European Review for Medical and Pharmacological Sciences 2019; 23: 7163-7182

The new era of vaccines: the "nanovaccinology"

A. FACCIOLÀ[1], G. VISALLI[2], P. LAGANÀ[2], V. LA FAUCI[2], R. SQUERI[2], G.F. PELLICANÒ[3], G. NUNNARI[1], M. TROVATO[1], A. DI PIETRO[2]

[1]Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy
[2]Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy
[3]Department of Human Pathology of the Adult and the Developmental Age "G. Barresi", University of Messina, Messina, Italy

Abstract. – Vaccinations are the most effective preventive methods against infectious diseases and represent one of the most relevant successes of medicine. Vaccine development is constantly evolving; therefore, the number of vaccine candidates is progressively increasing. However, most of new potential vaccines are characterized by a lower immunogenicity, with the inability to stimulate powerful and long-lasting immune responses. Hence, to get modern and effective vaccines, we need of adjuvants and innovative delivery systems that increase their immunogenicity. The use of nanotechnology in vaccinology is providing the opportunity to contrast these difficulties and develop effective vaccines. Particularly, nanoparticles used as vehicles of vaccine components, are able to increase the host's immune responses and, due to their size, to reach specific cellular districts. To date, a certain number of nanovaccines has been approved for human health and many are studied in clinical or pre-clinical trials. There are several types of nanoparticles considered as possible delivers of vaccine antigens. These nanoparticles-based synthetic delivery systems, in the size range of 20-200 nm, protect antigen from degradation, enhance its presentation and facilitate its uptake by professional antigen presenting cells. Virus-like particles, self-assembled proteins, micelles, liposomes, inorganic nanoparticles, and polymers are the most studied of these systems. In this review, we provide a general overview of different types, methods of synthesis, characterizations, properties and applications of nanoparticles in vaccine production.

Key Words:
Vaccines, Nanotechnology, Nanoparticles, Nanovaccinology.

Introduction

Vaccinations are the most effective preventive weapon against infectious diseases and represent one of the principal topics for human health. In about two hundred years, vaccinations have allowed to the worldwide eradication of smallpox, the elimination of poliomyelitis in the majority of countries and the decrease in the morbidity and mortality of a number of vaccine-preventable diseases (VPDs). Nevertheless, vaccine hesitancy is spreading in many countries, especially due to an unjustified fear of hypothetic side effects of vaccines and a lack of trust in vaccine safety and efficacy[1,2]. Consequently, some outbreaks of VPDs occurred in many parts of the world[3-5] and important public health targets, such as eradication of poliomyelitis and congenital rubella, are not still reached, as demonstrated by a lot of international studies[6-9]. Unexpectedly, vaccine hesitancy is a condition concerning even healthcare workers that often are under or no vaccinated against the main VPDs because of doubts about vaccine safety and efficacy[10-14].

Because vaccine development is constantly evolving, the number of candidate vaccines is progressively increasing[15,16]. However, most potential vaccines have an essential composition, generally characterized by a lower immunogenicity, with the inability to elicit powerful and long-lasting immune responses[17,18]. Therefore, to obtain modern and effective vaccines, we need of adjuvants and innovative delivery systems that enhance their immunogenicity. This research topic is placed in a context of technological innovations aimed at reduce the burden of infectious diseases in all their aspects[19].

The first idea of nanotechnology was born in the mind of the great physicist Richard P. Feynman in 1959[20]. In 1986, Eric Drexler introduced the term "nanotechnology" in his book "Engines of Creation" to describe the approach of molecular manufacturing and some of its consequen-

Corresponding Author: Alessio Facciolà, MD; e-mail: afacciola@unime.it

ces[21]. Nanotechnology is an important discovery applied in many industrial and scientific fields. Particularly in the latter, nanotechnologies have allowed the development of "nanomedicine"[22]. Nanotechnology has been part of conventional scientific theories with potential medical applications since the early 1990s. From then to today, several "nanostructures", such as nanoparticles, nanorods, nanospheres, nanofibers, nanotubes, and others, have been considered for numerous applications to biologic systems[20].

In its strictest definition from the National Nanotechnology Initiative, nanotechnology refers to structures of about 1−1000 nm of size in at least one dimension with variable composition, size, shape, and surface properties[23,24]. Nanoparticles, because of their size similar to some cellular components, can enter into living cells through the mechanism of endocytosis, in particular pinocytosis[25]. The clinical application of these structures is revolutionizing some medical fields, including diagnosis, prevention and treatment, as carriers and delivers of biologically active compounds. Indeed, some approved nano-sized vaccines and drug delivery systems are making a real revolution in disease prevention and management[24,26-28]. Natural or synthetic materials able to incorporate exogenous drugs and self-assemble to form nanoparticles (NPs) in aqueous media have been planned and studied as drug delivery carriers[29-34]. In parallel to researches about possible medical applications of nanoparticles, several studies have been carried out to assess their biocompatibility and potential cellular toxicity[35-43].

The use of nanotechnology in vaccinology is providing the opportunity to contrast some difficulties and to develop effective vaccines[44,45]. Particularly, nanoparticles used as vehicles of vaccine components, are able to play an important role in the development of new vaccines both in the increase of the host's immune responses and in the possibility, due to their size, to reach specific cellular districts. We can distinguish a therapeutic and a prophylactic nanovaccinology. Therapeutic nanovaccinology is primarily addressed to the therapy of cancer[46,47] but is more and more studied to treat other diseases or conditions, such as Alzheimer's disease[48], hypertension[49] and nicotine addiction[26]. Prophylactic nanovaccinology is instead addressed to the prevention of several infectious diseases. A certain number of prophylactic nanovaccines has been already approved for human use and many are studied in clinical or pre-clinical trials[50-52]. Vaccine antigen, placed on the surface or encapsulated inside nanoparticles, is presented to the immune system in the same way in which it would be presented by pathogen[53]. Three are the key factors for an effective and successful vaccine: (1) a good antigen to activate immunity, (2) an immune adjuvant to co-stimulate innate immune system and (3) a carrier system to allow the achievement of the antigen presenting cells (APCs) by the two previous components. To achieve these goals, it is necessary that the design of nanoparticles concerns especially composition, size, surface properties (all factors influencing link and delivery of antigen), biodistribution, and immunostimulatory capacity of the used nanostructures[29,54-56]. Some studies have reported that nanoparticles facilitate the cellular entry by endocytosis, in particular pinocytosis, of the vaccine compounds improving the effectiveness of vaccines[25,57]. To well optimize the application of nanoparticles in vaccinology, it is important to determine their size, physic-chemical compositions and modifications of the materials, as these parameters determine their biological effects "*in vivo*"[58]. Other factors to consider when talking about nanoparticle-based vaccines, are shape and dose of nanoparticles. Therefore, vaccine nanoparticles with various compositions, sizes, shapes and surface properties are to be produced to meet different requirements[59].

In this review, we provide a general overview of different types, methods of synthesis, characterizations, properties and applications of nanotechnology in vaccine production, focusing the attention only on prophylactic nanovaccines used to prevent infectious diseases.

Different Types of Nanoparticles Used as Potential Vaccine Delivery Vehicles

There are several types of nanoparticles considered as possible delivers of vaccine antigens. Particularly, several researches has focused their attention on the development of nanoparticles-based synthetic delivery systems in the viral size range (20-200 nm) that protect antigen from degradation, enhance its presentation and facilitate its uptake by professional APCs[60-67]. The principal of these systems are virus-like particles, self-assembled proteins, micelles, liposomes, inorganic nanoparticles, and polymers.

Virus-like particles (VLPs)

Virus-like particles (VLPs) are non-infectious self-assembling nanoparticles formed by a structured protein capsid lacking of genetic mate-

rial, already used for development of viral vaccines[51,68,69]. VLPs are brilliant nanoparticles because they are equipped by an external viral casing, characterized by repetitive epitopes, immediately recognizes as a non-self-structure by immune system. Therefore, VLPs share with viruses the positive capacity to stimulate immune responses strongly but they do not have the harmful ability to induce infection. The naturally-mimicking size, the repetitive structural order, and a fast and effective processing of these nanoparticles leads to elicitation of rapid and long-term host immune responses even in the absence of adjuvant[68,70-72].

VLPs size normally ranges between 20-800 nm[28,73], they can derive from a number of viruses[68], and they can be produced with technologies using different cell systems, such as *Escherichia coli*, yeasts, Baculovirus, mammalian, plant and cell free systems[74,75] (Figure 1).

VLPs manufacturing proceeds through an "*in vivo*" methodology, with a first step in which the spontaneous assembly of viral capsid proteins occurs directly inside the expression cell vector. Then, a second step aims to purify the new formed particles from both adhered to the surface and incorporated cellular contaminants. Sometimes, to obtain well-purified VLPs, it is necessary to disassemble and, therefore, reassemble nanoparticles[74]. However, an emerging and recent approach proceeds through a cell-free *in vitro* assembly processing[76-78]. This process consists in the reversal of the traditional "assemble-then-purify" methodology. In particular, after expression, the capsid proteins are purified from contaminants and then reassembled "*in vitro*". In this way, there is no need to disassemble VLPs after their assembly into cell. However, further approaches of VLPs producing are available[28,51,79,80].

Already available VLPs-based vaccines consist of self-assembled viral protein particles deriving from virus towards which vaccine was produced. Moreover, VLPs are also able to carry a target antigen linked to the surface and belonging to an unrelated virus[81-83]. Many VLP-based vaccines are in clinical or pre-clinical trials, so we will assist surely to an increase in the number of these type of vaccines[28,51].

Hepatitis B VLP-Based Vaccine

The first VLP-based vaccine produced and commercialized in 1986 for human use was that against hepatitis B, containing the Hepatitis B surface antigen (HBsAg) produced in yeast *via* recombinant DNA technology. The recombinant HBsAg can form 22 nm spherical VLPs that are further adsorbed on an aluminum hydroxide gel[84]. These nanoparticles contains also host cell-derived lipids (30-50%) and about 70 molecules of S protein; intra- and intermolecular disulfide bonds stabilize the structure. The S protein contained in these yeast-derived nanoparticles is highly hydrophobic and not glycosylated, differently to naturally mammalian-derived HBsAg particles. Moreover, it is strongly associated with lipids and it is responsible for the antigenic capacities of HBsAg VLPs. Thus, these VLPs can be considered as lipoproteins formed by a well-organized and rather stiff lipid interface surrounding a hydrophobic and more fluid inner core. The protein components are absorbed in this lipid material and exhibits an outer part and one inserted into the inner core[85]. It was shown that this vaccine is able to stimulate the activation of CD4+ and CD8+ T cells[86].

Human Papillomavirus VLP-Based Vaccine

After the HBV vaccine, the second VLPs-based vaccine approved in 2006 for human use, was that against Human Papillomavirus (HPV)[87]. HPVs are a numerous group of oncogenic viruses

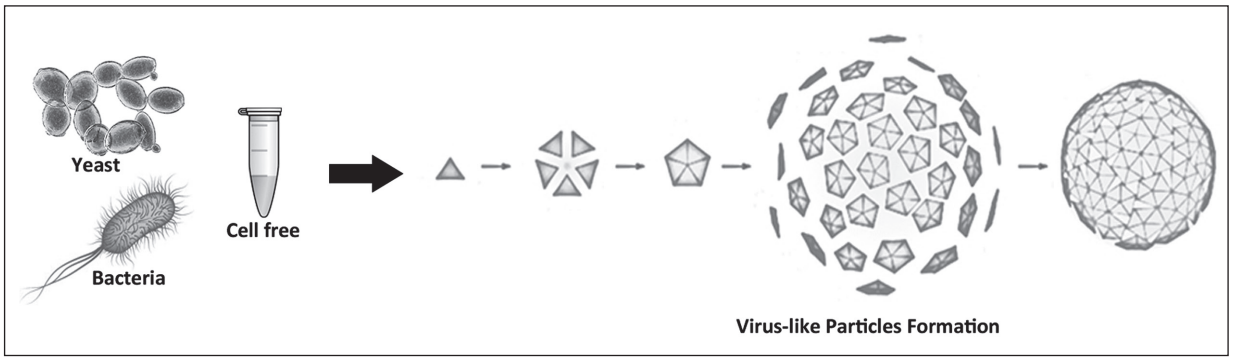

Figure 1. Manufacturing scheme of VLPs.

associated with both benign and malignant lesions of skin and mucous membranes. Nowadays, it is well known the role of these viruses in causing cervical cancers and several other types of malignancies, such as "typical" (anal, oropharyngeal, penile, vulvar, and vaginal) and atypical ones (prostate, bladder, rectal)[88-93]. Particularly, according to National Cancer Institute, HPVs cause virtually all cervical, 70% of oropharyngeal, 90% of anal, 75% of vaginal and 70% of vulvar cancers[94]. For these reasons, the increase of HPV vaccination coverage could remarkably reduce the burden of these types of malignancies. Particularly, the spread of HPV vaccination is potentially able to reduce cervical cancer incidence worldwide up to 90%[95,96]. In addition, reducing the need for screening and medical care (especially biopsies and invasive procedures), this practice can help to reduce healthcare costs and concerns related to follow-up procedures[97].

