over-expressed normal or embryonic proteins from fetal development, are intensively investigated for cancer vaccines. Direct administration of autologous DCs activated by tumor antigens ex-vivo may be an efficient approach for vaccination against cancers. Recent studies have demonstrated immunogenicity of dying cancer cells under certain chemotherapies or radiotherapy. Adaptive cell therapy for T-cells and immune checkpoint inhibition also use full in cancer immunotherapy.

INTRODUCTION

Nanotechnology is the study of extremely small structures, having size of 0.1 to 100 nm. Nano medicine is a relatively new field of science and technology. At present nano, medicine is very useful in severe diseases like CNS disorders, Cancer etc [1, 2]. Cancer is the name given for these diseases in which the body cells become abnormal and divide without control. Cancer cells may invade nearby tissues. And they may spread through the bloodstream and lymphatic control. Cancer cells may invade nearby tissues. And they may spread through the bloodstream and lymphatic characteristics are uncontrolled proliferation of the cells in the human body and ability of these cells to migrate from the original site and spread to distant sites (metastasis). If the spread is not controlled, cancer can result in death. The purpose of cancer vaccines is to stimulate the immune system to be able to recognize cancer cells as abnormal and destroy them. Some vaccines for particular cancers have been developed and are being tested to see whether they can treat cancer, or help to stop it from coming back after cancer treatment [3, 4]. Harashima*et al* describes, *ex-vivo* siRNA delivery to primary mouse bone marrow-derived dendritic cells (BMDCs) for potential use as a cancer vaccine. An exciting approach to cancer treatment has been offered by the progress in the development of nanoparticle drug delivery systems [5]. An exceptional characteristic of these nano systems lies in their capacity to stimulate the immune system, which could be the foundation for the design of a cancer vaccine [6-9]. Kranz *et al.* describe a vaccination strategy against cancer that targets existing tumours by recruiting immune mechanisms normally used against viral infection. They used nanoparticles carrying tumour RNA to simulate the intrusion of a viral pathogen into the bloodstream. When the nanoparticles reach lymphoid tissues, including the spleen and lymph nodes, they activate antiviral defense mechanisms in immune cells such as dendritic cells [10].

PRINCIPLES OF ADAPTIVE IMMUNITY AGAINST CANCER [11]:

Immune responses to foreign pathogens can be classified into two categories, namely innate and adaptive immunity. Innate immunity provides rapid defense against pathogens while adaptive immunity requires processing of pathogens by APCs, presentation of immunogenic antigen. To T cells and B cells, and elicitation of cellular and humoral immune responses. APCs play a pivotal role at the interface of innate and adaptive immune responses. In theory, immune system can inhibit oncogenesis by actively identifying and eliminating cancerous cells, a process referred to as immune surveillance. However, tumor cells have devised

mechanisms to evade immune responses, including down-regulation of tumor antigens and promotion of immunosuppression. Established tumor microenvironment is generally immunosuppressive due to upregulation of inhibitory molecules against T cells. Activated T cells up-regulate cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that binds to costimulatory molecules on DCs with higher affinity than CD28. Although CTLA-4 naturally serves as a peripheral inhibitory signal to prevent over-reactivity of T cells, it also dampens anti-tumor immune responses. Besides, subsets of tumor cells highly express programmed death-ligand 1 (PD-L1) that binds to programmed death-1 (PD-1) on T cells and inhibit their effector functions. Tumor cells can also secrete cytokines such as IL-10 and TGF-β, which both directly inhibit the proliferation of CTLs and drive the differentiation of T_{regs} that provide an additional source of these immunosuppressive cytokines. In addition to T_{regs}, tumor cells can recruit other inhibitory immune cells such as macrophages and myeloid-derived suppressor cells (MDSCs) to further dampen cytotoxic functions of CTLs. Thus, tumor cells can promote immunosuppressive tumor microenvironment and shield themselves from CTLs by hijacking normal negative feedback loops designed to guard against excessive activation of T cell responses.

NANOTECHNOLOGY BASED APPROACHES IN CANCER VACCINES [12-14]:

1. Nanotechnology Based Systems for Delivery of Tumor Antigens [15]: Tumor antigens can be categorized broadly into subunit antigens and whole-cell antigens. Subunit antigens include altered cell-surface polysaccharides, peptides, oncoproteins, and DNA and mRNA that encode those proteins, while tumor-cell lysate and immunogenically dying tumor cells can serve as the source of whole-cell antigens. Table 1 presents major advantages and challenges for each class of tumor antigens utilized for cancer vaccination.

