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# Carbon nanotubes as vaccine scaffolds

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## Abstract

Carbon nanotubes display characteristics that are potentially useful in their development as scaffolds for vaccine compositions. These features include stability *in vivo*, lack of intrinsic immunogenicity, low toxicity, and the ability to be appended with multiple copies of antigens. In addition, the particulate nature of carbon nanotubes and their unusual properties of rapid entry into antigen-presenting cells, such as dendritic cells, make them especially useful as carriers of antigens. Early attempts demonstrating carbon nanotube-based vaccines can be used in both infectious disease settings and cancer are promising.

## 1.0 Introduction

Vaccines are drugs designed to elicit a specific, active immune response in a host that will prevent a disease from starting, or lessen the effects of a disease after it has begun. In the field of infectious diseases, nearly all vaccines are administered prophylactically. Such approaches have provided some of the most important advances in human health over the last century. Many attempts also have been made to generate “therapeutic” cancer vaccines for patients who already have a cancer, which could be used to reduce tumor burden, or prevent or slow recurrence of cancers. No such effective specific cancer vaccines are marketed in the USA today.

The immune system is designed to recognize and react with foreign antigens and vaccines are drugs that are engineered to mimic these foreign molecules, so as to direct the specific immune response for therapeutic purposes. Antigens presented by microbes and bacteria are typically more potently immunogenic than self-antigens derived form cancers. In cancer vaccines, the antigens are not foreign, and are usually over-expressed proteins or sugars on the surface or inside the tumor cell. Intracellular proteins from microbes, viruses or cancers can be presented on the cell surface in the context of MHC molecules for T cell recognition[1, 2]. Presentation by dendritic cells (DCs), which are the most effective antigen presenting cells (APCs)[3, 4], drives the initiation of a strong response. Self-antigens are generally weakly immunogenic and the immune system of animals or patients with cancer either does not recognize the antigen, is tolerant to it, or can not mount an adequate cytocidal response[5, 6]. Soluble molecules are usually weak immunogens; therefore, delivery formulations that include carrier molecules and adjuvants can make more effective immunization strategies. Adjuvants promote a more potent immune response, such as



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cytokines[7], saponins, CpG motifs[8], and heat shock proteins[9]. Synthetic and natural materials, such as dendrimers or keyhole limpet hemocyanin (KLH), have been used as carrier molecules[10, 11].

Vaccine carriers that efficiently deliver the antigens into professional antigen presenting cells such as dendritic cells, would be most useful, as presentation of peptide antigens by the MHC molecules of dendritic cells (APC's) is essential to mounting a potent immune response. Particulate vaccines are a promising approach to improve the immunogenicity of proteins, as the particles improve immune responses. Interestingly, the response is highest when the particles are on the nano-scale, which may relate to the ability of cells to best interact with the vaccine particle of this size[12,13,14]. Nanoparticulate formulations also can serve as an extracellular or intracellular depot of antigen, which prolongs immune activation.

Carbon nanotubes (CNT) offer a number of features that make them interesting candidate materials for vaccine compositions, as a scaffold to carry the specific antigenic target and to facilitate its presentation to the immune system. CNT are relatively inert and non-immunogenic and non-toxic[15-18] by themselves. They are highly stable on the shelf and in vivo. Their unique structures allow highly efficient conjugation of many antigens, simultaneously, and to multiple different antigens at once, to their surfaces. Interestingly, 100% of the structure of single wall CNT is surface atoms. In addition, CNT can be made particulate and insoluble, thereby prolonging effects *in vivo* as a depot, and promoting engulfment by phagocytic cells involved in the generation of the immune response. Finally, CNT have the interesting property of rapid entry into cells, including dendritic cells, which are essential to the stimulation of effective immune responses[19-21].

In this review we discuss various approaches to vaccine development, the current methods to isolate and purify CNT for this purpose, methods to covalently functionalize them with biologically active molecules such as protein antigens, and the early attempt to make vaccines with CNT. The biomedical applications of CNT, including their use as carries for antigens, as immune stimulants, or as inflammatory molecules, have been reviewed several times recently or in this issue[15, 18, 22-24]. We encourage readers to seek these papers for a broader look at the biomedical applications of CNT.

## 2.0 Vaccines and adjuvants

Development and application of vaccines against pathogens have achieved a significant success in controlling and preventing life-threatening infectious diseases in the past century[25,26]. Similarly, the development of cancer vaccines has been of intense research interest in the past few decades and has provided approaches to adjuvant therapy in conjunction with other anti-cancer therapies[27]. Vaccines normally consist of three components. The first component is represented by one or more specific antigens that can be encoded by DNAs, or are peptides and proteins, or carbohydrates, derived from immune-dominant epitopes identified in pathogens or cancer cells. Upon vaccination, these specific antigens are able to generate specific and long-lasting immune responses against the host cells, whereby destroying either pathogens or cancer cells. The second component of vaccines, which is not always necessary, is a carrier. This is a scaffold, which may be immunogenic on its own and that is used to deliver the antigen to appropriate cells in vivo or retain it at a site. The third important component of vaccines is the adjuvant, which is required for effective vaccine delivery and for inducing robust inflammatory responses.

Effective adjuvants act through multiple mechanisms, including the generation of antigen depots, activation of antigen-presenting cells (APCs) via pattern recognition receptors

(PRRs) such as toll-like receptors (TLRs) and enhancing the presentation of vaccine antigens by APCs[28,29]. In addition to adjuvants with biological functions, the carrier delivery systems included in the vaccine formulation can also help to achieve desired targeting ability, depot and inflammation[30]. Adjuvants can be generally divided into two categories: immune-stimulatory molecules and antigen delivery vehicles. However, they can exhibit both characteristics simultaneously. Vaccines and adjuvants used in earlier studies are either attenuated pathogens (for example: Coley's toxins) or adjuvants that are the mixtures of bacterial walls and mineral oil, such as complete or incomplete Freund's adjuvants. Although it had been a wide-spread practice for immunologists to use adjuvants together with specific vaccines to generate effective immune responses, the mechanisms of their action were poorly understood. The discovery of the TLRs in 1990s, significantly increased the understating of how adjuvants stimulate innate immunity and bridge it to adaptive immunity. Recent advances in nanotechnology have also made it possible for specific, effective and controlled delivery of the vaccines to immune system[30].