HPV vaccines are based on VLPs formed by assembly of 72 pentamers of L1 protein, the HPV major capsid protein, expressed in yeast in high amount through recombinant DNA technology, purified, and combined to make the vaccine[98]. To date, the Food and Drug Administration (FDA) has approved three different HPV vaccines: a tetravalent (2006), a bivalent (2009) and, very recently, a nonavalent vaccine (2014). All three vaccines are able to prevent infections caused by 16 and 18 HPV types, the two widespread high-risk HPVs (HR-HPVs) that cause about 70% of cervical cancers and a high percentage of some of the other HPV-related malignancies[99,100]. Tetravalent vaccine also prevents infections caused by 6 and 11 HPV types, which determine 90% of genital warts[101]. Finally, the new nonavalent vaccine prevents infection caused by the same four HPV types contained in previous vaccines with the addition of other five HR-HPVs (31, 33, 45, 52, and 58). These vaccines are adjuvant with aluminum hydroxide, AS04 and monophosphoryl lipid A (MPL) in bivalent, amorphous aluminum hydroxyphosphate sulfate (AAHS) in tetravalent and nonavalent[102]. The potential public health impact and cost-effectiveness of nonavalent HPV vaccine has been investigated by mathematical models in different efficacy, cost, and vaccine coverage settings. Particularly, Largeron et al[103] demonstrated that the universal vaccination with nonavalent HPV vaccine in Germany extended to boys would be associated after 100 years with a reduction of 24% in the incidence of cervical cancer, 30% and 14% of anal cancer in males and females respectively, and finally a million cases of genital warts avoided. Moreover, this strategy would be associated with an incremental cost-effectiveness ratio (ICER) of 22,987€ per QALY gained. Despite this important evidence, HPV vaccines have been associated to some alleged side effects that several scientific evidences have refused but that have slowed down its widespread diffusion[104].

Hepatitis E VLP-Based Vaccine

Hepatitis E virus (HEV) is an RNA virus belonging to the Hepeviridae family, normally causing a self-limited acute hepatitis (with a mortality rate <0.1% in healthy patients), and a chronic infection in immunocompromised patients. However, this virus can be highly pathogen if contracted during pregnancy, especially in the first trimester[105]. In the US it is estimated that the HEV seroprevalence is approximately 9% while in Europe the rate ranges between 5% and 20%[106-109].

Because the production of high amount of viruses using cell cultures is difficult, many experiments have been performed to obtain recombinant HEV VLPs[110,111]. In previous years, a variety of systems for the study of HEV have been developed. VLPs formed by the self-assembly of the core protein (ORF2) have been one of the first model used to study the structure of the virus, its entry process, and its vaccine potential. Indeed, the antigenic property of HEV core protein was clarified very early, and different approaches for the development of an effective HEV vaccine have been investigated. In a study conducted by Purcell et al[112], *Rhesus* monkeys inoculated with 1 or 10 μg doses of the 56 kDa HEV capsid protein were 100% protected from the hepatitis and 63% from infection after the first inoculation, when challenged with an infectious dose of 10^4 of HEV. In a subsequent phase 2, randomized, double-blind, placebo-controlled trial in Nepal enrolling 2000 participants, the same vaccine based on the 56 kDa capsid protein protected effectively from HEV[113].

A second approach is based on a protein named p239 (aa 368-606 of the capsid protein) recombinantly produced in bacteria and purified under denaturing conditions that maintain unaltered the antigenic epitopes[114,115]. Li et al[114] tested the p239 as vaccine candidate in *Rhesus* monkeys with a prime/boost approach of 5, 10, and 20 μg. The vaccinated monkeys were protected after challenge with HEV genotypes 1 and 4. The vaccine showed a 100% efficacy in protecting monkeys

from hepatitis and infection when a viral dose of 10^4 HEV was administered. The same percentage of efficacy against hepatitis was obtained by using a 10^7 viral dose. Using the same vaccine candidate, a randomized, controlled phase II clinical trial enrolling 457 adults, showed an encouraging 100% seroconversion rate in vaccinated subjects[116]. Finally, a large randomized, double-blind, placebo-controlled, single center phase III clinical trial was performed from August 2007 to June 2009 in China. This study enrolled 112,604 people, of which half received the p239 vaccine while the other half (placebo group) received a HBV vaccine in a three doses schedule. The results showed that the p239 vaccine was immunogenic with an efficacy of 100% and well tolerated[117]. After this trial, p239 vaccine was approved in China in 2012. Despite the positive results, the vaccine was not approved in other countries because of several critical points moved to the trial, among which not having enrolled people <16 years and >65 years of age, as well as the two principal risk groups represented by pregnant women and immunosuppressed patients.

The third vaccine candidate tested in clinical trials is a bacterially produced self-assembling core protein (aa 439-617 of the capsid protein) named p179, experimented first in rabbits to confirm its protective potential. The animals were inoculated with three different doses (20, 30, and 40μg) of p179 and they were protected from the infection by the production of high titers (10^3-10^4) of anti-HEV; however, some challenged rabbits showed viral shedding. After these evidences in animals, in 2017 a Chinese phase I trial using a p179 vaccine was carried out on 120 participants to study its efficacy and potential side effects. The vaccine resulted well tolerated and appeared to be safe[118].

Self-Assembled Proteins

Similar to VLPs, some supramolecular structures have been formed from self-assembled proteins and, recently, self-assembling systems carrying higher levels of antigen have studied for the preparation of nanoparticle-based vaccines[119]. The principal difference with VLPs is that self-assembled proteins are not be formed by viral components.

Self-assembled peptide nanoparticles (SAPNs) have been studied as drug delivery systems[120,121]. These nanoparticles consist of two helical coiled-coil domains connected by a short linker region into a single peptide chain. One helix forms a trimeric coiled coil while the other forms a pentameric coiled coil[122]. These nanostructures have a self-assembling tendency in aqueous medium due to their physiochemical features. Chung et al[123] reported that amphiphilic peptides show no evidence of toxicity in liver, spleen, kidney, bladder, bowel, lung, and heart. It was demonstrated that APCs are able to uptake APNPs and to present antigen epitopes more effectively compared to naked antigens[124]. In 2012, Wahome et al[125] carried out an *in vivo* experiment using APNPs exhibiting on surface two important HIV epitopes, 4E10 and 2F5, which are critical targets in the production of neutralizing antibodies. The results showed that these nanostructures induced potent immune responses against these crucial HIV epitopes.

Another system is that based on the use of ferritin, a protein able to self-assemble into nearly spherical 10 nm structure. Kanekiyo et al[126] fused influenza virus haemagglutinin (HA) to ferritin to obtain a recombined protein that spontaneously assemble to form an octahedrally-symmetric particle showing an 8 trimeric HA spikes. The authors demonstrated that these nanovaccine determine a higher immune response than normal influenza vaccine. Moreover, Lee et al[127] used engineered human ferritin nanoparticles to deliver tumor antigens to lymph nodes and to develop a possible therapeutic vaccine in cancer treatment.

Another self-assembling protein-based system is that based on the use of the Major Vault Protein (MVP), an intracellular self-assembling protein ubiquitously present[128]. Particularly, ninety-six units of MVP can self-assemble to form a barrel-shaped vault nanoparticle, with a size of about 40 nm of width and 70 nm of length. Antigens are attached with an interaction domain of the protein and, then, packaged inside the self-assembling vault nanoparticles. Champion et al[129] used these nanostructures to deliver the Major Outer Membrane Protein (MOMP) of *Chlamydia muridarum* and stimulate mucosal immunity.

Micelles

Micelles are nanoparticles formed by spontaneous self-assembly in water of single amphiphilic (hydrophobic/hydrophilic) molecules with the subsequent formation of two distinct portions, a hydrophobic core and a hydrophilic surface named "corona"[130]. Historically studied and used as drug delivery vehicles, through a process of encapsulation of hydrophobic drugs in the core[131], micellar nanoparticles have been also considered in recent years as adjuvants for vaccine delivery.

Thanks to their chemical characteristics, micelles have a double way to transport vaccine candidates. First, antigens can be covalently associated to the hydrophilic corona. This approach has been used to associate HIV antigens to adjuvant-loaded polymer-based micelles obtaining an effective antigen able to strongly stimulate the APCs *in vitro*[132]. A second way is the production of peptide amphiphiles (PAs) a class of peptide-based biomaterials consisting of peptides linked to hydrophobic alkyl tails, which self-assemble in aqueous medium to form micellar structures[133]. Many studies have used PA micelles as vaccine candidates to improve peptide immunogenicity[134-136]. Several PA micelle characteristics, such as size, shape, and composition, play a crucial role to increase the capacity of these nanoparticles to determine immune responses[135-138]. For example, it has been demonstrated that cylindrical or spherical-shaped PA micelles contain the right amounts of antigen and amphiphilic adjuvants, and are able to efficiently reach the lymph nodes[133,136,139]. In two different researches[135,136], PAs were used as vaccine candidates to induce a stronger antigen-specific IgG1 antibody response against group A streptococcus in mice after subcutaneous inoculation.

Thanks to their small size (generally <100 nm) micelles are able to ease the antigen delivery to APCs, especially dendritic cells (DCs) present in large amount in lymph nodes compared to periphery. Indeed, these nanoparticles are not only capable to interact with DCs in the injection site, but they travel through lymphatic system up to lymph nodes where promote germinal center formation[140]. Micelles can easily acquire appropriate surface properties through the right choice of hydrophilic segments of the "corona". Moreover, through appropriate design of the hydrophobic and hydrophilic portions, a number of additional immunostimulatory molecules (such as, for example, Toll Like Receptor ligands or mannose receptor ligands) can be easily integrated in these nanoparticles to induce an enhanced activation of the DCs, which play an essential role in the onset of immune responses.

Finally, micelles provide a unique platform to enhance the immunostimulating capacity of the antigen because, by choosing specific hydrophobic molecules, micelle can easily determine endosome disruption with the subsequent release of the peptide vaccines in the cytosol of cells, antigenic presentation and strong cytotoxic T cell responses *in vivo*[141].

Liposomes

Liposomes are spherical vesicles formed by a double lipid layer surrounding an aqueous core that have received a lot of attention in the development of vaccines[142]. These nanostructures can be distinguished by number of layers in unilamellar, multilamellar or polymerized types[143]. Liposomes are used as drug delivery system, transporters of genes, and are particularly useful for applications in biomedicine because of their immunogenic power[144]. In liposome, antigen may be transported in the aqueous core, embedded in the lipid bilayer or absorbed in the surface. Antigen incorporation can be secondary to covalent lipid linking (at pre- or post-vesicle formation), non-covalent surface attachment, encapsulation, electrostatic interactions (with lipids of opposite charge) or surface adsorption. Liposome acts like an adjuvant, strengthening immune responses of the vaccine and increasing its efficacy. Liposomal vaccines are characterized by low reactogenicity, they are biodegradable and flexible. Many intrinsic features need to be considered when designing liposomal vaccines (Figure 2).