Table 1. Major advantages and remaining challenges for tumor antigens

Tumor antigens		Advantages	Challenges
Subunit Antigens	Polysaccharides	Defined chemical synthesis	Elicitation of humoral rather than cellular immune responses
	Peptides	Ease of production Stable vaccine formulations May not require antigen-processing by APCs	Poor delivery efficiency Monovalent immune response Subject to HLA-specificity
	Proteins	Broad-epitope immune responses Wide HLA-specificity	Poor delivery efficiency Suboptimal for CD8+ T cell responses Weak immunogenicity of self-antigens
	DNA and mRNA	Ease of production *In-situ* expression of full-length antigens Flexible to encode immune stimulators	Poor delivery efficiency Poor *in-vivo* stability Limited transfection efficiency
Whole-cell Antigens	Tumor-cell lysate	Broad-epitope immune responses Potential for "personalized" therapy	Requires tissue biopsy Manufacturing challenges Loss of antigenicity during production Presence of self-antigens
	Immunogenically dying tumor cells	Broad-epitope immune responses Full preservation of tumor antigens Potential for "personalized" therapy	Requires additional therapeutic interventions Presence of self-antigens and immunosuppressive molecules, e.g., PD-L1

Some viruses (e.g., Epstein-Barr virus (EBV), human papillomavirus (HPV) and hepatitis B and C viruses) contribute to cancer development, and virally encoded gene products can also serve as the potential targets of immunotherapy [16]. Among different types of tumor antigens, oncoproteins, which are either mutated or over-expressed normal or embryonic proteins from fetal development, are intensively investigated for cancer vaccines due to their potential to elicit broad-epitope CD8+ and CD4+ T-cell responses. In comparison to full-length protein antigens that require cellular uptake and processing, peptide epitopes can directly bind to MHC molecules, and their stability is less affected during the preparation and storage of vaccine products. However, the major challenge facing cancer vaccination based on subunit antigens is their poor immunogenicity and limited therapeutic efficacy. In addition, conventional vaccine/adjuvant delivery systems have limited capability to target delivery of tumor antigens adjuvants to proper APCs and intracellular compartments.

1.1. Efficient Draining to Lymphoid Tissues [17]: Nanocarriers can improve the efficacy of subunit cancer vaccines by facilitating antigen presentation and T-cell activation. This is achieved by exploiting efficient draining of nano carriers to lymphoid tissues and their prolonged tissue residence as well as controlled release of antigens and adjuvants. Particle size is one of the primary factors determining the efficiency of lymphatic draining. Large particles (>500 nm in diameter) can be physically trapped at the injection site by interaction with extracellular matrix proteins, whereas ultra-small nanoparticles (<10 nm in diameter) or soluble antigen molecules can rapidly diffuse into and out of lymph nodes, thus minimizing the chance of APCs phagocytizing sufficient amount of vaccine particles. On the other hand, particles of an intermediate size (10–100 nm in diameter) can both efficiently drain to regional draining lymph nodes and become retained there, thereby increasing the chance of antigen uptake and presentation by APCs.

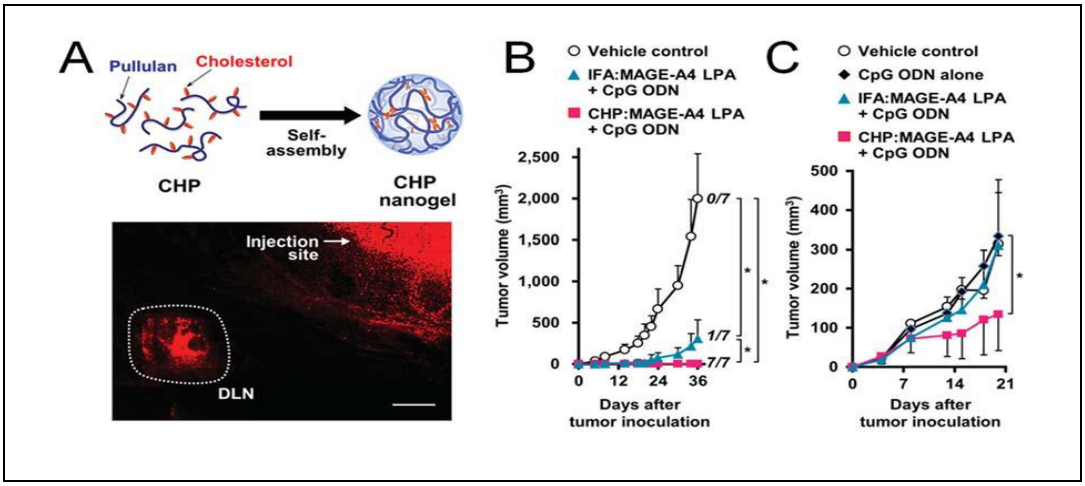

Figure 1. Anti-tumor efficacy improved by efficient lymphoid draining and retention of nanoparticle-based cancer vaccines. (A) Lymphoid draining of a fluorophore-labeled pullulan nano gel 6 h post subcutaneous injection to mice. Scale bar, 1mm. The nano gel loaded with a long peptide antigen (LPA) MAGE-A4 achieved better prophylactic (B) and therapeutic(C) efficacy compared to the soluble antigen; (B) Mice were immunized on day -7, followed by inoculation of tumor on day 0; (C) mice were inoculated with tumor on day 0, followed by immunization on day 4 and 11.