## 2.1 Immuno-stimulatory adjuvants

**2.1.1 TLR agonists—**The immune system recognizes pathogen-associated molecular patterns (PAMPs) expressed by wide variety of infectious microorganisms, by PRRs, which include TLRs, NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs). The use of TLR agonists as adjuvants is based on the knowledge that several TLR ligands are known as PAMPs, and engagement of receptor-ligands activate DCs, macrophages and other innate immune systems that express TLRs on their cell surface or in their intracellular endosomes. Activation of DCs and macrophages results in enhanced phagocytosis of antigens, cytokine production, and up-regulation of co-stimulatory molecules. Subsequently, the host mounts an adaptive immune response, characterized by the expansion of antigen-specific T and B cells, activation of T helper and cytotoxic T cells and production of antibodies, that insure long-lasting immunological memory to protect against infection and cancers. Twelve TLRs and their ligands have been identified in humans[31]. For example: TLR-3 binds to double strand RNA (dsRNA) in virus, which has been known to induce type I interferons, enhances antigen presentation and the cytotoxicity of NK and T cells. The synthetic dsRNA polyinosinic-polycytidylic acid (Poly I:C) has been tested in pre-clinical studies and in numbers of human clinical trials as an adjuvant to anti-cancer vaccines[32,33]. TLR4 binds to bacteria lipopolysaccharide (LPS), which is a potent non-specific immunostimulator and is presented in various adjuvants. TLR5 binds to flagellin [34] and TLR9 to unmethylated CPG oligodeoxynucleotide bacterial DNA. TLR-9 agonist CPG-OD represents the most studied and advanced adjuvant candidate[35]. When used as an adjuvant, CPG motifs stimulate cells that express TLR-9, primarily plasmacytoid DC and B cells, to produce Th1 cytokines, enhances antigen presentation and induction of long-lasting CD8 T cells and Ab production. CPG-ODN has been evaluated as adjuvant in a wide variety of preclinical models and in human clinical trials in both infectious diseases and cancer vaccine therapies[35-40].

**2.1.2 Cytokines—**Cytokines produced by immune cells are important immune-modulators in both innate and adaptive immune responses. Therefore, a number of cytokines have been used as adjuvants to enhance vaccine efficacy[41]. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is the most widely used adjuvant in human trials, primarily because it can activate macrophages and induce DC differentiation, thus to help initiating an effective Ag-specific immune response. Some reports showed that GM-CSF may induce myeloid suppressor cells, however, the vast majority of studies have shown that GM-CSF enhances cancer vaccine-induced immune responses, when used as an adjuvant[42,43]. We have evaluated the effects of GM-CSF in MHC class I peptide vaccines derived from BCR.ABL in a mouse model, in combination with TiterMax adjuvant. The mice immunized with peptide/TiterMax plus GM-CSF showed stronger IFN-γ secretion by CD8 T cells than

the group without GM-CSF (unpublished). In addition, type I IFNs (IFN-α/β) are pleoitropic innate cytokines that are secreted in response to viral infection. IFN–α/β can up-regulate MHC class I and II expression, induce Th1 T cell helper responses and activate NK cells and CD8 T cell cytotoxicity and therefore, can be the most powerful natural adjuvants of the immune system. IFN-α has successfully been shown to improve the protective immunity of peptide and vector-based vaccines in experimental models. When type I IFNs were used an adjuvant in clinical trials in patients with cancers, both immunological and clinical responses were observed[36,44]. In addition, interleukin -2 and -12 (IL-2 and IL-12) have also been used as adjuvants in cancer therapy, as both cytokines are potent stimulators for cytotoxicity of CD8 T cells. However, the cytokines have short half-life *in vivo*, and cause toxicity if administered repeatedly and systemically[45-47].

**2.1.3 Other adjuvants—**QS-21 is a saponin-derived soluble adjuvant. It interacts with cholesterol of cell membrane leading to pore-formation on the membranes thereby enhancing antigen-uptake. QS-21 has been shown to enhance both cellular and humoral immune responses and has been used as an adjuvant in a number of clinical trials, in cancer vaccines including chronic myeloid leukemia (CML), breast, prostate and melanoma, as well as in infectious diseases, such as malaria, influenza and hepatitis B[45]. However, it can cause pain at injection sites and hemolysis, which limits its use. Interestingly, in our work, when mice were immunized with class I peptide vaccines with QS-21 via footpads, the CD8 T cell response was significantly weaker, compared to the vaccines that included TiterMax (unpublished). This study suggests that better uptake of the antigens is not sufficient; the antigenic depot effect and immune-stimulatory effects provided by TiterMax are also required for inducing a robust vaccine-induced cellular immune response. In addition, aluminum salt-based adjuvants (alum) have been used in many vaccine formulation and are the most widely used adjuvants in humans, including hepatitis B virus (HBV), hepatitis A virus, diphtheria, tetanus, human papilloma virus (HPV), etc[48,49]. Similarly, Montanide, a mineral oil-based adjuvant, has been widely used in human trials in both cancer vaccines and infectious diseases[50]. The mechanisms of the action for these adjuvants have been attributed to their ability to provide a long-lasting depot effect, effective Ag uptake and presentation by APCs and activation of APCs[50].

## 2.2 Delivery vehicles as adjuvants

Besides the addition of adjuvants with biological functions, vaccine delivery by use of a carrier system can significantly impact vaccine efficacy, by achieving target specificity, controlling the quantity end timing of vaccines, reducing the unwanted non-specific immune response and enhancing the vaccine-specific immunogenicity. Carriers, such as liposomes, microspheres, proteasomes, virosomes and virus-like particles (VLPs), antigen cochleates, dendrimers and carbon nanotubes have been widely explored for vaccine delivery[30,52-54]. Single-wall carbon nanotubes (SWNTs) have emerged as a potential vaccine delivery vehicle, given their unique physical and chemical properties. (see discussion in section 5 below.) In choosing a nanomaterial as an adjuvant or carrier one needs to carefully consider the properties of the material as they relate to depot effects (that is, does it retain the antigen in the same site for long periods and does it release the material and by what kinetics?) and inflammatory effects (is the carrier immunogenic by itself or will it enhance or detract from the immunogenicity of the carried antigen?) In principle, an optimal carrier would carry large numbers of antigens and many types of antigens, would retain antigens with slow release over time to allow inflammation at the site, would promote internalization of the antigen into the antigen presenting cell such as a dendritic cell and would not incite a dominant response to itself,

## 3.0 Purification of Nanotubes

Medical applications of CNT require highly pure and well characterized materials. Whether selecting for length, diameter, chirality, number of walls, metallicity, degree of modification or fidelity, the purification of carbon nanotubes has been a challenge since their discovery, and is today the largest obstacle to their swift translation into useful products. In the following section a number of techniques will be examined in the effective purification of carbon nanotubes that could be useful for vaccines.