Previous reviews have investigated the different factors that could play a role in the functions and efficacy of liposomes as vaccine candidates[145,146]. These factors include liposome size, lamellarity, surface charge, fluidity of the bilayer, and the addition of immunostimulatory lipids. Regarding size, it has been showed that vesicles with a diameter >2 μm displaying a tuberculosis (TB) antigen, are more effective to induce cell proliferation and low IL-10 production, compared to vesicles of 500 nm diameter[147]. The lamellarity nature of the liposomal vesicle could also influence immune responses. Beck et al[148] studied the efficacy of a recombinant HIV protein named CN54 gp140 using small unilamellar (SUV) and large multilamellar vesicles (MLV). The liposomes contained different mixtures of monophosphoryl lipid A (MPLA) and lipids, with or without the addition of saponin QS21. The authors showed that SUVs without QS21 were able to induce a TH2 cell-mediated response to the antigen secondary to a high antibody production, contrary to MLVs. Surface charge of liposomes plays a key role for correct vaccine design because it can heavily affect the interaction of antigen with liposome (e.g. anionic antigens better interact with cationic lipids) and the subsequent antigen loading. Hussain et al[149] showed that the change of the cationic lipid dimethyl dioctadecyl ammonium (DDA) with the neutral distearoyl-sn-glycero-3-phosphocholine

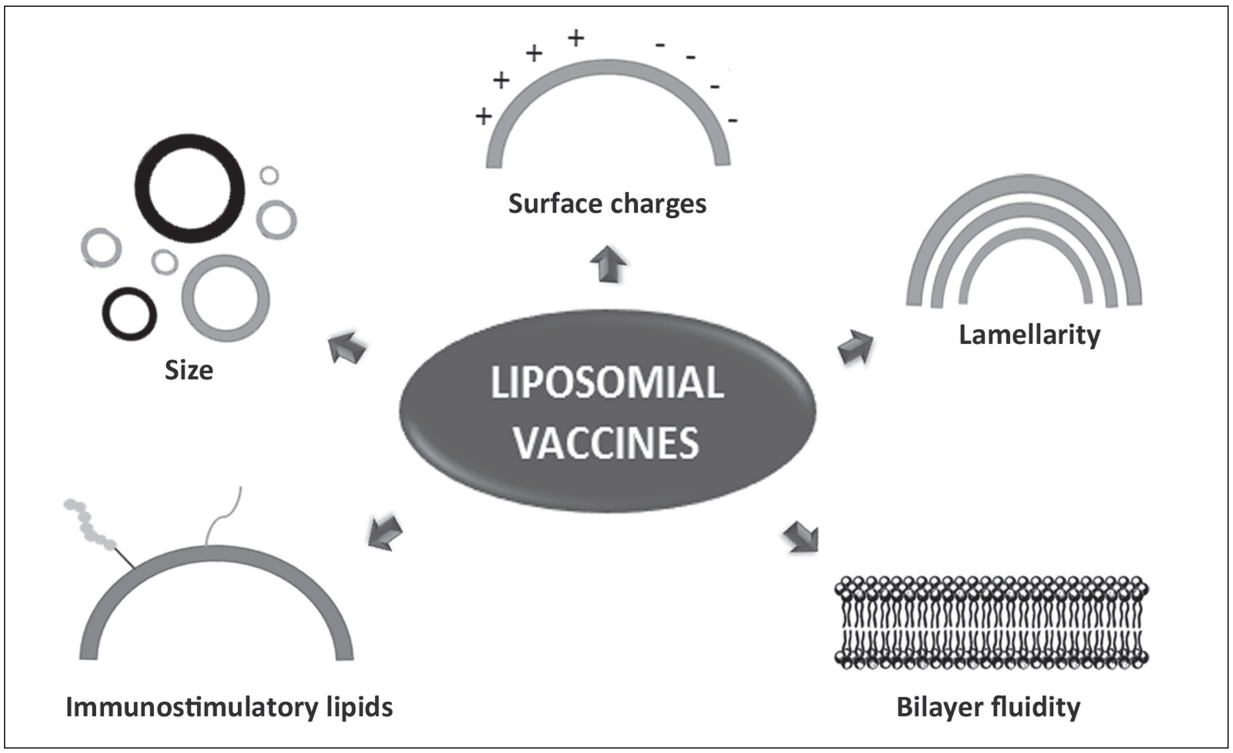

Figure 2. Physicochemical and morphological factors to consider in liposomal vaccine design.

(DSPC) decreased the amount of the TB recombinant antigen H56 from 84% to 15%. Moreover, Joseph et al[150] studied the efficacy of an intranasal influenza vaccine model liposomal-based containing haemagglutinin neuraminidase (HN) antigen linked to polycationic sphingolipid ceramide carbamoyl-spermine (CCS) or other monocationic, neutral and anionic lipids. The authors showed that neutral and anionic lipid-based preparations were not effective after intranasal administration in a murine model while two monocationic-based liposomal formulations (containing 1,2-dimyristoyl-3-trimethylammonium-propane, DMTAP and 1,2-dioleoyl-3-trimethylammonium-propane, DOTAP) induced strong local and systemic immune responses (both TH1 and TH2). Finally, many published papers have investigated and described the importance of bilayer fluidity in the design of effective liposomal vaccines. Kaur et al[151] showed how cholesterol influences the bilayer fluidity and, consequently, the antigenic capacity of liposome-based vaccine. Particularly, less IgG production was observed when cholesterol increased in DDA:TDB (trehalose dibehenate) liposomal formulations used for immunized mice. This effect might be subsequent to the loss of antigen in liposomes containing high cholesterol concentration and, thus, more fluid. Moreover, the level of IFN-γ was more elevated when cholesterol was not present in the lipid bilayer.

Several liposome-based vaccines against viral, bacterial, fungal and parasitic diseases have been considered for human use. Particularly viral liposomes, named "virosomes", have been extensively studied. Virosomes are liposomes that exhibit viral proteins on their surface that allow them to effectively merge with the membrane of target cells[152]. More recently, a study was carried out to investigate immune responses to virosomes and liposomes and their internalization in a triple culture model of human respiratory tract. Particularly, the used culture model was formed by cells derived from the epithelial cell line 16HBE that were grown with monocyte-derived macrophages (MDMs) and DCs. Cells were exposed to both the nanoparticles to evaluate the immune responses. Virosomes were prepared with solubilized influenza A/Brisbane/59/2007 H1N1 membrane proteins included in the neutral lipids 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine, POPE. Liposomes were formed by the same neutral lipids, but they did not contain any

influenza soluble membrane proteins. The results showed that virosomes were internalized more efficiently by all cell types in mono- and co-cultures, with MDMs and DCs presenting the highest capacity of internalization observed with flow cytometry and laser scanning microscopy. Moreover, DCs were discreetly activated by liposomes and virosomes in monocultures, and produced elevated levels of cytokine such as IL-1β and IL-8 in co-cultures. Finally, virosomes were internalized at higher levels in epithelial cells compared to liposomes[153].

Inorganic Nanoparticles

Inorganic nanoparticles are nanocarriers consisting of an inorganic solid nucleus, which have been used in vaccinology as both adjuvants and carriers of antigens to increase immune responses. The positive aspects of these nanoparticles are their stiff structure and controllable synthesis; the negative one is the frequent non-biodegradability[154,155]. The most studied inorganic nanoparticles as vaccine platforms are carbon, gold, silica and calcium nanoparticles.

Carbon Nanoparticles

Many studies have been conducted to evaluate the use of carbon nanomaterials as adjuvants or carriers for different kinds of vaccines especially because they are internalized into a wide variety of cell types[156-160]. Many structural and physical features of these nanosystems effect the capacity of carrying antigens and stimulating immune responses, among which their surface modifications[161].

Among carbon nanoparticles, carbon nanotubes (CNTs) have received a great attention because of their exceptional features that make them usable in many industrial fields. CNTs are engineered nanoparticles formed by a thick sheet of graphene that rolls up to form a hollow cylinder named single-walled CNT (SWCNT). If more graphene sheets are present, the CNT it will be formed by concentric multiple sheets (from 2 to 50, linked together by van der Waals interactions); this CNT is named multi-walled CNT (MWCNT)[162] (Figure 3).

Their lengths vary from 100 to 1000 nm and their diameter range between 0.4-2 nm and 2-100

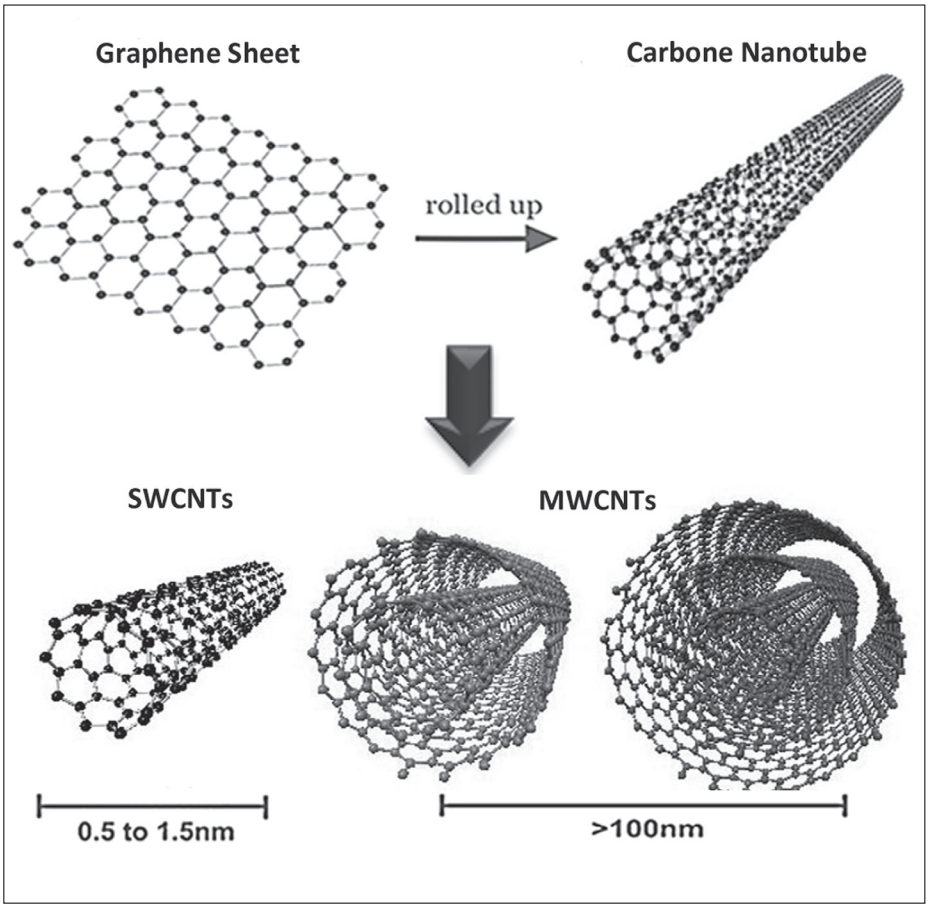

Figure 3. Structure and types of Carbon nanotubes (CNTs).

nm for SWCNTs and MWCNTs, respectively[163]. Some of the first investigations using CNTs as carriers for vaccine candidates were carried out by Pantarotto et al[156,157] in 2003. The study design consisted in the covalent attachment of hand-foot-and-mouth disease viral (HFMDV) envelope peptides to CNTs. The results showed that the epitope structure maintained its immunogenic capacity following the attachment to CNTs. Indeed, the CNT-viral protein complexes were capable of eliciting specific neutralizing IgG immune responses in animal models[156,157].

In 2009, Zeinali et al[164] investigated the immunological and cytotoxicological aspects of tuberculin purified protein derivative (PPD) conjugated to SWCNTs. In this study, male BALB/c mice were challenged with bacillus Calmette-Guérin (BCG), PPD, SWCNT-PPD conjugate and PPD in complete Freund's adjuvant (CFA). The induction of cellular immune responses was analyzed by measuring the levels of Th1 (IFN-gamma and IL-12) and Th2 cytokines (IL-10 and IL-5). The authors found that the immunization with PPD or PPD in CFA induced a Th2 cytokine response while immunization with BCG determined a mixed Th1/Th2 cytokine response. Conversely, SWCNT-PPD generated preferentially a Th1-type cytokine response without potential cytotoxic effects.

In 2016, Hassan et al[165] demonstrated that CNTs' surface properties affects their potency as vaccine nanocarriers *in vitro* and *in vivo*. Particularly, the authors used MWCNTs-antigen conjugates, modifying their length and surface charge and determining the variations in the internalization by DCs. The results showed that the reduction in charge negativity of MWCNT-antigen conjugates increase its cellular uptake and thus the stimulation of immune responses.

Finally, in 2016, Calegari et al[166] carried out a study about the use of MWCNTs as carriers of viral DNA for the stimulation of immune responses against Dengue fever. The study aimed to assess the ability of carboxylated and not carboxylated MWCNTs in increasing the expression of the tetravalent vaccine candidate (TVC) plasmid vector for dengue virus *in vitro* using Vero cells and carrying a parallel *in vivo* challenge through the intramuscular route in animals to evaluate the immunological responses. The results showed the ability of MWCNTs to penetrate target cells reaching both cytoplasm and cell nucleus and to increase the amount of B-cells producing antibodies in comparison to naked plasmid.

Gold Nanoparticles

Gold nanoparticles (GNs) are particles of different shapes (rod, spherical, cubic, etc.), sizing from 1 nm to 150 nm, and whose surface can be modified with the attachment of carbohydrates residues. Both size and shape are the most important factors affecting the immunity properties of gold nanoparticles, which lead to an enhancement in immune response *via* different cytokine pathways[167,168]. Safari et al[167] used gold glyconanoparticles displaying a synthetic tetrasaccharide epitope related to *Streptococcus pneumoniae* type 14 capsular polysaccharide (Pn14PS) complexed with the ovalbumin 323-339 peptide (OVA(323-339)) and D-glucose to induce specific immune responses in mice. The authors found that the used nanoplatform generated a Th cell activation confirmed by the production of specific anti-Pn14PS IgG antibodies and elevated cytokine levels. Moreover, the produced antibodies promoted the phagocytosis of bacteria by human leukocytes, indicating the efficacy of the induced immune response.