Furthermore, enhanced cytotoxic CD8+ T-cell responses and inhibition of tumor growth have been achieved by targeting nano vaccines to tumor-draining lymph nodes rather than non-tumour draining lymph nodes, suggesting that antigen-primed but immune-suppressed lymphoid tissues can serve as ideal sites of immune activation. These studies rely on various imaging techniques with nanoparticles labeled with fluorescent dyes or contrast agents to track and quantify lymphatic draining. For example, a polyester nanoparticle system loaded with ovalbumin (OVA) and labeled with a near-infrared probe has been utilized to demonstrate co-transport of the antigen and nanoparticles to draining lymph nodes. In an alternative approach, poly(lactic-co-glycolic acid) (PLGA) nanoparticles designed to carry iron oxide particles conjugated with fluorophore-labeled peptide antigen permitted bimodal tracking of the nanocarriers with MRI and fluorescent imaging. In addition, lymphatic draining of particulate vaccines also depends on the material composition, morphology, and surface chemistry of particles.

1.2. Targeted Delivery to Dendritic Cells[18]: Once in contact with immune cells, tumor antigens have to be engulfed and processed by APCs, preferably DCs, to activate adaptive immune responses. Therefore, vaccine delivery targeted to DCs may be beneficial.

Nanoparticles encapsulating tumor antigens can also be modified with targeting moieties on their surfaces to achieve DC-specific delivery. Such delivery systems specifically targeted to DEC-205, DC-SIGN, mannose receptor, Fc receptor, CD40, or CD11c have been reported. Although these systems have been shown to induce stronger DC activation, compared with their non-targeted counterparts, it remains to be determined whether a particular targeting ligand is optimal for the DC-targeting approach. Interestingly, despite differential levels of DC-targeting, these various nano vaccine formulations induced similar levels of CD8+ T cell responses. It should be noted that the extent of DC-targeting, particle uptake, and subsequent immune activation depends on specific physicochemical properties of the nanocarrier itself as well as adjuvants employed in the vaccine system. In particular, different DC subsets have distinctive sites of tissue residence, receptor expression profiles, and functions, and nano vaccines designed to target DC subsets with high efficiency of antigen cross-presentation.

1.3. Promotion of Cross-Presentation[19]: Extracellular antigens are usually processed and presented via MHC-II by APCs to CD4+ T cells; however, tumor antigens engulfed by APCs need to be presented via MHC-I to activate CTLs, which are the main effector cells against tumor cells. Thus, traditional vaccine approaches relying on soluble protein or peptide tumor antigens may skew immune responses to CD4+ T cell responses while failing to induce sufficient CTL responses. In contrast, tumor antigens delivered by functional nanomaterials designed to promote endosomal escape (*i.e.*, translocation of antigens from endosomes/phagosomes to cytosol) may induce cross-presentation and favorably elicit CD8+ T cell responses. To this end, extensive research efforts have been focused on pH-sensitive delivery systems that can retain their cargo under the physiological pH condition while triggering release of antigens and disruption of endocytic vacuoles at the acidic (~pH 6) endosomal microenvironment. For example, a liposomal antigen delivery system modified with a pH-sensitive dextran derivative has been shown to promote cytosolic delivery of antigens. In addition, a micellar system composed of an amphiphilic polymer with a pH-sensitive building block forming the particle core has been devised to induce fusion of the nanomaterials to endosomal vesicles, thus transporting protein antigen surface-displayed on micelles from endosome to cytosol and promoting antigen cross-presentation and CD8+ T cell responses. An alternative approach includes an oxidation-sensitive polymer some that can respond to the oxidative environment of endosomes and deliver antigens and adjuvants to cytosol. Additionally, liposomes modified with a cell-penetrating peptide octa-arginine or gold nanoparticles displaying tumor antigens were also shown to promote cross-presentation.

1.4. Co-Delivery of Adjuvants[20]: Another major advantage of nanoparticle delivery systems lies in their ability to co-deliver antigens together with adjuvants, thereby enhancing cross-presentation and/or skewing immune responses to desired CD4+ T helper phenotypes. Agonists for Toll-like receptors (TLRs) have been widely investigated as adjuvants for cancer vaccines. Although TLRs are mainly involved in innate immunity by sensing pathogenic danger signals, they are crucial for induction of adaptive immune responses as they can promote cross-presentation in APCs to activate CD8+ T cells or prime APCs to release cytokines that can polarize CD4+ TH cells to specific phenotypes. Since the TH1 responses elicited by activation of TLR3, TLR7, or TLR9 contribute to CD8+ T cell responses, agonists of these TLRs have been widely examined for cancer nano vaccines. CpG, which is an unmethylated oligonucleotide containing CpG motif, is a potent TLR9 agonist. CpG has been complexed with cationic polymers via the electrostatic interaction or conjugated with nanocarriers, which improved immune activation compared with administration of free soluble adjuvant **(Figure 2)**. The charge-mediated entrapment was also exploited to co-load an anionic TLR3 agonist poly I:C and cationic antigen peptides onto gold nanoparticles via the "layer-by-layer" strategy, leading to elicitation of robust antigen-specific CD8+ T cells when tested with a model antigen *in-vivo* **(Figure 2)**.