### 3.1 Synthetic Purity

The goal of obviating purification altogether through a uniform synthesis has eluded scientists for the last two decades; however, a number of partially selective syntheses have been developed. Nanotubes are produced by arc discharge using carbon based electrodes impregnated with metal catalysts, or by catalytic methods such as chemical vapor deposition (CVD) and high pressure carbon monoxide (HiPCO) process[55]. Other techniques such as laser-vaporization and high-temperature heat treatments also exist, but are not as commercially popular[56].

These techniques have important general differences in the products they generate. For instance, multi-walled tubes generated by arc discharge tend to have a higher fidelity (fewer defects) than other methods, but their synthesis is not as scalable for mass production as are catalytic synthetic methods. Single wall tubes, however, have similar fidelity regardless of production method[57]. Such general differences are a starting point for choosing which tubes to purchase.

Within these categories, there are many options for modifying the product. The modification of atmosphere, pressure, temperature, flow rates, catalyst, distance between electrodes, voltage, and electrode doping, among others have influences on the qualities of the nanotubes generated[58].

One aspect of selection that has met with success through synthetic modifications is the number of walls in tubes. This has permitted the preferential creation of single, double, and less defined multi-walled tubes. As an example, the selective creation of double-walled tubes was discovered by the inclusion of sulphur in anode catalyst mixtures at low pressures[59,60].

Single-walled tubes can be created at greater than 90% exclusivity with control of pressure and the addition of Nickel and Cobalt to Iron catalysts[61,62], or by restricting the size of tube seeding nanodots[63] . Though far from completely selective, nanotubes have been synthesized with preferential chiralities as well[64]. Chirality of nanotubes, especially single-walled tubes, determines their electronic properties, and must be controlled if they are to be used in computer chips.

Nanotubes are generated by a variety of methods; each approach has its own profile of properties, proportions and residual impurities. Ultimately, one of the more important aspects of planning a nanotube purification is deciding which type of tubes to purchase initially. If residual metals are more of a concern HiPCO tubes should be avoided as they are furnished with a higher iron content[65]. If amorphous carbon contamination to multi-walled tubes is an issue then CVD[66] tubes should be substituted with those manufactured by arc-discharge.

### 3.2 The Removal of Amorphous and Graphitic Carbon, and Catalysts from Nanotubes

For medical applications the CNT must be rigorously pure, reproducible and well defined before regulatory agencies will allow their use in humans. The method by which nanotubes are generated, as discussed above, not only determines the properties of the tubes, but also the proportions of metals and other impurities that may need to be removed before the nanotubes can be employed. For instance, metals can interfere with conductivity and radiolabelling, and inclusion of amorphous carbon weakens the mechanical and thermal conductivity properties of nanofibers. Myriad methodologies have been developed to separate carbon nanotubes from metals, $sp^3$ carbon species, graphitic carbon species, and multi-form amorphous carbon. Techniques taking advantage of physical, chemical and electrical differences between tubes and their contaminants have met with success and are often used in combination to produce “pure” (but generally heterogeneous in length and chirality). These methods include acid treatments to remove metals[67,68], oxidations to open end caps[69,70] and to break the tubes at points of imperfection[71,72], filtrations to remove small, often imperfect carbon fragments[73], centrifugation[74], and covalent and non-covalent modifications that allow separations on the basis of solubility through extraction, gel permeation, filtration, and chromatography[75-81]. Nanotubes have been heated (annealing)[82] or LASERed to high temperatures which also works to remove imperfections and restore conjugation to bent or broken regions through isomerization. A technique currently being development by the Scheinberg lab takes advantage of the bundling properties of functionalized tubes in order to remove impurities through aqueous dialysis (unpublished). In the case of vaccines, once a immunoreactive peptide has been attached to the nanotube, purification of successfully labeled nanotubes could be accomplished through use of affinity columns, though no such work has yet been published on this method.

### 3.3 Purifications of Chirality

Purifications of a mixture of zigzag, armchair and helical nanotubes are perhaps the most challenging of all separations. It should be noted that perfect armchair and zigzag tubes are not chiral species as they have a mirror plane, but are often spoken of as such in comparing chiralities. Small amounts of tubes of a single chirality can now be separated through ultracentrifugation[83] and gel filtration[84]. Other processes have been developed to create asymmetric tubes out of symmetric ones. Hongjie Dai suspended nanotubes in water in a vertical monolayer and selectively oxidized the ends of the bundles beneath the surface of the water creating a uniformly asymmetric species[85]. Finally, electric fields can be used to align and order nanotubes both during and after their growth controlling conformation in this case rather than chirality[86].

We propose an effective way to separate tubes by their chiralities through chromatographic separation using tubes of a set chirality as a stationary phase. A hefty investment of chiral tubes into a chromatography column with a nanotube stationary phase of set chirality could provide a mechanism for the mass production of chiral tubes. One such product is already in development.

### 3.4 Assessment of Purity

A number of methods are available to assess the purity of nanotubes and nanotube constructs with the requisite standard being direct visualization through HRTEM, SEM and AFM. Dynamic light scattering[87] is used to determine the length distribution of nanotubes; Raman spectroscopy[88] elucidates the degree of fullerene-like carbon versus amorphous, graphitic, or $sp^3$ carbon impurities. Near infrared studies[89] are best used to determine the gross purity of a sample for which direct visualization studies may be too myopic. The characteristic absorption slope of nanotubes also provides supporting information as to the degree of nanotube modifications. Degrees of modification can also be assessed through

chemical tests such as the Kaiser/Sarin assay. When nanotubes are made soluble through modification, HPLC is very useful in the assessment of purity, and can produce discreet peaks even for substances as heterogeneous in structure as carbon nanotubes. Mechanical techniques may also be used to appraise nanotube purity. Measurement of the Young's modulus, or thermal conductivity may be used as a gross assessment of nanotube purity, but does not clearly identify the problematic factor. Thermogravimetic analysis can provide information on long term stability and potential for oxidation under extreme conditions[90-92].

## 4.0 Functionalization of carbon nanotubes

Pristine carbon nanotubes are chemically inert and extensively aggregate into particulates in aqueous solution under physiological conditions. Chemical modification must be performed to introduce multiple reactive moieties onto either the single-walled and multi-walled CNT surfaces. These reactive functionalities can be employed to further append antigenic peptides, proteins and biologics. Chemical functionalization may also lead to better dispersion of the vaccine and consequently improve antigen presentation in vivo. Needless to say, chemical functionalization provides a means to covalently attach antigens to the CNT platform which yields more stable vaccine composition and unique pharmacokinetic and pharmacodynamic properties.