GNs can also be used as carriers of viral antigens, such as influenza, or as a DNA vaccine adjuvant for HIV. The AIDS pandemic has determined remarkable efforts to produce effective HIV vaccines. Indeed, the introduction of the highly active antiretroviral therapy (HAART) in the treatment of HIV infection has changed the natural history of this infection, remarkably reducing the mortality rate but turning it into a chronic condition burdened by cardiovascular, neurological, renal and bone diseases and malignancies[169-177]. However, the treatment is not able to eliminate the virus that persists in latency for all life of infected subjects. As a result, HIV vaccines have been extensively studied but have failed, especially due to concerns of safety or efficacy in humans. In 2012, Xu et al[178] used a surface-engineered gold nanorods (NRs) used as carriers and adjuvant of a promising DNA vaccine for HIV treatment. Particularly, the authors used three different modified gold NRs carrying, on their surface, cetyltrimethylammonium bromide (CTAB), poly(diallydimethylammonium chloride) (PDDAC), and polyethyleneimine (PEI) respectively, and they found that PDDAC- or PEI-modified gold NRs significantly activated APCs, promoting cellular and humoral immunity, as well as T cell proliferation, compared to naked HIV-1 Env plasmid DNA treatment *in vivo*.

Even in 2012, Gregory et al[179] used the conjugation of *Yersinia pestis* F1-antigen to gold na-

noparticles to improve antigen immunogenicity, compared to normal alumin adjuvants (alhidrogel). Particularly, the results showed that gold NPs-F1 in alhydrogel elicited the greatest IgG antibody response against the antigen, compared to gold NPs-F1 in PBS or unconjugated F1-antigen in PBS or alhydrogel. Moreover, compared with unconjugated F1-antigen, the IgG2a response was increased in mice challenged with gold NPs-F1 in PBS ($p<0.05$) but not in mice immunised with gold NPs-F1 in alhydrogel.

Finally, in 2015 Kumar et al[180] investigated the immunological properties of GNS of different shapes and size, evaluating their capacity to deliver the codon-harmonized recombinant *Plasmodium falciparum* surface protein (CHrPfs25) antigen expressed in *Escherichia coli*, and the induction of specific neutralizing antibodies compared to same antigen in presence of conventional adjuvant alum. The results showed that the vaccine platform used elicited strong production of neutralizing antibodies, and suggested that GNs can be considered as promising vaccine delivery vehicles to enhance the immunogenicity of vaccine antigens.

Silica Nanoparticles

Silica nanoparticles (SiNPs) are brilliant nanocarriers used for many applications such as vaccine delivery and selective tumor targeting[181]. Chemical modification of SiNPs can be obtained adding on their surface abundant silanol groups, which can improve cell recognition, interaction with cells, cellular uptake and absorption of specific biomolecules[182]. The most interesting aspect regarding vaccine use of SiNPs is that antigenic conjugation was not required to have an immunostimulatory effect[183]. It is possible to produce high efficiency nanocarriers of 50-200 nm, named mesoporous silica nanoparticles (MSNs), which can work as both nanocarriers and adjuvants for an effective delivery of antigens[184,185]. Very recently, a new generation antigen carriers and adjuvants named silica vesicles (SVs) are used against the bovine viral diarrhoea virus (BVDV), which is disseminated in cattle industry worldwide. These nanoparticles showed a high charging capacity and controlled release of the codon-optimized E2 (oE2) protein, which is the major antigen of the BVDV[186]. However, the most critical concern about the application of SiNPs in biomedical uses is their toxicity derived from the reducing agents and stabilizers used for their synthesis.

Calcium Nanoparticles

Calcium phosphate nanoparticles (CaPNPs) are formed by mixing sodium citrate, calcium chloride and dibasic sodium phosphate. They are promising candidates for vaccine applications especially because they are biodegradable and their characteristic surface structure allows simple modifications[187]. Once phagocytized, CaPNPs melt into cytoplasm when the pH values change from neutral to acidic during cellular uptake, releasing their encapsulated materials. Therefore, CaPNPs can naturally release their content without any external help, which makes them perfect antigens carrier. Furthermore, CaPNPs are stable under physiological conditions and have an excellent biocompatibility, being easily biodegraded[188]. A previous study showed that surface-modified CaP-NPs of 80 nm size could carry genes in liver protecting the inner DNA from external DNase both *in vitro* and *in vivo*[189]. Previous investigations[187,190] have proved that CaP-NPs can be used as adjuvants for DNA vaccines and mucosal immunity. Finally, previous *in vivo* experiments have showed that micrometer-sized CaP aggregates could elicit high titers of neutralizing antibody, and have proved high more potent protection against viral infections than the aluminium adjuvant[191].

Polymers

Polymer-based nanoparticles are solid structures with a size of 10-500 nm characterized by high biological safety and good biodegradability, in which bioactive materials can be internalized through dissolving, packaging, or surface adsorption[192]. These nanomaterials have studied as new vehicle for transport and controlled release of drugs and/or antigens because, inside them, they are protected from degradation. Polymer-based nanomaterials are distinguished in natural and synthetic. Among the first, chitosan and γ-polyglutamic acid (γ-PGA) are the most important while, among the second, polylactic acid (PLA), and poly(lactic-glycolic acid) (PLGA), are the principal[193].

Chitosan

Chitosan (CS) is a natural polyaminosaccharide derived from deacetylation of chitin, a molecule formed by polymeration of D-glucosamine and N-acetyl-D-glucosamine monomers bound with β-(1,4) links. This polymer is easy obtained from the exoskeletons of crustaceans and squids and from fungal cellular wall. CS nanoparticles

are non-toxic and they can be easily modified in shape and size. These features make them suitable for the use in new generations vaccines. Particularly, CS can link negative charged protein or plasmid DNA through electrostatic interaction, protecting them from degradation[194]. Through various chemical modifications, such as acylation, alkylation, sulfation, hydroxylation, quaternization, and carboxymethylation, it is possible to obtain CS derivatives. These compounds have received a great attention especially for their antimicrobial and repairing activity[195,196]. Moreover, CS has become an interesting vaccine adjuvant and carrier. In 2010, Prego et al[197] used CS-based nanoparticles to elicit immune responses against HBV infection. The study showed the adjuvant capacity of the used nanovaccine platform, highlighted by the induction of important seroprotection rates (up to 5,500 mIU/ml, values 9-fold higher than the conventional alum-adsorbed vaccine) after intramuscular administration. Li et al[198] reviewed the literature regarding the use of CS as delivery carrier of vaccine antigens and drugs. Particularly, the authors included sixty-three papers of which thirty-five showed the advantage in the use of CS nanoparticles applied as vaccine carrier.

Gamma Poly-Glutamic Acid

Gamma poly-glutamic acid (γ-PGA) nanoparticles are negatively charged polyelectrolytes used as both antigen carrier systems and vaccine adjuvants to induce strong humoral and cellular immune responses. γ-PGA nanoparticles are water soluble and they exhibit intrinsic immunostimulatory properties because they are capable to active TLR4 and MyD88 signaling pathways without any external adjuvant[199,200]. γ-PGA has been also used complexed with CS as suitable material for preparation of nanogel (NG). CS-γ-PGA nanogels are an ideal choice for vaccine carriers to improve the immune effect of loaded antigen. As demonstrated by Wang et al[201], nanogels not only act as carriers of antigen vaccines, but also exhibit adjuvant effects, especially in promoting cellular immune responses. The authors investigated the influence of nanogel carriers on the prophylactic effect of a loaded HBsAg vaccine. Particularly, positively charged HBsAg NG (HBsAg NG (+)) and negatively charged HBsAg NG (HBsAg NG (−)) were tested, and the efficiency of this HBsAg NG in protection against pAAV/HBV1.2 plasmid challenge was evaluated. The results showed that a single dose of a HBsAg NG (+) could induce both humoral immunity and cellular immunity, and provided long-term immune protection against HBV.

Poly-Lactic-Co-Glycolic Acid

Poly-lactic-co-glycolic acid (PLGA) is a biodegradable polymer with good biocompatibility and degradability, widely used in the production of microspheres, microcapsules, nanoparticles, pellets, implants, and film preparation. Recently, it was studied as drug carrier, especially chemotherapy, because drugs encapsulated into these nanoparticles or microspheres easily reach tumor site with a reduction of the adverse reaction; they also prolong the lifetime of drugs *in vivo*, and improve their pharmacokinetic properties[202]. Very recently a vaccine against *Campylobacter jejuni* PLGA-based was used to induce protective immunity in poultry. *Campylobacter* spp are commensal microorganisms of the gastrointestinal tract of many wild and farm animals (especially birds such as poultry and ducks), that have long been recognized among the most common responsible agents of enteritis and gastroenteritis in humans, both adults and in pediatric patients[203]. The study aimed to investigate the protective effects of soluble and PLGA-encapsulated oligodeoxynucleotides (ODN) containing unmethylated CpG motifs (E-CpG ODN) as well as *C. jejuni* lysate as a multi-antigen vaccine against colonization with *C. jejuni*. The results showed that oral administration of a low (5 μg) or high (50 μg) dose of CpG determined a significant reduction in cecal *C. jejuni* colonization[204].

Conclusions

New generation vaccinology has greatly benefited and it will benefit from use of nanoparticles used as delivery vehicles and/or immune potentiators. Nanoparticles are able to improve not only antigen uptake from APCs but also immunogenicity and slow release of antigens. A wide range of these particles varying in size, composition, and surface charge, are today available and they are capable of modulate bio-distribution, cellular trafficking, and overall immune responses. Moreover, most nanoparticles are characterized by biodegradability, biocompatibility and minimal toxicity and, therefore, they can offer safe and effective alternatives to traditional vaccines. However, many aspects still remain to be considered and investigated to increase the application

of these systems in vaccine as well as in drug delivery, including a lack of understanding of how their physical properties affect biodistribution and targeting, and how these properties influence their interactions with biological system at all levels.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Salmon DA, Dudley MZ, Glanz JM, Omer SB. Vaccine hesitancy: causes, consequences, and a call to action. Vaccine 2015; 33: D66-71. doi: 10.1016/j.vaccine.2015.09.035.
2) Facciolà A, Visalli G, Orlando A, Bertuccio MP, Spataro P, Squeri R, Picerno I, Di Pietro A. Vaccine hesitancy: an overview on parents' opinions about vaccination and possible reasons of vaccine refusal. J Public Health Res 2019; 8: 1436. doi: 10.4081/jphr.2019.
3) Yakovenko ML, Gmyl AP, Ivanova OE, Eremeeva TP, Ivanov AP, Prostova MA, Baykova OY, Isaeva OV, Lipskaya GY, Shakaryan AK, Kew OM, Deshpande JM, Agol VI. The 2010 outbreak of poliomyelitis in Tajikistan: epidemiology and lessons learnt. Euro Surveill 2014; 19: 20706.
4) Genovese C, La Fauci V, Costa GB, Buda A, Nucera S,Antonuccio GM, Alessi V, Carnuccio S, Cristiano P, Laudani N, Spataro P, Di Pietro A, Squeri A, Squeri R. A potential outbreak of measles and chickenpox among healthcare workers in a university hospital. EuroMed Biomed J 2019; 14: 45-48.
5) Palamara MA, Visalli G, Picerno I, DI Pietro A, Puglisi G, Marano F, D'Andrea G, Facciolà A. Measles outbreak from February to August 2017 in Messina, Italy. J Prev Med Hyg 2018; 59: E8-E13. doi: 10.15167/2421-4248/jpmh2018.59.1.853.
6) Lo Giudice D, Capua A, La Fauci V, Squeri R, Grillo OC, Calimeri S. Congenital rubella syndrome and immunity status of immigrant women living in southern Italy: a cross-sectional, seroepidemiological investigation. Travel Med Infect Dis 2014; 12: 253-257. doi: 10.1016/j.tmaid.2014.01.003.
7) Calimeri S, Capua A, La Fauci V, Squeri R, Grillo OC, Lo Giudice D. Prevalence of serum anti-rubella virus antibodies among pregnant women in southern Italy. Int J Gynaecol Obstet 2012; 116: 211-213. doi: 10.1016/j.ijgo.2011.10.029.
8) Facciolà A, Squeri R, Genovese C, Alessi V, La Fauci V. Perception of rubella risk in pregnancy: an epidemiological survey on a sample of pregnant women. Ann Ig 2019; 31: 65-71. doi: 10.7416/ai.2019.227.
9) La Fauci V, Squeri R, Genovese C, Alessi V, Facciolà A. The 'Dangerous Cocktail': an epidemiological survey on the attitude of a population of pregnant women towards some pregnancy risk factors. J Obstet Gynaecol 2019; Article in press.
10) La Fauci V, Riso R, Facciolà A, Ceccio C, Lo Giudice D, Calimeri S, Squeri R. Response to anti-HBV vaccine and 10-year follow-up of antibody levels in healthcare workers. Public Health 2016; 139: 198-202. doi: 10.1016/j.puhe.2016.08.007.
11) Squeri R, Riso R, Facciolà A, Genovese C, Palamara MA, Ceccio C, La Fauci V. Management of two influenza vaccination campaign in health care workers of a university hospital in the south Italy. Ann Ig 2017; 29: 223-231. doi: 10.7416/ai.2017.2150.
12) Gualano MR, Bert F, Voglino G, Buttinelli E, D'Errico MM, De Waure C, Di Giovanni P, Fantini MP, Giuliani AR, Marranzano M, Masanotti G, Massimi A, Nante N, Pennino F, Squeri R, Stefanati A, Signorelli C, Siliquini R; Collaborating Group. Attitudes towards compulsory vaccination in Italy: results from the NAVIDAD multicentre study. Vaccine 2018; 36: 3368-3374. doi: 10.1016/j.vaccine.2018.04.029.
13) Genovese C, Picerno IAM, Trimarchi G, Cannavò G, Egitto G, Cosenza B, Merlina V, Icardi G, Panatto D, Amicizia D, Orsi A, Colosio C, Marsili C, Lari C, Palamara MAR, Vitale F, Casuccio A, Costantino C, Azara A, Castiglia P, Bianco A, Currà A, Gabutti G, Stefanati A, Sandri F, Florescu C, Marranzano M, Giorgianni G, Fiore V, Platania A, Torre I, Cappuccio A, Guillari A, Fabiani L, Giuliani AR, Appetiti A, Fauci V, Squeri A, Ragusa R, Squeri R. Vaccination coverage in healthcare workers: a multicenter cross-sectional study in Italy. J Prev Med Hyg 2019; 60: E12-E17. doi: 10.15167/2421-4248/jpmh2019.60.1.1097.
14) Ferrera G, Squeri R, Genovese C. The evolution of vaccines for early childhood: the MMRV. Ann Ig 2018; 30: 33-37. doi: 10.7416/ai.2018.2232.
15) Oberg AL, Kennedy RB, Li P, Ovsyannikova IG, Poland GA. Systems biology approaches to new vaccine development. Curr Opin Immunol 2011; 23: 436-443. doi: 10.1016/j.coi.2011.04.005.
16) Rappuoli R, Mandl CW, Black S, De Gregorio E. Vaccines for the twenty-first century society. Nat Rev Immunol 2011; 11: 865-872. doi: 10.1038/nri3085.
17) Almeida JP, Lin AY, Langsner RJ, Eckels P, Foster AE, Drezek RA. In vivo immune cell distribution of gold nanoparticles in naïve and tumor bearing mice. Small 2014; 10: 812-819. doi: 10.1002/smll.201301998.
18) Yang YW, Luo WH. Cellular biodistribution of polymeric nanoparticles in the immune system. J Control Release 2016; 227: 82-93. doi: 10.1016/j.jconrel.2016.02.011.
19) Caselli E, Brusaferro S, Coccagna M, Arnoldo L, Berloco F, Antonioli P, Tarricone R, Pelissero G, Nola S, La Fauci V, Conte A, Tognon L, Villone G, Trua N, Mazzacane S; SAN-ICA Study Group. Reducing healthcare-associated infections incidence by a probiotic-based sanitation system: a multicentre,