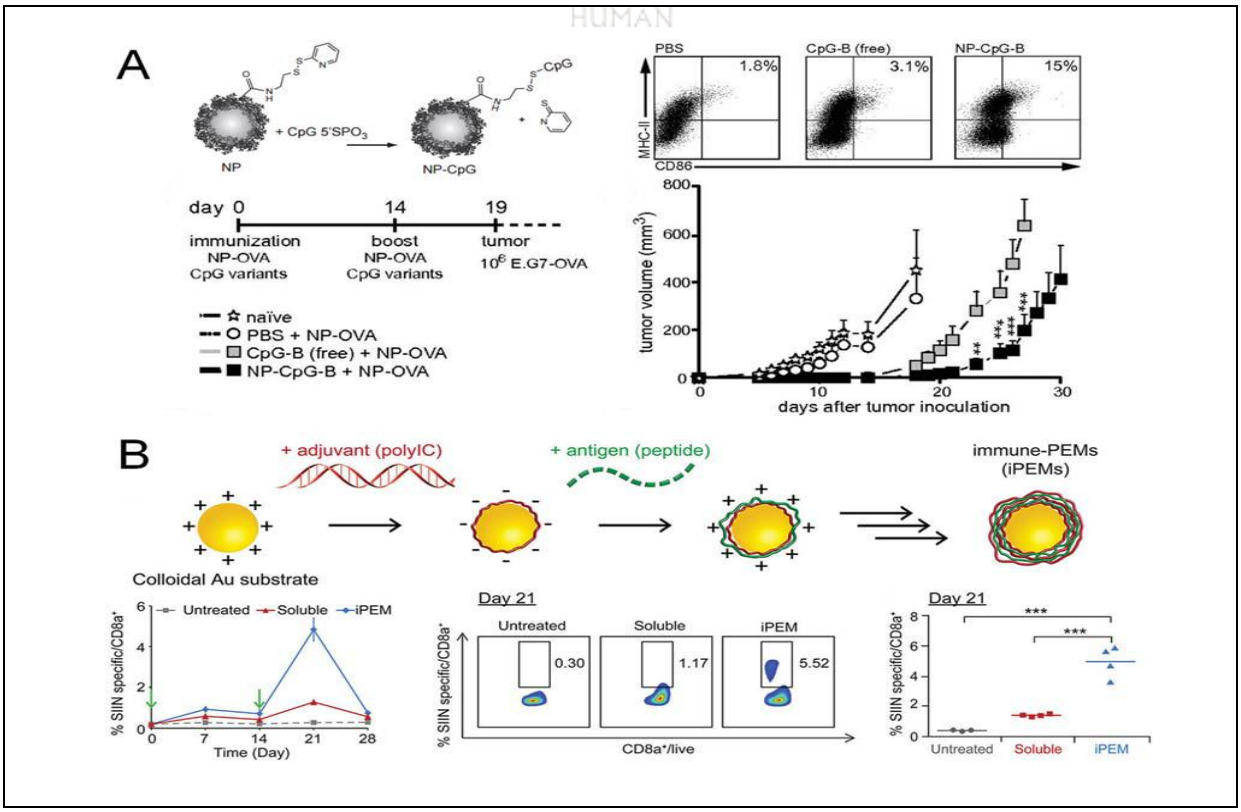

Figure 2. Co-delivery of antigens and adjuvants by nanoparticles. (A) TLR9 agonist CpG was conjugated on the surface of polymeric nanoparticles via disulfide exchange. The particulate adjuvant improved DC activation *in-vitro* as well as prophylactic efficacy against tumour *in-vivo*, compared with the soluble CpG; (B) TLR3 agonist poly I:C and an antigen peptide were complexed onto gold nanoparticles via electrostatic interactions, and elicited more antigen-specific CD8+ T cells compared with the soluble vaccine.