A rich synthetic organic chemistry of multi-wall and single-wall CNT scaffold modification has been reviewed by Singh et al.[93] The introduction of carboxylic acid moieties can be readily accomplished by treating the CNT with strong acid under oxidizing conditions. These acidic groups can further undergo amidation or esterification reactions with thionyl chloride or carbodiimide reagents, respectively to append other groups of biological value. This approach has been used to covalently attach many copies of the polyelectrolyte polyethylene imine[94] or proteins obtained from tumor lysates[95]. Another versatile route is the addition of primary amine groups employing the 1,3-dipolar cycloaddition of reactive azomethine ylides with the carbon-carbon double bonds of the CNT sidewall surface.[96] The ylides are generated in situ by thermal condensation of aldehydes and α-amino acids that upon reaction with the CNT surface yield pyrrolidine rings. This latter approach yields water soluble, stable and well dispersed functionalized CNT. The CNT-$(NH_2)_x$ platform has been used to append peptides via (i) condensation[97,98]; (ii) chemoselective ligation using bifunctional coupling reagents[97-99]; and (iii) bio-orthogonal coupling through a hydrazone linkage[100]. Interestingly, the resultant hydrazone linkage formed between the CNT and the peptide yields a chromophore that can be quantified spectrophotometrically ($\lambda_{max}$ = 354 nm, $\varepsilon$ = 29,000 $M^{-1}cm^{-1}$) and provides information on the amount of peptide attached per CNT.

CNT-based vaccines that have been built from the ground-up often require several synthetic and purification steps over the course of the production. Thorough characterization of the chemical identities of the starting materials, intermediates, and final products is important in obtaining reproducible and interpretable biological results, given the inherent variability that is observed in immunotherapeutic studies.

## 5.0 Use of CNT as vaccines *in vivo*

The ability to link multiple copies of antigens or immune stimulants simultaneously to CNT allows the design of diverse approaches for the use of CNT in vaccine construction. Viral, bacterial and protozoal antigens as well as CpG adjuvants have been appended [94,99,101]. One of the early attempts to apply CNT as a scaffold for vaccine development involved the covalent attachment of foot-and-mouth disease viral envelope peptides to CNT[97,98]. This work showed that it was possible to retain the epitope structure in an immunogenic form when attached to the CNT. Indeed, the CNT-viral protein molecular complexes were

capable of generating specific immune responses in animal models. Pantarotto showed that only the peptide-CNT elicited IgG responses which are neutralizing[97,98].

Meng et al. used a tumor cell lysate conjugated to single-wall CNT as a therapeutic cancer vaccine in mouse model hepatoma [95.] The conjugated vaccine improved cure rates as compared to lysates alone, apparently by improved activation of cytolytic T cells.

Single-wall CNT-PPD antigen was used to study the character of the T cell responses in mice[101]. Interestingly, while traditional adjuvants such as PPD in Freund's adjuvant generated a predominately Th-2 response, the single-wall CNT-PPD response was biased toward a Th-1 cytokine response (interferon and IL-12.)

Mocan and others[102] compared the effects of multi-wall CNT and embryonic stem cells, injected separately into the same mice to suppress the growth of murine colon carcinoma. This combination was more effective than either agent administered alone, and both CD4 and CD8 activation was enhanced, but whether there is an induction of a specific immune response to the cancer cells was not studied.

The Wilm's tumor protein (WT1) is over-expressed in many human leukemias and cancers and it is widely used in human trials as a cancer vaccine[50,104, 105]. Villa et al. aimed to demonstrate that carbon nanotube-peptide constructs could improve the immunogenicity of this weakly immunogenic, clinically relevant cancer-associated peptide[100]. They used spectrally quantifiable chemical approaches to covalently append large numbers of a 19 amino acid peptide onto solubilized single-wall carbon nanotubes. The nanotube scaffold itself was non-cytotoxic to dendritic cells in vitro and appeared non-immunogenic in mice. The peptide alone with an adjuvant did not induce an immune response in mice. The peptide-single-wall CNT vaccine was internalized into dendritic cells and macrophages within minutes *in vitro*, which should serve to promote immunization. Mice immunized with the single-wall CNT-peptide vaccine and an adjuvant induced specific IgG responses against the peptide.

In addition they observed that uptake of the vaccine into the dendritic cells or macrophages occurred in 5 min, followed 15 min later by accumulation of the peptide-single wall CNT in a diffuse perinuclear compartment. Microscopic analyses suggested that CNT-peptide constructs were concentrated in intracellular vesicles potentially enhancing peptide delivery into APCs[100].

## 6.0 Conclusions

Numerous approaches to vaccine development are under study including the use of nanoparticles as carriers. Nanomaterials have features that allow them to be effective carriers including multivalency, stability, and a likelihood of internalization into antigen presenting cells. The use of CNT in this regard is just beginning. As carriers alone, or with small molecules attached to them, they clear the blood via the kidney[105]. However, with larger appended moieties or proteins they are retained within the blood stream. Their unusual property of rapidly internalizing into antigen presenting cells while carrying their cargo is a distinctive advantage to CNT. A better understanding of the specifications that control this process, such as length, charge, hydrophobicity, are required to optimize their use in this regard. Due to their stealthy characteristics, SWNT may not adequately stimulate innate immunity alone as do classic adjuvants or carriers. Therefore, immunologic adjuvants appear necessary for more potent immune responses to CNT-based vaccines, which is not surprising, as adjuvants are necessary for eliciting antibody responses for other vaccines. One advantage though is the ability to conjugate different molecules simultaneously to the

nanotube. For example, one could attach both the antigen and the adjuvant or an immune-stimulatory cytokine to the same construct. Importantly, conjugation of the antigen to CNT appears to avoid the use of carrier proteins such as keyhole limpet hemocyanin (KLH). KLH has some features in common with a nanomaterial, such large size and insolubility, but is a biologic material and difficult to access from the sea. Considerable investigation remains to understand the role of CNT in vaccine compositions in comparison to more traditional carriers.