prospective, intervention study. PLoS One 2018; 13: e0199616. doi: 10.1371/journal.pone.0199616.

20) Saunders SA. Current practicality of nanotechnology in dentistry. Part 1: focus on nanocomposite restoratives and biomimetics. Clin Cosmet Investig Dent 2009; 1: 47-61.

21) Drexler EK. Nanosystems: molecular machinery, manufacturing, and computation. New York, NY, USA. ISBN:0-471-57518-6, John Wiley & Sons, Inc., 1992.

22) Hulla JE, Sahu SC, Hayes AW. Nanotechnology: history and future. Hum Exp Toxicol 2015; 34: 1318-1321. doi: 10.1177/0960327115603588.

23) Moghimi SM, Hunter AC, Murray JC. Nanomedicine: current status and future prospects. FASEB J 2005; 19: 311-330.

24) Couvreur P, Vauthier C. Nanotechnology: intelligent design to treat complex disease. Pharm Res 2006; 23: 1417-1450.

25) Treuel L, Jiang X, Nienhaus GU. New views on cellular uptake and trafficking of manufactured nanoparticles. J R Soc Interface 2013; 10: 20120939. doi: 10.1098/rsif.2012.0939.

26) Maurer P, Jennings GT, Willers J, Rohner F, Lindman Y, Roubicek K, Renner WA, Müller P, Bachmann MF. A therapeutic vaccine for nicotine dependence: preclinical efficacy, and phase I safety and immunogenicity. Eur J Immunol 2005; 35: 2031-2040.

27) Dobrovolskaia MA, McNeil SE. Immunological properties of engineered nanomaterials. Nat Nanotechnol 2007; 2: 469-478. doi: 10.1038/nnano.2007.223.

28) Roldão A, Mellado MCM, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. Expert Rev Vaccines 2010; 9: 1149-1176. doi: 10.1586/erv.10.115.

29) Yu C, Qian L, Uttamchandani M, Li L, Yao SQ. Single-vehicular delivery of antagomir and small molecules to inhibit mir-122 function in hepatocellular carcinoma cells by using "smart" mesoporous silica nanoparticles. Angew Chem Int Ed Engl 2015; 54: 10574-10578. doi: 10.1002/anie.201504913.

30) Yu Z, Yan B, Gao L, Dong C, Zhong J, D Ortenzio M, Nguyen B, Seong Lee S, Hu X, Liang F. Targeted delivery of bleomycin: a comprehensive anticancer review. Curr Cancer Drug Targets 2016; 16: 509-521.

31) Yu Z, Cai Z, Chen Q, Liu M, Ye L, Ren J, Liao W, Liu S. Engineering β-sheet peptide assemblies for biomedical applications. Biomater Sci 2016; 4: 365-374. doi: 10.1039/c5bm00472a.

32) Fang T, Dong Y, Zhang X, Xie K, Lin L, Wang H. Integrating a novel SN38 prodrug into the PEGylated liposomal system as a robust platform for efficient cancer therapy in solid tumors. Int J Pharm 2016; 512: 39-48. doi: 10.1016/j.ijpharm.2016.08.036.

33) Iannazzo D, Pistone A, Salamò M, Galvagno S, Romeo R, Giofré SV, Branca C, Visalli G, Di Pietro A. Graphene quantum dots for cancer targeted drug delivery. Int J Pharm 2017; 518: 185-192. doi: 10.1016/j.ijpharm.2016.12.060.

34) Iannazzo D, Pistone A, Celesti C, Triolo C, Patané S, Giofré SV, Romeo R, Ziccarelli I, Mancuso R, Gabriele B, Visalli G, Facciolà A, Di Pietro A. A smart nanovector for cancer targeted drug delivery based on graphene quantum dots. Nanomaterials (Basel) 2019; 9. pii: E282. doi: 10.3390/nano9020282.

35) Zoroddu MA, Medici S, Ledda A, Nurchi VM, Lachowicz JI, Peana M. Toxicity of nanoparticles. Curr Med Chem 2014; 21: 3837-3853.

36) Sarkar A, Ghosh M, Sil PC. Nanotoxicity: oxidative stress mediated toxicity of metal and metal oxide nanoparticles. J Nanosci Nanotechnol 2014; 14: 730-743.

37) Visalli G, Bertuccio MP, Iannazzo D, Piperno A, Pistone A, Di Pietro A. Toxicological assessment of multi-walled carbon nanotubes on A549 human lung epithelial cells. Toxicol In Vitro 2015; 29: 352-362. doi: 10.1016/j.tiv.2014.12.004.

38) Huang YW, Cambre M, Lee HJ. The toxicity of nanoparticles depends on multiple molecular and physicochemical mechanisms. Int J Mol Sci 2017; 18. pii: E2702. doi: 10.3390/ijms18122702.

39) Visalli G, Currò M, Iannazzo D, Pistone A, Pruiti Ciarello M, Acri G, Testagrossa B, Bertuccio MP, Squeri R, Di Pietro A. In vitro assessment of neurotoxicity and neuroinflammation of homemade MWCNTs. Environ Toxicol Pharmacol 2017; 56: 121-128. doi: 10.1016/j.etap.2017.09.005.

40) Visalli G, Facciolà A, Iannazzo D, Piperno A, Pistone A, Di Pietro A. The role of the iron catalyst in the toxicity of multi-walled carbon nanotubes (MWCNTs). J Trace Elem Med Biol 2017; 43: 153-160. doi: 10.1016/j.jtemb.2017.01.005.

41) Trovato MC, Andronico D, Sciacchitano S, Ruggeri RM, Picerno I, Di Pietro A, Visalli G. Nanostructures: between natural environment and medical practice. Rev Environ Health 2018; 33: 305-317. doi: 10.1515/reveh-2017-0036.

42) Visalli G, Facciolà A, Currò M, Laganà P, La Fauci V, Iannazzo D, Pistone A, Di Pietro A. Mitochondrial impairment induced by sub-chronic exposure to multi-walled carbon nanotubes. Int J Environ Res Public Health 2019; 16. pii: E792. doi: 10.3390/ijerph16050792.

43) Facciolà A, Visalli G, La Maestra S, Ceccarelli M, D'Aleo F, Nunnari G, Pellicanò GF, Di Pietro A. Carbon nanotubes and central nervous system: Environmental risks, toxicological aspects and future perspectives. Environ Toxicol Pharmacol 2019; 65: 23-30. doi: 10.1016/j.etap.2018.11.006.

44) Liao W, Ning Z, Chen L, Wei Q, Yuan E, Yang J, Ren J. Intracellular antioxidant detoxifying effects of iosmetin on 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidative stress through inhibition of reactive oxygen species generation. J Agric Food Chem 2014; 62: 8648-8654. doi: 10.1021/jf502359x.

45) Liao W, Li W, Zhang T, Kirberger M, Liu J, Wang P, Chen W, Wang Y. Powering up the molecular therapy of RNA interference by novel nanoparticles. Biomater Sci 2016; 4: 1051-1061. doi: 10.1039/c6bm90031c.

46) Bolhassani A, Safaiyan S, Rafati S. Improvement of different vaccine delivery systems for cancer therapy. Mol Cancer 2011; 10: 3. doi: 10.1186/1476-4598-10-3.

47) Hamdy S, Haddadi A, Hung RW, Lavasanifar A. Targeting dendritic cells with nano-particulate PLGA cancer vaccine formulations systems for cancer therapy. Adv Drug Deliv Rev 2011; 63: 943-955. doi: 10.1016/j.addr.2011.05.021

48) Chackerian B. Virus-like particle based vaccines for Alzheimer disease. Hum Vaccin 2010; 6: 926-930. doi: 10.4161/hv.7.1.12655.

49) Tissot AC, Maurer P, Nussberger J, Sabat R, Pfister T, Ignatenko S, Volk HD, Stocker H, Müller P, Jennings GT, Wagner F, Bachmann MF. Effect of immunisation against angiotensin II with CYT006-AngQb on ambulatory blood pressure: a double-blind, randomized, placebo-controlled phase IIa study. Lancet 2008; 371: 821-827. doi: 10.1016/S0140-6736(08)60381-5.

50) Plummer EM, Manchester M. Viral nanoparticles and virus-like particles: platforms for contemporary vaccine design. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2011; 3: 174-196. doi: 10.1002/wnan.119.

51) Kushnir N, Streatfield SJ, Yusibov V. Virus-like particles as a highly efficient vaccine platform: diversity of targets and production systems and advances in clinical development. Vaccine 2012; 31: 58-83. doi: 10.1016/j.vaccine.2012.10.083.

52) Correia-Pinto JF, Csaba N, Alonso MJ. Vaccine delivery carriers: insights and future perspectives. Int J Pharm 2013; 440: 27-38. doi: 10.1016/j.ijpharm.2012.04.047.

53) Gregory AE, Titball R, Williamson D. Vaccine delivery using nanoparticles. Front Cell Infect Microbiol 2013; 3: 13. doi: 10.3389/fcimb.2013.00013.

54) Treuel L, Docter D, Maskos M, Stauber RH. Protein corona - from molecular adsorption to physiological complexity. Beilstein J Nanotechnol 2015; 6: 857-873. doi: 10.3762/bjnano.6.88.

55) Xu Q, Lv Y, Dong C, Sreeprased TS, Tian A, Zhang H, Tang Y, Yu Z, Li N. Three-dimensional micro/nanoscale architectures: fabrication and applications. Nanoscale 2015; 7: 10883-10895. doi: 10.1039/c5nr02048d.

56) Niu Y, Stadler FJ, Song J, Chen S, Chen S. Facile fabrication of polyurethane microcapsules carriers for tracing cellular internalization and intracellular pH-triggered drug release. Colloids Surf B Biointerfaces 2017; 153: 160-167. doi: 10.1016/j.colsurfb.2017.02.018.