In addition to efficient loading of adjuvants, co-entrapment of an antigen and adjuvant within the same particles can also enhance the efficiency of cross-presentation and induction of CD8+ T cells, compared with soluble vaccine components admixed together. Moreover, nanoparticles designed for multifaceted drug loading can support a combinational use of adjuvants, thus permitting exploitation of synergy among certain TLR agonists. For example, CpG and poly I:C have been co-loaded into polyester nanoparticles, while the TLR4 agonist glucopyranosyl lipid A and TLR7 agonist imiquimod have been co-encapsulated into liposomes. In both cases, the TH1 response was significantly improved by the dual TLR agonists-loaded particles, compared with that elicited by a single adjuvant. Alternatively, TLR agonists can be combined with siRNAs inhibiting the immunosuppressive pathways. Co-delivery of CpG and siRNA targeting IL-10, the inducer of TH2 and T_{reg} cells, skewed immune responses to the TH1 type. The combination of peptide epitope of tyrosine-related protein 2 (Trp2) and CpG-based nano vaccine with siRNA against TGF-β, which is one of the major cytokines responsible for induction and maintenance of immunosuppressive tumor microenvironment, has significantly improved the therapeutic efficacy of nanoparticle-based cancer vaccine in a late-stage murine melanoma model.

1.5. Delivery of DNA and mRNA Tumor Antigens[21]: DNA and mRNA encoding oncogenic proteins or peptides are appealing tumor antigens due to the ease of manufacturing scale-up and their potential for further modification with nucleic acid sequences that encode for proteins with immunostimulatory functions (e.g., flagellin). However, previous clinical trials on DNA cancer vaccines, majority of which were administered as naked DNA via the intramuscular route, showed generally poor response rates. Although viral vectors and electroporation have been employed to improve the transfection of DNA vaccines, they are subject to safety and compliance issues. Alternatively, synthetic delivery systems can be used to deliver DNA and mRNA therapeutics due to several advantages: (1) synthetic materials such as cationic lipids and polymers are safer alternatives to viral vectors; (2) gene

therapeutics can be stabilized and protected from nuclease-mediated degradation by particulate carriers, and DNA and RNA have also been designed to self-assemble into distinctive nanostructures with improved colloidal stability; (3) injection-free gene delivery routes can be exploited with DNA- and RNA-loaded nanocarriers, such as microneedles, pH-sensitive polymeric nanoparticles, or lipoplexes for non-parenteral routes of delivery; and (4) nanocarriers can be modified with targeting moieties, e.g., mannose, to achieve DC-targeted delivery and transfection.

1.6. Delivery of Whole-Cell Cancer Vaccines [22]: Compared with a single peptide or protein antigen, whole-cell cancer vaccines may elicit multivalent immune responses by broadening epitope recognition and help to realize personalized immunotherapy. Whole-cell antigen can be obtained from tumor cell lysates with necrotic features or inactivated whole tumor cells with apoptotic features. Similar to subunit vaccine nano carriers, tumor cell lysates and TLR agonists have been co-encapsulated into particulate delivery systems, including liposomes or PLGA micro/nanoparticles. Whole-cell cancer vaccine has also been delivered by a biodegradable, "infection-mimicking" PLGA matrix containing tumor lysate as the source of tumor antigens, granulocyte macrophage colony-stimulating factor (GM-CSF) for recruitment of DCs *in-situ*, and CpG for activation of recruited DCs. This PLGA matrix-based whole-cell cancer vaccine successfully elicited antigen specific CD8+ T cells and improved both prophylactic and therapeutic anti-tumor efficacy, compared with a conventional whole-cell vaccine GVAX, composed of irradiated, GM-CSF-secreting tumor cells. In an alternative approach, plasma membrane of tumor cells has been extracted and coated on to polymeric nanoparticle cores along with the TLR4 agonist MPLA as a tumor cell-mimicking cancer vaccine.

2. Nanotechnology based Delivery Systems for DC-Based Cancer Vaccines[23]: Direct administration of autologous DCs activated by tumor antigens *ex-vivo* may be an efficient approach for vaccination against cancers. One study has employed antigen-loaded poly(γ-glutamic acid) nanoparticles to show that DCs activated by these particles released TH1 cytokines, elicited robust T-cell activation *in-vitro*, and enhanced protection against tumor challenge in mice. In another approach, antigen delivery by porous silica particles induced secretion of type I IFN cytokine from DCs, leading to reduced tumor growth in both therapeutic and prophylactic conditions. The benefits of multi-drug loading within nanoparticles were also demonstrated in a Tri Mix delivery system: the mixture of antigen

and adjuvant mRNAs was encapsulated in cationic liposomes which were then conjugated to micro bubbles to allow ultrasound-triggered transfection of DCs. DCs activated by this strategy exhibited enhanced therapeutic efficacy against established tumors when compared with DCs primed with antigen mRNA with or without lipopolysaccharide (LPS).There has been increasing interest in artificial APCs (aAPCs) surface-decorated with covalently conjugated tumor antigen/MHC complex and anti-CD28 antibody. The rationale is that direct activation of antigen-specific T cells by aAPCs will obviate the need for antigen delivery to APCs and antigen processing and presentation, while also avoiding potential activation of immune checkpoint molecules, such as CTLA-4, expressed on T cells via the use of agonist antibodies directed toward co-stimulatory pathways. Indeed, various particle platforms have been explored for aAPCs, including PLGA microparticles, liposomes, iron/dextran nanoparticles, and carbon nanotubes. In particular, aAPCs composed of an iron nanoparticle core and stimulatory molecules on the dextran shell was shown to induce T-cell receptor clustering when incubated with T cells under magnetic field, thus allowing external stimulus-induced proliferation of antigen-specific T cells *in-vitro* and *in-vivo*.