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## REFERENCES

1. Mackall CL. Spreading the wealth: Antigen discovery in adult tumors can help hone the search for pediatric tumor antigens. J. Immunother. 2001; 24:281. [PubMed: 11565827]
2. Farkas AM, Finn OJ. Vaccines based on abnormal self-antigens as tumor-associated antigens: Immune regulation. Semin. Immunol. 2010; 22:125. [PubMed: 20403708]
3. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature. 2007; 449:419. [PubMed: 17898760]
4. Melief CJM. Cancer immunotherapy by dendritic cells. Immunity. 2008; 29:372. [PubMed: 18799145]
5. Dyall R, Bowne WB, Weber LW, LeMaoult J, Szabo P, Moroi Y, Piskun G, Lewis JJ, Houghton AN, Nikolic-Zugic J. Heteroclitic immunization induces tumor immunity. J. Exp. Med. 1998; 188:155.
6. Houghton AN, Guevara-Patino JA. Immune recognition of self in immunity against cancer. J. Clin. Invest. 2004; 114:468. [PubMed: 15314682]
7. Heath AW, Playfair JHL. Cytokines as Immunological Adjuvants. Vaccine. 1992; 10:427. [PubMed: 1609545]
8. O'Hagan DT, MacKichan ML, Singh M. Recent developments in adjuvants for vaccines against infectious diseases. Biomol. Eng. 2001; 18:69. [PubMed: 11566599]
9. Srivastava PK, Menoret A, Basu S, Binder RJ, McQuade KL. Heat shock proteins come of age: Primitive functions acquire new roles in an adaptive world. Immunity. 1998; 8:657. [PubMed: 9655479]
10. Kojima C. Design of stimuli-responsive dendrimers. Expert Opin Drug Del. 2010; 7:307.
11. Livingston PO, Ragupathi G. Cancer vaccines targeting carbohydrate antigens. Hum. Vaccines. 2006; 2:137.
12. Xiang SD, Scalzo-Inguanti K, Minigo G, Park A, Hardy CL, Plebanski M. Promising particle-based vaccines in cancer therapy. Expert Rev Vaccines. 2008; 7:1103. [PubMed: 18767957]
13. Fifis T, Gamvrellis A, Crimeen-Irwin B, Pietersz GA, Li J, Mottram PL, McKenzie IFC, Plebanski M. Size-dependent immunogenicity: Therapeutic and protective properties of nano-vaccines against tumors. J. Immunol. 2004; 173:3148. [PubMed: 15322175]
14. Mottram PL, Leong D, Crimeen-Irwin B, Gloster S, Xiang SD, Meanger J, Ghildyal R, Vardaxis N, Plebanski M. Type 1 and 2 immunity following vaccination is influenced by nanoparticle size: Formulation of a model vaccine for respiratory syncytial virus. Mol. Pharmaceut. 2007; 4:73.
15. Kostarelos K, Bianco A, Prato M. Promises, facts and challenges for carbon nanotubes in imaging and therapeutics. Nat. Nanotechnol. 2009; 4:627. [PubMed: 19809452]
16. Dumortier H, Lacotte S, Pastorin G, Marega R, Wu W, Bonifazi D, Briand JP, Prato M, Muller S, Bianco A. Functionalized carbon nanotubes are non-cytotoxic and preserve the functionality of primary immune cells. Nano Lett. 2006; 6:3003.
17. Mutlu GM, Budinger GRS, Green AA, Urich D, Soberanes S, Chiarella SE, Alheid GF, McCrimmon DR, Szleifer I, Hersam MC. Biocompatible nanoscale dispersion of single-walled

carbon nanotubes mnimizes in vivo pulmonary toxicity. Nano Lett. 2010; 10:1664. [PubMed: 20377197]

18. Scheinberg DA, Villa CH, Escorcia FE, McDevitt MR. Conscripts of the infinite armada: systemic cancer therapy using nanomaterials. Nat. Rev. Clin. Oncol. 2010; 7:266. [PubMed: 20351700]
19. Kostarelos K, Lacerda L, Pastorin G, Wu W, Wieckowski S, Luangsivilay J, Godefroy S, Pantarotto D, Briand JP, Muller S, Prato M, Bianco A. Cellular uptake of functionalized carbon nanotubes is independent of functional group and cell type. Nat. Nanotechnol. 2007; 2:108. [PubMed: 18654229]
20. Kam NWS, O'Connell M, Wisdom JA, Dai HJ. Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. Proc. Natl. Acad. Sci. USA. 2005; 102:11600. [PubMed: 16087878]
21. Konduru NV, Tyurina YY, Feng WH, Basova LV, Belikova NA, Bayir H, Clark K, Rubin M, Stolz D, Vallhov H, Scheynius A, Witasp E, Fadeel B, Kichambare PD, Star A, Kisin ER, Murray AR, Shvedova AA, Kagan VE. Phosphatidylserine targets aingle-walled carbon nanotubes to professional phagocytes in vitro and in vivo. PLoS One. 2009; 4:e4398. [PubMed: 19198650]
22. Rosen Y, Elman NM. Carbon nanotubes in drug delivery: focus on infectious diseases. Expert Opin. Drug. Del. 2009; 6:517.
23. Prato M, Kostarelos K, Bianco A. Functionalized carbon nanotubes in drug design and discovery. Acc. Chem. Res. 2008; 41:60. [PubMed: 17867649]
24. Lacerda L, Bianco A, Prato M, Kostarelos K. Carbon nanotubes as nanomedicines: From toxicology to pharmacology. Adv. Drug Deliv. Rev. 2006; 58:1460. [PubMed: 17113677]
25. Gresser I. A. Chekhov, M.D., and Coley's toxins. New Engl. J. Med. 1987; 317:457. [PubMed: 3302707]
26. Wack A, Rappuoli R. Vaccinology at the beginning of the 21st century. Curr. Opin. Immunol. 2005; 17:411. [PubMed: 15950445]
27. Berzofsky JA, Terabe M, Wood LV. Strategies to use immune modulators in therapeutic vaccines against cancer. Seminars in oncology. 2012; 39:348. [PubMed: 22595057]
28. Medzhitov R, Janeway CA Jr. Innate immunity: Minireview the virtues of a nonclonal system of recognition. Cell. 1997; 91:295. [PubMed: 9363937]
29. Montomoli E, Piccirella S, Khadang B, Mennitto E, Camerini R, De Rosa A. Current adjuvants and new perspectives in vaccine formulation. Expert Rev.Vaccines. 2011; 10:1053. [PubMed: 21806399]
30. Leleux J, Roy K. Micro and nanoparticle-based delivery systems for vaccine immunotherapy: an immunological and materials perspective. Adv. Healthc. Mater. 2013; 2:72. [PubMed: 23225517]
31. Steinhagen F, Kinjo T, Bode C, Klinman DM. TLR-based immune adjuvants. Vaccine. 2011; 29:3341. [PubMed: 20713100]
32. Sabbatini P, Tsuji T, Ferran L, Ritter E, Sedrak C, Tuballes K, Jungbluth AA, Ritter G, Aghajanian C, Bell-McGuinn K, Hensley ML, Konner J, Tew W, Spriggs DR, Hoffman EW, Venhaus R, Pan L, Salazar AM, Diefenbach CM, Old LJ, Gnjatic S. Phase I Trial of Overlapping Long Peptides from a Tumor Self-Antigen and Poly-ICLC Shows Rapid Induction of Integrated Immune Response in Ovarian Cancer Patients. Clin. Cancer Res. 2012; 18:6497. [PubMed: 23032745]
33. Wong JP, Christopher ME, Viswanathan S, Dai X, Salazar AM, Sun LQ, Wang M. Antiviral role of toll-like receptor-3 agonists against seasonal and avian influenza viruses. Curr. Parm. Des. 2009; 15:1269.
34. Garaude J, Blander JM. "Flagellated" cancer cells propel anti-tumor immunity. Oncoimmunology. 2012; 1:71. [PubMed: 22720215]
35. Chu RS, Targoni OS, Krieg AM, Lehmann PV, Harding CV. CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (Th1) immunity. J. Exp. Med. 1997; 186:1623. [PubMed: 9362523]
36. Sun S, Zhang X, Tough DF, Sprent J. Type I interferon-mediated stimulation of T cells by CpG DNA. J. Exp. Med. 1998; 188:2335. [PubMed: 9858519]
37. Cooper CL, Davis HL, Angel JB, Morris ML, Elfer SM, Seguin I, Krieg AM, Cameron DW. CPG 7909 adjuvant improves hepatitis B virus vaccine seroprotection in antiretroviral-treated HIV-infected adults. Aids. 2005; 19:1473. [PubMed: 16135900]