57) Wen Y, Meng WS. Recent in vivo evidences of particle-based delivery of small-interfering RNA (siRNA) into solid tumors. J Pharm Innov 2014; 9: 158-173.

58) Ye M, Tang L, Luo M, Zhou J, Guo B, Liu Y, Chen B. Size- and time-dependent alteration in metabolic activities of human hepatic cytochrome P450 isozymes by gold nanoparticles via microsomal coincubations. Nanoscale Res Lett 2014; 9: 642. doi: 10.1186/1556-276X-9-642.

59) Wen Y, Collier JH. Supramolecular peptide vaccines: tuning adaptive immunity. Curr Opin Immunol 2015; 35: 73-79. doi: 10.1016/j.coi.2015.06.007.

60) Hubbell JA, Thomas SN, Swartz MA. Materials engineering for immunomodulation. Nature 2009; 462: 449-460. doi: 10.1038/nature08604.

61) Akagi T, Shima F, Akashi M. Intracellular degradation and distribution of protein-encapsulated amphiphilic poly(amino acid) nanoparticles. Biomaterials 2011; 32: 4959-4967.

62) Eby JK, Dane KY, O'Neil CP, Hirosue S, Swartz MA, Hubbell JA. Polymer micelles with pyridyl disulfide-coupled antigen travel through lymphatics and show enhanced cellular responses following immunization. Acta Biomater 2012; 8: 3210-3217. doi: 10.1016/j.actbio.2012.06.007.

63) Smith DM, Simon JK, Baker JR Jr. Applications of nanotechnology for immunology. Nat Rev Immunol 2013; 13: 592-605. doi: 10.1038/nri3488.

64) Kourtis IC, Hirosue S, De Titta A, Kontos S, Stegmann T, Hubbell JA, Swartz MA. Peripherally administered nanoparticles target monocytic myeloid cells, secondary lymphoid organs and tumors in mice. PLoS One 2013; 8: e61646. doi: 10.1371/journal.pone.0061646.

65) Wilson JT, Keller S, Manganiello MJ, Cheng C, Lee CC, Opara C, Convertine A, Stayton PS. pH-Responsive nanoparticle vaccines for dual-delivery of antigens and immunostimulatory oligonucleotides. ACS Nano 2013; 7: 3912-3925. doi: 10.1021/nn305466z.

66) Stano A, Scott EA, Dane KY, Swartz MA, Hubbell JA. Tunable T cell immunity towards a protein antigen using polymersomes vs. solid-core nanoparticles. Biomaterials 2013; 34: 4339-4346. doi: 10.1016/j.biomaterials.2013.02.024.

67) Kaminskas LM, Porter CJH. Targeting the lymphatics using dendritic polymers (dendrimers). Adv Drug Deliv Rev 2011; 63: 890-900.

68) Noad R, Roy P. Virus-like particles as immunogens. Trends Microbiol 2003; 11: 438-444.

69) Grgacic EV, Anderson DA. Virus-like particles: passport to immune recognition. Methods 2006; 40: 60-65.

70) Jeong H, Seong BL. Exploiting virus-like particles as innovative vaccines against emerging viral infections. J Microbiol 2017; 55: 220-230. doi: 10.1007/s12275-017-7058-3.

71) Zhang LF, Zhou J, Chen S, Cai LL, Bao QY, Zheng FY, Lu JQ, Padmanabha J, Hengst K, Malcolm K, Frazer IH. HPV6b virus like particles are potent immunogens without adjuvant in man. Vaccine 2000; 18: 1051-1058.

72) Bachmann MF, Jennings GT, Vaccine delivery: a matter of size, geometry, kinetics and molecularpatterns. Immunology 2010; 10: 787-796. doi: 10.1038/nri2868.

73) Pushko P, Pumpens P, Grens E. Development of virus-like particle technology from small highly symmetric to large complex virus-like particle structures. Intervirology 2013; 56: 141-165. doi: 10.1159/000346773.

74) Pattenden LK, Middelberg APJ, Niebert M, Lipin DI. Towards the preparative and large-scale precision manufacture of virus-like particles. Trends Biotechnol 2005; 23: 523-529.

75) Zeltins A. Construction and characterization of virus-like particles: a review. Mol Biotechnol 2013; 53: 92-107. doi: 10.1007/s12033-012-9598-4.

76) Sánchez-Rodríguez SP, Münch-Anguiano L, Echeverría O, Vázquez-Nin G, Mora-Pale M, Dordick JS, Bustos-Jaimes I. Human parvovirus B19 virus-like particles: in vitro assembly and stability. Biochimie 2012; 94: 870-878. doi: 10.1016/j.biochi.2011.12.006.

77) Liew MWO, Chuan YP, Middelberg APJ. High-yield and scalable cell-free assembly of virus-like particles by dilution. Biochem Eng J 2012; 67: 88-96.

78) Lu Y, Welsh JP, Chan W, Swartz JR. Escherichia coli-based cell free production of flagellin and ordered flagellin display on virus-like particles. Biotechnol Bioeng 2013; 110: 2073-2085. doi: 10.1002/bit.24903.

79) Chuan YP, Lua LHL, Middelberg APJ. Virus-like particle bioprocessing. Weinheim, Germany: Biopharmaceutical Production Technology: Wiley-VCH Verlag GmbH & Co. KGaA; 2012; pp. 139-163.

80) Lua LH, Connors NK, Sainsbury F, Chuan YP, Wibowo N, Middelberg AP. Bioengineering virus-like particles as vaccines. Biotechnol Bioeng 2014; 111: 425-440. doi: 10.1002/bit.25159.

81) Ionescu RM, Przysiecki CT, Liang X, Garsky VM, Fan J, Wang B, Troutman R, Rippeon Y, Flanagan E, Shiver J, Shi L. Pharmaceutical and immunological evaluation of human papillomavirus virus like particle as an antigen carrier. J Pharm Sci 2006; 95: 70-79.

82) Tissot AC, Renhofa R, Schmitz N, Cielens I, Meijerink E, Ose V, Jennings GT, Saudan P, Pumpens P, Bachmann MF. Versatile virus-like particle carrier for epitope based vaccines. PLoS One 2010; 5: e9809. doi: 10.1371/journal.pone.0009809.

83) Middelberg APJ, Rivera-Hernandez T, Wibowo N, Lua LHL, Fan Y, Magor G, Chang C, Chuan YP, Good MF, Batzloff MR. A microbial platform for rapid and low-cost virus-like particle and capsomere vaccines. Vaccine 2011; 29: 7154-7162.

84) Greiner VJ, Ronzon F, Larquet E, Desbat B, Estèves C, Bonvin J, Gréco F, Manin C, Klymchenko AS, Mély Y. The structure of HBsAg particles is not modified upon their adsorption on aluminium hydroxide gel. Vaccine 2012; 30: 5240-5245. doi: 10.1016/j.vaccine.2012.05.082.

85) Greiner VJ, Egelé C, Oncul S, Ronzon F, Manin C, Klymchenko A, Mély Y. Characterization of the lipid and protein organization in HBsAg viral particles by steady-state and time-resolved fluorescence spectroscopy. Biochimie 2010; 92: 994-1002. doi: 10.1016/j.biochi.2010.04.014.

86) Zhao Q, Li S, Yu H, Xia N, Modis Y. Virus-like particle-based human vaccines: quality assessment based on structural and func- tional properties. Trends Biotechnol 2013; 31: 654-663. doi: 10.1016/j.tibtech.2013.09.002.

87) Cutts FT, Franceschi S, Goldie S, Castellsague X, de Sanjose S, Garnett G, Edmunds WJ, Claeys P, Goldenthal KL, Harper DM, Markowitz L. Human papillomavirus and HPV vaccines: a review. Bull World Health Organ 2007; 85: 719-726.

88) Gillison ML, D'Souza G, Westra W. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst 2008; 100: 407-420. doi:10.1093/jnci/djn025.

89) Rezazadeh A, Laber DA, Ghim SJ, Jenson AB, Kloecker G. The role of human papilloma virus in lung cancer: a review of the evidence. Am J Med Sci 2009; 338: 64-67. doi: 10.1097/MAJ.0b013e3181a393ba.

90) Visalli G, Currò M, Facciolà A, Riso R, Mondello P, Laganà P, Di Pietro A, Picerno I, Spataro P. Prevalence of human papillomavirus in saliva of women with HPV genital lesions. Infect Agent Cancer 2016; 11: 48. doi: 10.1186/s13027-016-0096-3.

91) Visalli G, Riso R, Facciolà A, Mondello P, Caruso C, Picerno I, Di Pietro A, Spataro P, Bertuccio MP. Higher levels of oxidative DNA damage in cervical cells are correlated with the grade of dysplasia and HPV infection. J Med Virol 2016; 88: 336-344. doi: 10.1002/jmv.24327.

92) Ceccarelli M, Rullo EV, Facciolà A, Madeddu G, Cacopardo B, Taibi R, D'Aleo F, Pinzone MR, Picerno I, di Rosa M, Visalli G, Condorelli F, Nunnari G, Pellicanò GF. Head and neck squamous cell carcinoma and its correlation with human papillomavirus in people living with HIV: a systematic review. Oncotarget 2018; 9: 17171-17180. doi: 10.18632/oncotarget.24660.

93) Visalli G, Facciolà A, D'Aleo F, Pinzone MR, Condorelli F, Picerno I, Nunnari G, Pellicanò GF, Ceccarelli M, Venanzi Rullo E. HPV and urinary bladder carcinoma: a review of the literature. World Cancer Res J 2018; 5: e1038.

94) National Cancer Institute. HPV and cancer; 2019. Available from: https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-and-cancer

95) Kavanagh K, Pollock KG, Cuschieri K, Palmer T, Cameron RL, Watt C, Bhatia R, Moore C, Cubie H, Cruickshank M, Robertson C. Changes in the prevalence of human papillomavirus following a national bivalent human papillomavirus vaccination programme in Scotland: a 7-year cross-sectional study. Lancet Infect Dis 2017; 17: 1293-1302. doi: 10.1016/S1473-3099(17)30468-1.

96) Huh WK, Joura EA, Giuliano AR, Iversen OE, de Andrade RP, Ault KA, Bartholomew D, Cestero RM, Fedrizzi EN, Hirschberg AL, Mayrand MH, Ruiz-Sternberg AM, Stapleton JT, Wiley DJ, Ferenczy A, Kurman R, Ronnett BM, Stoler MH, Cuzick J, Garland SM, Kjaer SK, Bautista OM, Haupt R, Moeller E, Ritter M, Roberts CC, Shields C, Luxembourg A. Final efficacy, immunogenicity, and safety analyses of a nine-valent human papillomavirus vaccine in women aged 16-26 years: a randomised, double-blind trial. Lancet 2017; 390: 2143-2159. doi: 10.1016/S0140-6736(17)31821-4.

97) Steinbrook R. The potential of human papillomavirus vaccines. N Engl J Med 2006; 354: 1109-1112.

98) Garland SM, Smith JS. Human papillomavirus vaccines: current status and future prospects. Drugs 2010; 70: 1079-1098. doi: 10.2165/10898580-000000000-00000.

99) Gillison ML, Chaturvedi AK, Lowy DR. HPV prophylactic vaccines and the potential prevention of noncervical cancers in both men and women. Cancer 2008; 113: 3036-3046. doi: 10.1002/cncr.23764.

100) Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, Jiang B, Goodman MT, Sibug-Saber M, Cozen W, Liu L, Lynch CF, Wentzensen N, Jordan RC, Altekruse S, Anderson WF, Rosenberg PS, Gillison ML. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 2011; 29: 4294-4301. doi: 10.1200/JCO.2011.36.4596.

101) Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, Chiacchierini LM, Jansen KU; Proof of Principle Study Investigators. A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med 2002; 347: 1645-1651.

102) Lopalco PL. Spotlight on the 9-valent HPV vaccine. Drug Des Devel Ther 2016; 11: 35-44. doi: 10.2147/DDDT.S91018.

103) Largeron N, Petry KU, Jacob J, Bianic F, Anger D, Uhart M. An estimate of the public health impact and cost-effectiveness of universal vaccination with a 9-valent HPV vaccine in Germany. Expert Rev Pharmacoecon Outcomes Res 2017; 17: 85-98. doi: 10.1080/14737167.2016.1208087.

104) Genovese C, LA Fauci V, Squeri A, Trimarchi G, Squeri R. HPV vaccine and autoimmune diseases: systematic review and meta-analysis of the literature. J Prev Med Hyg 2018; 59: E194-E199. doi: 10.15167/2421-4248/jpmh2018.59.3.998.

105) Yang, C. Yu W, Bi Y, Long F, Li Y, Wei D, Hao X, Situ J, Zhao Y, Huang F. Increased estradiol in hepatitis E virus infected pregnant women promote viral replication. J Viral Hepat 2018; 25: 742-751. doi: 10.1111/jvh.12865.