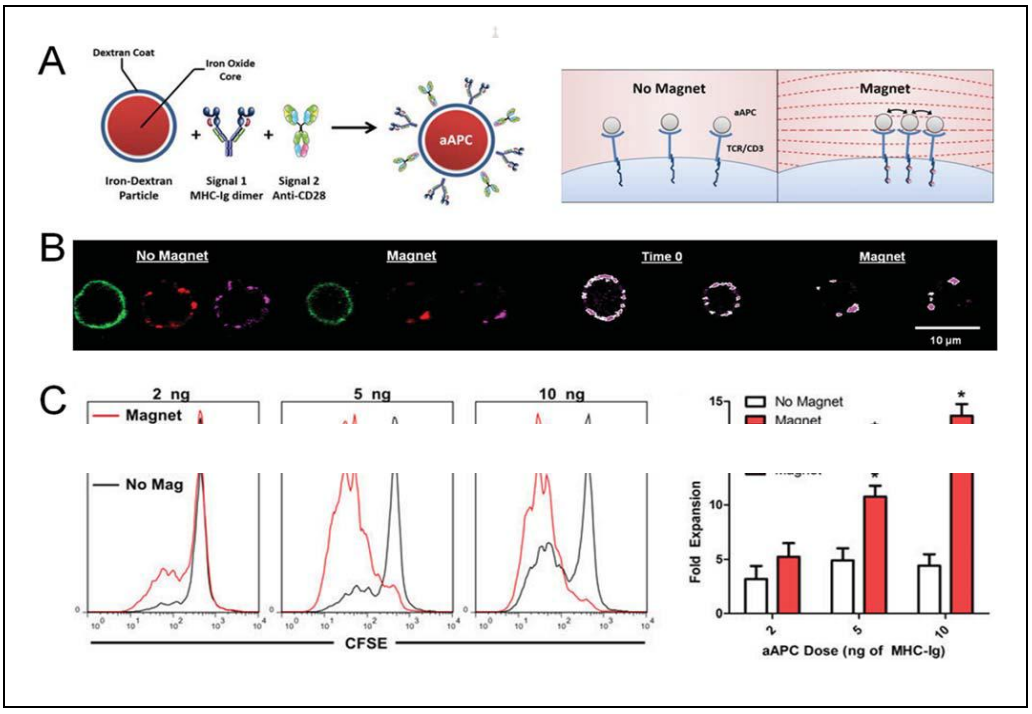

Figure 3. Artificial antigen-presenting cells (aAPCs) for activation of T cells. (A) aAPCs composed of an iron nanoparticle core and dextran shell conjugated with stimulatory molecules induced clustering of T-cell receptors (TCRs) under magnetic field; (B) TCR clustering was visualized by fluorescence imaging. Green, lymphocyte marker on T cells;

red: aAPCs; magenta: CD3ε on T cells; (C) Proliferation of T cells was enhanced by aAPC induced TCR clustering *in-vitro*.

Moreover, carbon nanotubes loaded with activation signals have been also developed as aAPCs to expand antigen-specific CD8+ T cells, which were then successfully used for adoptive T cell therapy. Interestingly, recent studies have revealed that ellipsoidal PLGA nano/micro particles were more efficient aAPCs than their spherical counterparts, demonstrating that biophysical parameters of aAPCs may play a crucial role in induction of T cell responses.

3. Nanotechnology based Delivery Approaches to Induce Immunogenic Cell Death (ICD) [24]: Recent studies have demonstrated immunogenicity of dying cancer cells under certain chemotherapies or radiotherapy. Although systemic administration of chemotherapeutics is generally immunosuppressive, *in-situ* treatments with certain chemo drugs especially anthracyclines, such as doxorubicin and mitoxantrone, have been shown to induce ICD. In addition, the abscopal effect observed during radiotherapy, *i.e.*, regression of distant, non-irradiated tumors, is also believed to be caused by systemic immune responses elicited by dying primary tumor cells. Since the initial discovery of ICD, anthracycline chemo drugs have been investigated widely for immune-mediated antitumor efficacy in addition to their direct tumor-killing effects, especially in combination with other cancer immunotherapies, such as vaccines, adoptive cell transfer, and checkpoint inhibitors. Indeed, ICD suggests an alternative approach for whole-cell vaccination based on *ex-vivo* generated immunogenically dying tumor cells or induction of ICD *in-situ*. In addition, co-delivery of adjuvants with ICD inducers may be helpful to potentiate anti-tumor immune responses, thus motivating the use of adjuvant-carrying particulate delivery systems for further enhancing ICD. For example, PLGA microparticles have been employed to encapsulate doxorubicin and CpG and intratumorally injected for induction of ICD. In addition, doxorubicin-based *in-situ* vaccination strategy combined with anti-CTLA-4 and anti-OX40, an agonistic antibody against the stimulatory checkpoint molecule OX40, has been shown to improve T cell infiltration into distant tumors, leading to tumor eradication and increased survival.