38. Sogaard OS, Lohse N, Harboe ZB, Offersen R, Bukh AR, Davis HL, Schonheyder HC, Ostergaard L. Improving the immunogenicity of pneumococcal conjugate vaccine in HIV-infected adults with a toll-like receptor 9 agonist adjuvant: a randomized, controlled trial. Clin. Infect. Dis. 2010; 51:42. [PubMed: 20504165]
39. Speiser DE, Lienard D, Rufer N, Rubio-Godoy V, Rimoldi D, Lejeune F, Krieg AM, Cerottini JC, Romero P. Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909. J. Clin. Invest. 2005; 115:739. [PubMed: 15696196]
40. Valmori D, Souleimanian NE, Tosello V, Bhardwaj N, Adams S, O'Neill D, Pavlick A, Escalon JB, Cruz CM, Angiulli A, Angiulli F, Mears G, Vogel SM, Pan L, Jungbluth AA, Hoffmann EW, Venhaus R, Ritter G, Old LJ, Ayyoub M. Vaccination with NY-ESO-1 protein and CpG in Montanide induces integrated antibody/Th1 responses and CD8 T cells through cross-priming. Proc. Natl. Acad. Sci. USA. 2007; 104:8947. [PubMed: 17517626]
41. Decker WK, Safdar A. Cytokine adjuvants for vaccine therapy of neoplastic and infectious disease. Cytokine Growth Factor Rev. 2011; 22:177. [PubMed: 21862380]
42. Parmiani G, Castelli C, Pilla L, Santinami M, Colombo MP, Rivoltini L. Opposite immune functions of GM-CSF administered as vaccine adjuvant in cancer patients. Ann. Oncol. 2007; 18:226. [PubMed: 17116643]
43. Clive KS, Tyler JA, Clifton GT, Holmes JP, Mittendorf EA, Ponniah S, Peoples GE. Use of GM-CSF as an adjuvant with cancer vaccines: beneficial or detrimental? Expert Rev. Vaccines. 2010; 9:519. [PubMed: 20450326]
44. O'Brien L, Perkins S, Williams A, Eastaugh L, Phelps A, Wu J, Phillpotts R. Alpha interferon as an adenovirus-vectored vaccine adjuvant and antiviral in Venezuelan equine encephalitis virus infection. J. Gen. Virol. 2009; 90:874. [PubMed: 19264673]
45. Reed SG, Bertholet S, Coler RN, Friede M. New horizons in adjuvants for vaccine development. Trends Immunol. 2009; 30:23. [PubMed: 19059004]
46. Del Vecchio M, Bajetta E, Canova S, Lotze MT, Wesa A, Parmiani G, Anichini A. Interleukin-12: biological properties and clinical application. Clin. Cancer Res. 2007; 13:4677. [PubMed: 17699845]
47. Rosenberg SA, Lotze MT. Cancer immunotherapy using interleukin-2 and interleukin-2-activated lymphocytes. Annu. Rev. Immunol. 1986; 4:681. [PubMed: 3518753]
48. De Gregorio E, Tritto E, Rappuoli R. Alum adjuvanticity: unraveling a century old mystery. Eur. J. Immunol. 2008; 38:2068. [PubMed: 18651701]
49. Marrack P, McKee AS, Munks MW. Towards an understanding of the adjuvant action of aluminium. Nat. Rev. Immunol. 2009; 9:287. [PubMed: 19247370]
50. Maslak PG, Dao T, Krug LM, Chanel S, Korontsvit T, Zakhaleva V, Zhang R, Wolchok JD, Yuan J, Pinilla-Ibarz J, Berman E, Weiss M, Jurcic J, Frattini MG, Scheinberg DA. Vaccination with synthetic analog peptides derived from WT1 oncoprotein induces T-cell responses in patients with complete remission from acute myeloid leukemia. Blood. 2010; 116:171. [PubMed: 20400682]
51. Jäger E, Jäger D, Knuth A. Antigen-specific immunotherapy and cancer vaccines. Int. J. Cancer. 2003; 106:817. [PubMed: 12918057]
52. Gregoriadis G, Davis D, Davies A. Liposomes as immunological adjuvants: antigen incorporation studies. Vaccine. 1987; 5:145. [PubMed: 3604394]
53. Henriksen-Lacey M, Korsholm KS, Andersen P, Perrie Y, Christensen D. Liposomal vaccine delivery systems. Expert opin. drug deliv. 2011; 8:505. [PubMed: 21413904]
54. Plante M, Jones T, Allard F, Torossian K, Gauthier J, St-Felix N, White GL, Lowell GH, Burt DS. Nasal immunization with subunit proteosome influenza vaccines induces serum HAI, mucosal IgA and protection against influenza challenge. Vaccine. 2001; 20:218. [PubMed: 11567767]
55. Teo KBK, Singh C, Chhowalla M, Milne WI. Catalytic synthesis of carbon nanotubes and nanofibres. Encyclopedia of nanoscience and nanotechnology. 2003; 10:1.
56. Kingston CT, Simard B. Recent advances in laser synthesis of single-walled carbon nanotubes. J. Nanosci. Nanotech. 2006; 6:1225.
57. Fan Y, Goldsmith BR, Collins PG. Identifying and Counting Point Defects in Carbon Nanotubes. Nat. Mater. 2005; 4:906. [PubMed: 16267574]
58. Ebbesen, TW. Carbon Nanotubes: Preparation and Properties. CRC Press Llc; 1997.