106) Hartl J, Otto B, Madden RG, Webb G, Woolson KL, Kriston L, Vettorazzi E, Lohse AW, Dalton HR, Pischke S. Hepatitis E seroprevalence in Europe. A meta-analysis. Viruses 2016; 8. pii: E211. doi: 10.3390/v8080211.

107) La Fauci V, Sindoni D, Grillo OC, Calimeri S, Lo Giudice D, Squeri R. Hepatitis E virus (HEV) in sewage from treatment plants of Messina University Hospital and of Messina City Council. J Prev Med Hyg 2010; 51: 28-30.

108) La Fauci V, Facciolà A, Riso R, Calimeri S, Lo Giudice D, Squeri R. Seroprevalence of hev antibodies in a sample of pregnant women in the city of Messina. Ann Ig 2017; 29: 232-238. doi: 10.7416/ai.2017.2151.

109) Horvatits T, Ozga AK, Westhölter D, Hartl J, Manthey CF, Lütgehetmann M, Rauch G, Kriston L, Lohse AW, Bendall R, Wedemeyer H, Dalton HR, Pischke S. Hepatitis E seroprevalence in the Americas. A systematic review and meta-analysis. Liver Int 2018; 38: 1951-1964. doi: 10.1111/liv.13859.

110) Guu TS, Liu Z, Ye Q, Mata DA, Li K, Yin C, Zhang J, Tao YJ. Structure of the hepatitis E virus-like particle suggests mechanisms for virus assembly and receptor binding. Proc Natl Acad Sci U S A 2009; 106: 12992-12997. doi: 10.1073/pnas.0904848106.

111) Yamashita T, Mori Y, Miyazaki N, Cheng RH, Yoshimura M, Unno H, Shima R, Moriishi K, Tsukihara T, Li TC, Takeda N, Miyamura T, Matsuura Y. Biological and immunological characteristics of hepatitis E virus-like particles based on the crystal structure. Proc Natl Acad Sci USA 2009; 106: 12986-12991.

112) Purcell RH, Nguyen H, Shapiro M, Engle RE, Govindarajan S, Blackwelder WC, Wong DC, Prieels JP, Emerson SU. Pre-clinical immunogenicity and efficacy trial of a recombinant hepatitis E vaccine. Vaccine 2003; 21; 2607-2615.

113) Makar GA. Safety and efficacy of a recombinant hepatitis E vaccine. Yearbook Gastroenterol 2007; 247-248.

114) Li SW, Zhang J, He ZQ, Gu Y, Liu RS, Lin J, Chen YX, Ng MH, Xia NS. Mutational analysis of essential interactions involved in the assembly of hepatitis E virus capsid. J Biol Chem 2005; 280: 3400-3406.

115) Wei M, Zhang X, Yu H, Tang ZM, Wang K, Li Z, Zheng Z, Li S, Zhang J, Xia N, Zhao Q. Bacteria expressed hepatitis E virus capsid proteins maintain virion-like epitopes. Vaccine 2014; 32: 2859-2865. doi: 10.1016/j.vaccine.2014.02.025.

116) Zhang J, Liu CB, Li RC, Li YM, Zheng YJ, Li YP, Luo D, Pan BB, Nong Y, Ge SX, Xiong JH, Shih JW, Ng MH, Xia NS. Randomized-controlled phase II clinical trial of a bacterially expressed recombinant hepatitis E vaccine. Vaccine 2009; 27: 1869-1874. doi: 10.1016/j.vaccine.2008.

117) Zhu FC, Zhang J, Zhang XF, Zhou C, Wang ZZ, Huang SJ, Wang H, Yang CL, Jiang HM, Cai JP, Wang YJ, Ai X, Hu YM, Tang Q, Yao X, Yan Q, Xian YL, Wu T, Li YM, Miao J, Ng MH, Shih JW, Xia NS. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults. A large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 2010; 376: 895-902. doi: 10.1016/S0140-6736(10)61030-6.

118) Cao YF, Tao H, Hu YM, Shi CB, Wu X, Liang Q, Chi CP, Li L, Liang ZL, Meng JH, Zhu FC, Liu ZH, Wang XP. A phase 1 randomized open-label clinical study to evaluate the safety and tolerability of a novel recombinant hepatitis E vaccine. Vaccine 2017; 35: 5073-5080. doi: 10.1016/j.vaccine.2017.05.072.

119) Wang H, H Xie, J Wang, J Wu, X Ma, L Li, X Wei, Q Ling, P Song, L Zhou, Xu X, Zheng S. Self-assembling prodrugs by precise programming of molecular structures that contribute distinct stability, pharmacokinetics, and antitumor efficacy. Adv Funct Mat 2015; 25: 4956-4965. doi:10.1002/adfm.201501953.

120) Sun L, Fan Z, Wang Y, Huang Y, Schmidt M, Zhang M. Tunable synthesis of self-assembled cyclic peptide nanotubes and nanoparticles. Soft Matter 2015; 11: 3822-3832. doi: 10.1039/c5sm00533g.

121) Liao W, Zhang R, Dong C, Yu Z, Ren J. Novel walnut peptide-selenium hybrids with enhanced anticancer synergism: facile synthesis and mechanistic investigation of anticancer activity. Int J Nanomedicine 2016; 11: 1305-1321. doi: 10.2147/IJN.S92257.

122) Yang Y, Dai M, Wilson TM, Omelchenko I, Klimek JE, Wilmarth PA, David LL, Nuttall AL, Gillespie PG, Shi X. Na+/K+-ATPase α1 identified as an abundant protein in the blood-labyrinth barrier that plays an essential role in the barrier integrity. PLoS One 2011; 6: e16547. doi: 10.1371/journal.pone.0016547.

123) Chung EJ, Mlinar LB, Sugimoto MJ, Nord K, Roman BB, Tirrell M. In vivo biodistribution and clearance of peptide amphiphile micelles. Nanomedicine 2015; 11: 479-487. doi: 10.1016/j.nano.2014.08.006.

124) Nochi T, Yuki Y, Takahashi H, Sawada S, Mejima M, Kohda T, Harada N, Kong IG, Sato A, Kataoka N, Tokuhara D, Kurokawa S, Takahashi Y, Tsukada H, Kozaki S, Akiyoshi K, Kiyono H. Nanogel antigenic protein-delivery system for adjuvant-free intranasal vaccines. Nat Mater 2010; 9: 572-578. doi: 10.1038/nmat2784.

125) Wahome N, Pfeiffer T, Ambiel I, Yang Y, Keppler OT, Bosch V, Burkhard P. Conformation-specific display of 4E10 and 2F5 epitopes on self-assembling protein nanoparticles as a potential HIV vaccine. Chem Biol Drug Des 2012; 80: 349-357. doi: 10.1111/j.1747-0285.2012.01423.x.

126) Kanekiyo M, Wei CJ, Yassine HM, McTamney PM, Boyington JC, Whittle JR, Rao SS, Kong WP, Wang L, Nabel GJ. Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies. Nature 2013; 499: 102-106. doi: 10.1038/nature12202.

127) Lee BR, Ko HK, Ryu JH, Ahn KY, Lee YH, Oh SJ, Na JH, Kim TW, Byun Y, Kwon IC, Kim K, Lee J. Engineered human ferritin nanoparticles for direct delivery of tumor antigens to lymph node and cancer immunotherapy. Sci Rep 2016; 6: 35182. doi: 10.1038/srep35182.

128) Das D, Wang YH, Hsieh CY, Suzuki YJ. Major vault protein regulates cell growth/survival signaling through oxidative modifications. Cell Signal 2016; 28: 12-18. doi: 10.1016/j.cellsig.2015.10.007.

129) Champion CI, Kickhoefer VA, Liu G, Moniz RJ, Freed AS, Bergmann LL, Vaccari D, Raval-Fernandes S, Chan AM, Rome LH, Kelly KA. A vault nanoparticle vaccine induces protective mucosal immunity. PLoS One 2009; 4: e5409.

130) Trimaille T, Verrier B. Micelle-based adjuvants for subunit vaccine delivery. Vaccines (Basel) 2015; 3: 803-813. doi: 10.3390/vaccines3040803.

131) Isoglu IA, Ozsoy Y, Isoglu SD. Advances in micelle-based drug delivery: cross-linked systems. Curr Top Med Chem 2017; 17: 1469-1489. doi: 10.2174/1568026616666161222110600.

132) Jiménez-Sánchez G, Pavot V, Chane-Haong C, Handké N, Terrat C, Gigmes D, Trimaille T, Verrier B. Preparation and in vitro evaluation of imiquimod loaded polylactide-based micelles as potential vaccine adjuvants. Pharm Res 2015; 32: 311-320. doi: 10.1007/s11095-014-1465-5.

133) Trent A, Marullo R, Lin B, Black M, Tirrell M. Structural properties of soluble peptide amphiphile micelles. Soft Matter 2011; 7: 9572-9582. doi: 10.1039/c1sm05862b.

134) Black M, Trent A, Kostenko Y, Lee JS, Olive C, Tirrell M. Self-assembled peptide amphiphile micelles containing a cytotoxic T-cell epitope promote a protective immune response in vivo. Adv Mater 2012; 24: 3845-3849. doi:10.1002/adma.201200209

135) Trent A, Ulery BD, Black MJ, Barrett JC, Liang S, Kostenko Y, David NA, Tirrell MV. Peptide amphiphile micelles self-adjuvant group A streptococcal vaccination. AAPS J 2015; 17: 380-388. doi: 10.1208/s12248-014-9707-3.

136) Barrett JC, Ulery BD, Trent A, Liang S, David NA, Tirrell MV. Modular peptide amphiphile micelles improving an antibody-mediated immune response to group A streptococcus. ACS Biomater Sci Eng 2016; 3: 144-152. doi: 10.1021/acsbiomaterials.6b00422

137) Black M, Trent A, Tirrell M, Olive C. Advances in the design and delivery of peptide subunit vaccines with a focus on Toll-like receptor agonists. Expert Rev Vaccines 2010; 9: 157-173. doi: 10.1586/erv.09.160.

138) Liu H, Irvine DJ. Guiding principles in the design of molecular bioconjugates for vaccine applications. Bioconjug Chem 2015; 26: 791-801. doi: 10.1021/acs.bioconjchem.5b00103

139) Acar H, Srivastava S, Chung EJ, Schnorenberg MR, Barrett JC, LaBelle JL, Tirrell M. Self-assembling peptide-based building blocks in medical applications. Adv Drug Deliv Rev 2017; 110-111: 65-79. doi: 10.1016/j.addr.2016.08.006.

140) Luo Z, Shi S, Jin L, Xu L, Yu J, Chen H, Li X. Cationic micelle based vaccine induced potent humoral immune response through enhancing antigen uptake and formation of germinal center. Colloids Surf B Biointerfaces 2015; 135: 556-564. doi: 10.1016/j.colsurfb.2015.07.079.

141) Keller S, Wilson JT, Patilea GI, Kern HB, Convertine AJ, Stayton PS. Neutral polymer micelle carriers with pH-responsive, endosome-releasing activity modulate antigen trafficking to enhance CD8+ T cell responses. J Control Release 2014, 191: 24-33. doi: 10.1016/j.jconrel.2014.03.041.

142) Moon JJ, Suh H, Bershteyn A, Stephan MT, Liu H, Huang B, Sohail M, Luo S, Um SH, Khant H, Goodwin JT, Ramos J, Chiu W, Irvine DJ. Interbilayer-crosslinked multilamellar vesicles as synthetic vaccines for potent humoral and cellular immune responses. Nat Mater 2011; 10: 243-251. doi: 10.1038/nmat2960.

143) Schwendener RA. Liposomes as vaccine delivery systems: a review of the recent advances. Ther Adv Vaccines 2014; 2: 159-182. doi: 10.1177/2051013614541440.

144) Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov 2005; 4: 145-160.

145) Watson DS, Endsley AN, Huang L. Design considerations for liposomal vaccines: influence of formulation parameters on antibody and cell-mediated immune responses to liposome associated antigens. Vaccine 2012; 30: 2256-2272. doi: 10.1016/j.vaccine.2012.01.070.

146) Perrie Y, Crofts F, Devitt A, Griffiths HR, Kastner E, Nadella V. Designing liposomal adjuvants for the next generation of vaccines. Adv Drug Deliv Rev 2016; 99: 85-96. doi: 10.1016/j.addr.2015.11.005.

147) Henriksen-Lacey M, Devitt A, Perrie Y. The vesicle size of DDA:TDB liposomal adjuvants plays a role in the cell-mediated immune response but has no significant effect on antibody production. J Control Release 2011; 154: 131-137. doi: 10.1016/j.jconrel.2011.05.019.