4. Nanotechnology based Delivery Systems Targeted to Immune Checkpoints [25, 26]: Cancer immunotherapy aiming to reverse immunosuppression has achieved striking success in recent years. The CTLA-4 inhibitory antibody Ipilimumab has improved the survival of patients with advanced, untreatable melanoma by 3.7 months, and gained FDA-approval as a

new category of cancer immunotherapy. However, treatment with Ipilimumab was also accompanied by adverse events and moderate response rates. The PD-1 inhibitory antibodies Nivolumab and Pembrolizumab were also approved for the treatment of malignant melanoma in late 2014. PD-1 is now considered a better target than CTLA-4 because antibody-mediated blockade of PD-1 among tumor-infiltrating T cells within the tumor microenvironment leads to mitigated side effects and higher response rates, especially among patients with PD-L1 positive tumors. In addition, dual inhibition of CTLA-4and PD-1 recently has been shown to be more efficacious than a single therapy, mainly due to their distinctive mechanisms of action: the main sites of action for antibodies against CTLA-4 and PD-1 are thought to be within lymphoid tissues and tumor regions, respectively. However, the current systemic administration route for these therapeutic antibodies may still cause off-target toxicity. Alternatively, targeted delivery of siRNA against PD-L1 has also been investigated with cationic lipoid and polymeric nanoparticles. PD-L1 expressed on cancer cells were efficiently silenced by siRNA complexed with folic acid-modified polyethyleneimine, resulting in enhanced *in-vitro* T-cell activation. In addition to immune checkpoint inhibitors covered above, there are also stimulatory checkpoint targets, such as OX40 (CD134) and 4-1BB (CD137), which can be activated to improve anti-tumor immunity. Both molecules belong to the receptor family of tumor necrosis factor (TNF) and directly induce T-cell activation. Ligation of OX40 on T cells with its ligand on APCs results in activation of both CD4+ and CD8+ T cells, leading to inhibition of tumor growth. Notably, expansion of T_{regs} following activation of OX40 remains a controversial topic. Since all CD4+ T cell subtypes can be activated by the OX40 pathway, it is likely that induction of T_{regs} depends on the particular polarizing cytokine milieus that the cells are exposed to. In contrast to OX40, 4-1BB preferentially activates CD8+ rather than CD4+ T cells. 4-1BB is upregulated as a surrogate for CD28 which cannot compete against CTLA-4 in binding to co-stimulatory molecules during the late or secondary immune response. Nanoparticle delivery systems have been developed for this antibody therapeutics, aiming to mimic the natural immune activation by antibodies surface-displayed on particles or to reduce systemic toxicity by localized administration. Anti-OX40 antibody was conjugated to the surfaces of PLGA nano particles via EDC/NHS chemistry and promoted antigen-specific killing by CD8+T cells *in-vitro*. In another combinational therapeutic approach, anti-4-1BB antibody and IL-2 were separately displayed on liposomal surfaces for localized tumor therapy, inducing robust antitumor CTL responses, while minimizing off-target side effects and preventing cytokine storm typically observed after systemic administration of immune therapeutics.