59. Hutchison JL, Kiselev NA, Krinichnaya EP, Krestinin AV, Loutfy RO, Morawsky AP, Muradyan VE, Obraztsova ED, Sloan J, Terekhov SV. Double-walled Carbon Nanotubes Fabricated by a Hydrogen Arc Discharge Method. Carbon. 2001; 39:761.
60. Li ZH, Wang M, Yang B, Xu YB. The Influence of Different Atmosphere Gases on the Growth and Structure of Double-walled Carbon Nanotubes. Inorg. Mater. 2007; 43:475.
61. Deck CP, Vecchio K. Prediction of carbon nanotube growth success by the analysis of carbon-catalyst binary phase diagrams. Carbon. 2006; 44:267.
62. Dupuis AC. The catalyst in the CCVD of carbon nanotubes – a review. Prog. Mater. Sci. 2005; 50:929.
63. Baker RTK, Barber MA, Harris PS, Feates S, Waite RJ. Nucleation and growth of carbon deposits from the nickel catalyzed decomposition of acetylene. J. Catalysis. 1972; 26:51.
64. Bachilo SM, Balzano L, Herrera JE, Pompeo F, Resasco DE, Weisman RB. Narrow (n, m)-distribution of single-walled carbon nanotubes grown using a solid supported catalyst. J. Amer. Chem. Soc. 2003; 125:11186. [PubMed: 16220926]
65. Chiang IW, Brinson BE, Huang AY, Willis P, Bronikowski M, Margrave J, Smalley R, Hauge R. Purification and characterization of single-wall carbon nanotubes (SWNTs) obtained from the gas-phase decomposition of CO (HiPco process). J. Phys. Chem. B. 2001; 105:8297.
66. Harris, PJF. Carbon Nanotube Science: Synthesis Properties and Applications. Cambridge University Press; Cambridge: 2009.
67. Rinzler A, Liu J, Dai H, Nikolaev P, Huffman C, Rodriguez-Macias F, Boul P, Lu AH, Heymann D, Colbert D. Large-scale purification of single-wall carbon nanotubes: process, product, and characterization. Appl. Phys. A Mater. Sci. Process. A. 1998; 67:29.
68. Park TJ, Banerjee S, Hemraj-Benny T, Wong SS. Purification strategies and purity visualization techniques for single-walled carbon nanotubes. J. Mater. Chem. 2006; 16:141.
69. Ebbesen TW, Ajayan PM, Hiura H, Tanigaki K. Purification of carbon nanotubes. Nature. 1994; 367:519.
70. Ikazaki F, Ohshima S, Uchida K, Kuriki Y, Hayakawa H, Yumura M, Takahashi K, Tojima K. Chemical purification of carbon nanotubes by the use of graphite intercalation compounds. Carbon. 1994; 32:1539.
71. Chen XH, Chen CS, Chen Q, Cheng FQ, Zhang G, Chen ZZ. Non-destructive purification of multi-walled carbon nanotubes produced by catalyzed CVD. Mater. Lett. 2002; 57:734.
72. Bonard J-M, Stora T, Salvetat J-P, Maier F, Stöckli T, Duschl C, Forró L, de Heer WA, Châtelain A. Purification and size-selection of carbon nanotubes. Adv. Mater. 1997; 9:827.
73. Shelimov KB, Esenaliev RO, Rinzler AG, Huffman CB, Smalley RE. Purification of single-wall nanotubes by ultrasonically assisted filtration. Chem. Phys. Lett. 1998; 282:429. R. E.
74. Yu A, Bekyarova E, Itkis ME, Fakhrutdinov D, Webster R, Haddon RC. Application of centrifugation to the large-scale purification of electric arc-produced single-walled carbon nanotubes. J. Amer. Chem. Soc. 2006; 128:9902. [PubMed: 16866549]
75. Niyogi S, Hu H, Hamon MA, Bhowmik P, Zhao B, Rozenzhak SM, Chen J, Itkis ME, Meier MS, Haddon RC. Chromatographic purification of soluble single-walled carbon nanotubes (s-SWNTs). J. Amer. Chem. Soc. 2001; 123:733. [PubMed: 11456587]
76. Duesberg GS, Burghard M, Muster J, Philipp G. Separation of carbon nanotubes by size exclusion chromatography. Chem. Commun. 1998:435.
77. Georgakilas V, Voulgaris D, Vazquez E, Prato M, Guldi DM, Kukovecz A, Kuzmany H. Purification of HiPco carbon nanotubes via organic functionalization. J. Amer. Chem. Soc. 2002; 124:14318. [PubMed: 12452701]
78. Banerjee S, Wong SS. Rational sidewall functionalization and purification of single-walled carbon nanotubes by solution-phase ozonolysis. J. Phys. Chem. B. 2002; 106:12144.
79. Hu H, Zhao B, Itkis ME, Haddon RC. Nitric acid purification of single-walled carbon nanotubes. J. Phys. Chem. B. 2003; 107:13838.
80. Duesberg GS, Muster J, Krstic V, Burghard M, Roth S. Chromatographic size separation of single-wall carbon nanotubes. Appl. Phys. A. 1998; 67:117.