5. Nanotechnology based Delivery Systems for Adoptively Transferred T Cells [27]: Adoptive cell therapy (ACT), based on autologous T cells expanded with tumor antigens and IL-2 *ex-vivo*, is envisioned to induce tumor regression as a "live drug". However, this approach is limited by moderate responses, due to insufficient expansion of transferred T cells and inefficient trafficking to tumor regions, as well as potential severe side effects characterized by "cytokine storm" with TNF, IFN-γ and IL-6 when IL-2 is systemically co-administered with T cells. To address these limitations, nanoparticle-based cellular engineering approaches have been examined to improve the therapeutic efficacy of ACT. Maleimide-modified synthetic nanoparticles were conjugated to the surfaces of CD8+ T cells via sulfhydryl groups exposed by cell-surface proteins. T-cell stimulating cytokine complexes, IL-15/IL-15Ra and IL-21, were encapsulated into nanoparticles to provide signals for T cell expansion *in-situ*. This strategy resulted in potent proliferation of transferred T cells and eradicated metastatic melanoma tumors in mice, whereas co-administration of T cells mixed with those cytokines failed to eliminate tumors. Tumor-specific T cells "equipped" with small-molecule inhibitors against T-cell exhaustion using the same method also showed therapeutic benefits. In an alternative strategy, *ex-vivo* expanded antigen-specific CD8+ T cells and T-cell activating particles were co-delivered via an implantable biodegradable hydrogel system to treat residual tumors as well as metastases in murine breast cancer models. Within the local implant, lipid-coated mesoporous silica particles encapsulating IL-15 and IL-15Ra while displaying stimulatory antibodies against CD3, CD28, andCD137 efficiently activated T cells, which were gradually released from the depot and more efficacious than systemic or local administration of T cells without stimulatory signals. In addition to CTL-mediated tumor-specific killing, T cells also have been utilized to shuttle therapeutics to diseased tissues. As shown by a recent study on a metastatic murine lymphoma model, autologous polyclonal T cells with the potential to home to tumor-bearing lymphoid tissues were primed *ex-vivo* and conjugated with chemo drug-loaded nanoparticles on the cell surfaces **(Figure 5A)**. This "Trojan horse" approach exploiting innate tropism of T cells to lymphoid tissues allowed selective delivery of the chemotherapeutic to disseminated lymphoma tumors, with 90-fold greater concentration of the drug accumulated in lymph nodes than free drug systemically injected at 10-fold higher doses **(Figure 5B)**. This T cell-mediated delivery significantly reduced tumor burden and improved survival, compared with administration of free drug or drug-loaded nanoparticles alone.

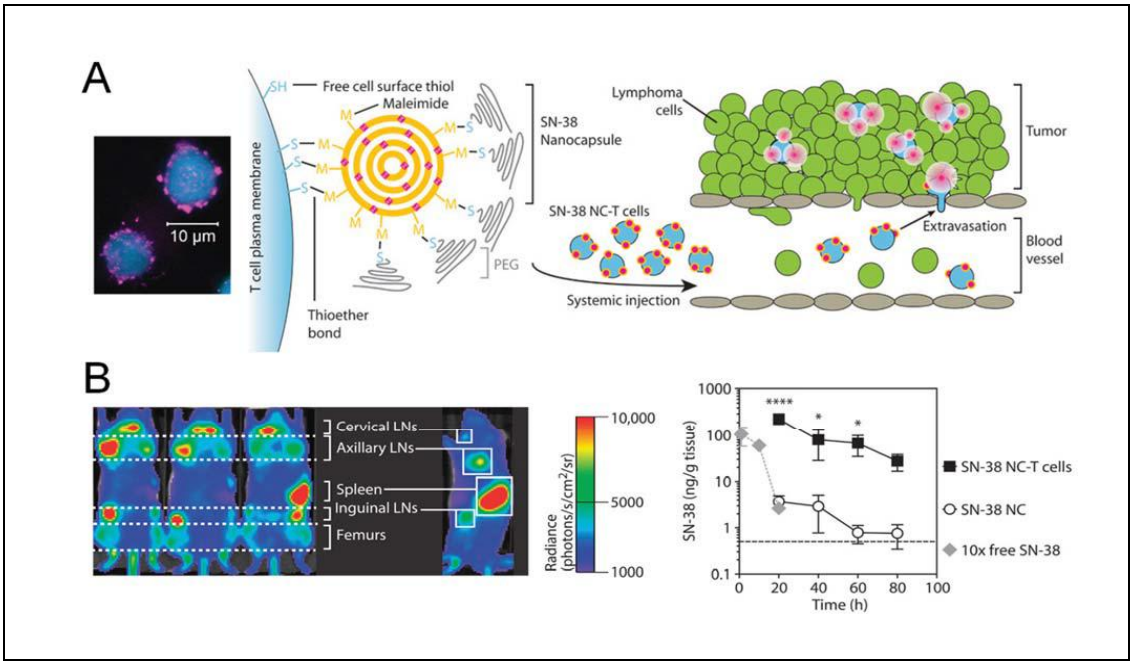

Figure 5. Engineered T cells for cancer therapy. (A) T cells (blue) conjugated with nanoparticles (magenta) loaded with a chemo drug (SN-38) were used for drug delivery to lymphoma; (B) T cells with surface-bound chemo drug preferentially accumulated in tumor-bearing lymphoid tissues following systemic administration, and significantly improved the drug distribution in lymph nodes, compared with equivalent or 10-fold higher dose of the nanoparticulate or soluble drug, respectively.

CONCLUSION

Cancer is one of the most life threatening diseases today, there is no much effective treatment because of lack of specificity. Immunotherapy can be a best approach**.** On the basis of present study, it was concluded, nanotechnology based systems are sounds much beneficial in cancer immunotherapy. At present, there are many researchers shows their interest on this topic. There are several approaches for vaccine delivery through nanotechnology. Such type's vaccines can be beneficial in future for prophylaxis as well as to cure cancer.

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