81. Chen RJ, Zhan Y, Wang D, Dai H. Noncovalent sidewall functionalization of single-walled carbon nanotubes for protein immobilization. J. Amer. Chem. Soc. 2001; 123:3838. [PubMed: 11457124]
82. Andrews R, Jacques D, Qian D, Dickey EC. Purification and structural annealing of multiwalled carbon nanotubes at graphitization temperatures. Carbon. 2001; 39:1681.
83. Green AA, Hersam MC. Nearly Single-Chirality Single-Walled Carbon Nanotubes Produced via Orthogonal Iterative Density Gradient Ultracentrifugation. Adv. Mater. 2011; 23:2185. [PubMed: 21472798]
84. Liu H, Nishide D, Tanaka T, Kataura H. Large-scale single-chirality separation of single-wall carbon nanotubes by simple gel chromatography. Nat. Commun. 2001; 2:309. [PubMed: 21556063]
85. Lee KM, Li L, Dai L. Asymmetric end-functionalization of multi-walled carbon nanotubes. J. Amer. Chem. Soc. 2005; 127:4122. [PubMed: 15783165]
86. Zhang Y, Chang A, Cao J, Wang Q, Kim W, Li Y, Morris N, Yenilmez E, Kong J, Dai H. Electric-field-directed growth of aligned single-walled carbon nanotubes. Appl. Phys. Lett. 2001; 79:3155.
87. Badaire S, Poulin P, Maugey M, Zakari C. In situ measurements of nanotube dimensions in suspensions by depolarized dynamic light scattering. Langmuir. 2004; 20:10367. [PubMed: 15544359]
88. Rao AM, Richter E, Bandow S, Chase B, Eklund PC, Williams KA, Fang S, Subbaswamy KR, Menon M, Thess A, Smalley RE, Dresselhaus G, Dresselhaus MS. Diameter-selective Raman scattering from vibrational modes in carbon nanotubes. Science. 1997; 275:187. [PubMed: 8985007]
89. Itkis ME, Perea DE, Jung R, Niyogi S, Haddon RC. Comparison of analytical techniques for purity evaluation of single-walled carbon nanotubes. J. Amer. Chem. Soc. 2005; 127:3439. [PubMed: 15755163]
90. Chiang IW, Brinson BE, Huang AY, Willis PA, Bronikowski MJ, Margrave JL, Smalley RE, Hauge RH. Purification and characterization of single-wall carbon nanotubes (SWNTs) obtained from the gas-phase decomposition of CO (HiPco process). J. Phys. Chem. B. 2001; 105:8297.
91. Chiang IW, Brinson BE, Smalley RE, Chiang IW, Brinson BE, Smalley RE, Margrave JL, Hauge RH. Purification and characterization of single-wall carbon nanotubes. J. Phys. Chem. B. 2001; 105:1157.
92. Dillon AC, Gennett T, Jones KM, Alleman JL, Parilla PA, Heben MJ. A simple and complete purification of single-walled carbon nanotube materials. Adv. Mater. 1999; 11:1354.
93. Singh P, Campidelli S, Giordani S, Bonifazi D, Bianco A, Prato M. Organic functionalisation and characterisation of single-walled carbon nanotubes. Chem. Soc. Rev. 2009; 38:2214. [PubMed: 19623345]
94. Carbone M, Valentini F, Caminiti R, Petrinca AR, Donia D, Divizia M, Palleschi G. Are PEI-coated SWCNTs conjugated with hepatitis A virus? A chemical study with SEM, Z-potential, EDXD and RT-PCR. Biomed. Mater. 2010; 5:035001.
95. Meng J, Meng J, Duan J, Kong H, Li L, Wang C, Xie S, Chen S, Gu N, Xu H, Yang XD. Carbon nanotubes conjugated to tumor lysate protein enhance the efficacy of an antitumor immunotherapy. Small. 2008; 4:1364. [PubMed: 18720440]
96. Georgakilas V, Tagmatarchis N, Pantarotto D, Bianco A, Briand JP, Prato M. Amino acid functionalisation of water soluble carbon nanotubes. Chem. Commun. 2002; 24:3050.
97. Pantarotto D, Partidos CD, Graff R, Hoebeke J, Briand JP, Prato M, Bianco A. Synthesis, structural characterization, and immunological properties of carbon nanotubes functionalized with peptides. J. Am. Chem. Soc. 2003; 125:6160. [PubMed: 12785847]
98. Pantarotto D, Partidos CD, Hoebeke J, Brown F, Kramer E, Briand JP, Muller S, Prato M, Bianco A. Immunization with peptide-functionalized carbon nanotubes enhances virus-specific neutralizing antibody responses. Chem. Biol. 2003; 10:961. [PubMed: 14583262]
99. Yandar N, Pastorin G, Prato M, Bianco A, Patarroyo ME, Manuel Lozano J. Immunological profile of a Plasmodium vivax AMA-1 N-terminus peptide-carbon nanotube conjugate in an infected Plasmodium berghei mouse model. Vaccine. 2008; 26:5864. [PubMed: 18771700]
100. Villa CH, Dao T, Ahearn I, Fehrenbacher N, Casey E, Rey DA, Korontsvit T, Zakhaleva V, Batt CA, Philips MR, Scheinberg DA. Single-walled carbon nanotubes deliver peptide antigen into

dendritic cells and enhance IgG responses to tumor-associated antigens. ACS Nano. 2011; 5:5300. [PubMed: 21682329]

101. Zeinali M, Jammalan M, Ardestani SK, Mosaveri N. Immunological and cytotoxicological characterization of tuberculin purified protein derivative (PPD) conjugated to single-walled carbon nanotubes. Immunol. Lett. 2009; 126:48. [PubMed: 19664657]
102. Mocan T, Iancu C. Effective colon cancer prophylaxis in mice using embryonic stem cells and carbon nanotubes. Int. J. Nanomedicine. 2011; 6:1945. [PubMed: 21976971]
103. May RJ, Dao T, Pinilla-Ibarz J, Korontsvit T, Zakhaleva V, Zhang RH, Maslak P, Scheinberg DA. Peptide epitopes from the Wilms' tumor 1 oncoprotein stimulate CD4+ and CD8+ T cells that recognize and kill human malignant mesothelioma tumor cells. Clin. Cancer Res. 2007; 13:4547. [PubMed: 17671141]
104. Krug LM, Dao T, Brown AB, Maslak P, Travis W, Bekele S, Korontsvit T, Zakhaleva V, Wolchok J, Yuan JD, Li H, Tyson L, Scheinberg DA. WT1 peptide vaccinations induce CD4 and CD8 T cell immune responses in patients with mesothelioma and non-small cell lung cancer. Cancer Immunol. Immun. 2010; 59:1467.
105. Ruggiero A, Villa CH, Bander E, Rey DA, Bergkvist M, Batt CA, Manova-Todorova K, Deen WM, Scheinberg DA, McDevitt MR. Paradoxical glomerular filtration of carbon nanotubes. Proc. Natl. Acad. Sci. USA. 2010; 107:12369. [PubMed: 20566862]