148) Beck Z, Matyas GR, Jalah R, Rao M, Polonis VR, Alving CR. Differential immune responses to HIV-1 envelope protein induced by liposomal adjuvant formulations containing monophosphoryl lipid A with or without QS21. Vaccine 2015; 33: 5578-5587. doi: 10.1016/j.vaccine.2015.09.001.

149) Hussain MJ, Wilkinson A, Bramwell VW, Christensen D, Perrie Y. Th1 immune responses can be modulated by varying dimethyldioctadecylammonium and distearoyl-sn-glycero-3-phosphocholine content in liposomal adjuvants. J Pharm Pharmacol 2014; 66: 358-366. doi: 10.1111/jphp.12173.

150) Joseph A, Itskovitz-Cooper N, Samira S, Flasterstein O, Eliyahu H, Simberg D, Goldwaser I, Barenholz Y, Kedar E. A new intranasal influenza vaccine based on a novel polycationic lipid--ceramide carbamoyl-spermine (CCS) I. Immunogenicity and efficacy studies in mice. Vaccine 2006; 24: 3990-4006.

151) Kaur R, Henriksen-Lacey M, Wilkhu J, Devitt A, Christensen D, Perrie Y. Effect of incorporating cholesterol into DDA:TDB liposomal adjuvants on bilayer properties, biodistribution, and immune responses. Mol Pharm 2014; 11: 197-207. doi: 10.1021/mp400372j.

152) Felnerova D, Gluck VJ, Moser RC. Liposomes and virosomes as delivery systems for antigens, nucleic acids and drugs. Curr Opin Biotechnol 2004; 15: 518-529.

153) Blom RA, Erni ST, Krempaská K, Schaerer O, van Dijk RM, Amacker M, Moser C, Hall SR, von Garnier C, Blank F. A triple co-culture model of the human respiratory tract to study immune-modulatory effects of liposomes and virosomes. PLoS One 2016; 11: e0163539. doi: 10.1371/journal.pone.0163539.

154) Kalkanidis M, Pietersz GA, Xiang SD, Mottram PL, Crimeen-Irwin B, Ardipradja K, Plebanski M. Methods for nano-particle based vaccine formulation and evaluation of their immunogenicity. Methods 2006; 40: 20-29.

155) He CF, Wang SH, Yu YJ, Shen HY, Zhao Y, Gao HL, Wang H, Li LL, Liu HY. Advances in biodegradable nanomaterials for photothermal therapy of cancer. Cancer Biol Med 2016; 13: 299-312.

156) 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: 6160-6164.

157) 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: 961-966. doi: 10.1016/j.chembiol.2003.09.011.

158) Yandar N, Pastorin G, Prato M, Bianco A, Patarroyo ME, Lozano JM. Immunological profile of a plasmodium vivax AMA-1 N-terminus peptide-carbon nanotube conjugate in an infected plasmodium berghei mouse model. Vaccine 2008; 26: 5864-5873. doi: 10.1016/j.vaccine.2008.08.014.

159) Scheinberg DA, McDevitt MR, Dao T, Mulvey JJ, Feinberg E, Alidori S. Carbon nanotubes as vaccine scaffolds. Adv Drug Deliv Rev 2013; 65: 2016-2022. doi: 10.1016/j.addr.2013.07.013.

160) Gottardi R, Douradinha B. Carbon nanotubes as a novel tool for vaccination against infectious diseases and cancer. J Nanobiotechnology 2013; 11: 30. doi: 10.1186/1477-3155-11-30.

161) Liu Y, Xu Y, Tian Y, Chen C, Wang C, Jiang X. Functional nanomaterials can optimize the efficacy of vaccines. Small 2014; 10: 4505-4520. doi: 10.1002/smll.201401707.

162) De Volder MF, Tawfick SH, Baughman RH, Hart AJ. Carbon nanotubes: present and future commercial applications. Science 2013; 339: 535-539. doi: 10.1126/science.1222453.

163) Yang W, Thordarson P, Gooding JJ, Ringer SP, Braet F. Carbon nanotubes for biological and biomedical applications. Nanotechnology 2007; 18: 412001. doi: 10.1088/0957-4484/18/41/412001.

164) 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: 48-53. doi: 10.1016/j.imlet.2009.07.012.

165) Hassan HA, Smyth L, Rubio N, Ratnasothy K, Wang JT, Bansal SS, Summers HD, Diebold SS, Lombardi G, Al-Jamal KT. Carbon nanotubes' surface chemistry determines their potency as vaccine nanocarriers in vitro and in vivo. J Control Release 2016; 225: 205-216. doi: 10.1016/j.jconrel.2016.01.030.

166) Calegari LP, Dias RS, de Oliveira MD, Pessoa CR, de Oliveira AS, Oliveira AF, da Silva CC, Fonseca FG, Versiani AF, De Paula SO. Multi-walled carbon nanotubes increase antibody-producing B

cells in mice immunized with a tetravalent vaccine candidate for dengue virus. J Nanobiotechnology 2016; 14: 61. doi: 10.1186/s12951-016-0196-7.

167) Safari D, Marradi M, Chiodo F, Th Dekker HA, Shan Y, Adamo R, Oscarson S, Rijkers GT, Lahmann M, Kamerling JP, Penadés S, Snippe H. Gold nanoparticles as carriers for a synthetic Streptococcus pneumoniae type 14 conjugate vaccine. Nanomedicine (Lond) 2012; 7: 651-662. doi: 10.2217/nnm.11.151.

168) Niikura K, Matsunaga T, Suzuki T, Kobayashi S, Yamaguchi H, Orba Y, Kawaguchi A, Hasegawa H, Kajino K, Ninomiya T, Ijiro K, Sawa H. Gold nanoparticles as a vaccine platform: influence of size and shape on immunological responses in vitro and in vivo. ACS Nano 2013; 7: 3926-3938. doi: 10.1021/nn3057005.

169) Facciolà A, Venanzi Rullo E, Ceccarelli M, D'Aleo F, Di Rosa M, Pinzone MR, Condorelli F, Visalli G, Picerno I, Fisichella R, Nunnari G, Pellicanò GF. Kaposi's sarcoma in HIV-infected patients in the era of new antiretrovirals. Eur Rev Med Pharmacol Sci 2017; 21: 5868-5869. doi: 10.26355/eurrev_201712_14036.

170) D'Aleo F, Ceccarelli M, Venanzi Rullo E, Facciolà A, Di Rosa M, Pinzone MR, Condorelli F, Visalli G, Picerno I, Berretta M, Pellicanò GF, Nunnari G. Hepatitis C-related hepatocellular carcinoma: diagnostic and therapeutic management in HIV-patients. Eur Rev Med Pharmacol Sci 2017; 21: 5859-5867. doi: 10.26355/eurrev_201712_14035.

171) D'Aleo F, Cama BAV, Paolucci IA, Venanzi Rullo E, Condorelli F, Facciolà A, Di Francia R, Savasta A, Pinzone MR, Picerno I, Visalli G, Nunnari G, Pellicanò GF , Ceccarelli M. New and old assumptions on lung cancer in people living with HIV. World Cancer Res J 2018; 5: e1036.

172) Facciolà A, Ceccarelli M, Venanzi Rullo E, d'Aleo F, Condorelli F, Visalli G, Cacopardo B, Pinzone MR, di Rosa M, Nunnari G, Pellicanò GF. Prostate cancer in HIV-positive patients: a review of the literature. World Cancer Res J 2018; 5: e1136.

173) Ceccarelli M, Venanzi Rullo E, Vaccaro M, Facciolà A, d'Aleo F, Paolucci IA, Cannavò SP, Cacopardo B, Pinzone MR, Pellicanò GF, Condorelli F, Nunnari G, Guarneri C. HIV-associated psoriasis: epidemiology, pathogenesis, and management. Dermatol Ther 2019; 32: e12806. doi: 10.1111/dth.12806.

174) D'Andrea F, Ceccarelli M, Facciolà A, Nunnari G, Pellicanò GF, Venanzi Rullo E. Breast cancer in women living with HIV. Eur Rev Med Pharmacol Sci 2019; 23: 1158-1164. doi: 10.26355/eurrev_201902_17007.

175) Facciolà A, Venanzi Rullo E, Ceccarelli M, d'Aleo F, D'Andrea F, Visalli G, Pinzone MR, Picerno I, Cacopardo B, Condorelli F, Madeddu G, Nunnari G, Pellicanò GF. Hodgkin's lymphoma in people living with HIV: epidemiology and clinical management. World Cancer Res J 2019; 6: e1295.

176) D'Andrea F, Pellicanò GF, Venanzi Rullo E, d'Aleo F, Facciolà A, Micali C, Coco M, Visalli G, Picerno I, Condorelli F, Pinzone MR, Cacopardo B, Nunnari G, Ceccarelli M. Cervical cancer in women living with HIV: a review of the literature. World Cancer Res J 2019; 6: e1224.

177) Trovato M, Ruggeri RM, Sciacchitano S, Vicchio TM, Picerno I, Pellicanò G, Valenti A, Visalli G. Serum interleukin-6 levels are increased in HIV-infected patients that develop autoimmune disease during long-term follow-up. Immunobiology 2018; 223: 264-268. doi: 10.1016/j.imbio.2017.10.039.

178) Xu L, Liu Y, Chen Z, Li W, Liu Y, Wang L, Liu Y, Wu X, Ji Y, Zhao Y, Ma L, Shao Y, Chen C. Surface-engineered gold nanorods: promising DNA vaccine adjuvant for HIV-1 treatment. Nano Lett 2012; 12: 2003-2012. doi: 10.1021/nl300027p.

179) Gregory AE, Williamson ED, Prior JL, Butcher WA, Thompson IJ, Shaw AM, Titball RW. Conjugation of Y. pestis F1-antigen to gold nanoparticles improves immunogenicity. Vaccine 2012; 30: 6777-6782. doi: 10.1016/j.vaccine.2012.09.021.

180) Kumar R, Ray PC, Datta D, Bansal GP, Angov E, Kumar N. Nanovaccines for malaria using Plasmodium falciparum antigen Pfs25 attached gold nanoparticles. Vaccine 2015; 33: 5064-5071. doi: 10.1016/j.vaccine.2015.08.025.

181) Yu Z, Xu Q, Dong C, Lee SS, Gao L, Li Y, D'Ortenzio M, Wu J. Self-assembling peptide nanofibrous hydrogel as a versatile drug delivery platform. Curr Pharm Des 2015; 21: 4342-4354.

182) Alshamsan A, Haddadi A, Incani V, Samuel J, Lavasanifar A, UludaĐ H. Formulation and delivery of siRNA by oleic acid and stearic acid modified polyethylenimine. Mol Pharm 2009; 6: 121-133. doi: 10.1021/mp8000815.

183) Wibowo N, Chuan YP, Seth A, Cordoba Y, Lua LH, Middelberg AP. Co-administration of non-carrier nanoparticles boosts antigen immune response without requiring protein conjugation. Vaccine 2014; 32: 3664-3669. doi: 10.1016/j.vaccine.2014.04.043.

184) Zhai W, He C, Wu L, Zhou Y, Chen H, Chang J, Zhang H. Degradation of hollow mesoporous silica nanoparticles in human umbilical vein endothelial cells. J Biomed Mater Res B Appl Biomater 2012; 100: 1397-1403. doi: 10.1002/jbm.b.32711.

185) Chen K, Zhang J, Gu H. Dissolution from inside: a unique degradation behaviour of core-shell magnetic mesoporous silica nanoparticles and the effect of polyethyleneimine coating. J Mater Chem 2012; 22: 22005-22012. doi: 10.1039/C2JM34364A.

186) Mody KT, Mahony D, Cavallaro AS, Zhang J, Zhang B, Mahony TJ, Yu C, Mitter N. Silica vesicle nanovaccine formulations stimulate long-term immune responses to the bovine viral diarrhoea virus E2 protein. PLoS One 2016; 11: e0146631. doi: 10.1371/journal.pone.0146631.

187) Temchura VV, Kozlova D, Sokolova V, Uberla K, Epple M. Targeting and activation of antigen-specific B-cells by calcium phosphate nanoparticles loaded with protein antigen. Biomaterials 2014; 35: 6098-6105. doi: 10.1016/j.biomaterials.2014.04.010.

188) Epple M, Ganesan K, Heumann R, Klesing J, Kovtun A, Neumann S, Sokolova V. Application of calcium phosphate nanoparticles in biomedicine. J Mater Chem 2010; 20: 18-23. doi: 10.1039/B910885H.

189) Morgan TT, Muddana HS, Altinoglu EI, Rouse SM, Tabaković A, Tabouillot T, Russin TJ, Shanmugavelandy SS, Butler PJ, Eklund PC, Yun JK, Kester M, Adair JH. Encapsulation of organic molecules in calcium phosphate nanocomposite particles for intracellular imaging and drug delivery. Nano Lett 2008; 8: 4108-4115. doi: 10.1021/nl8019888.

190) Knuschke T, Bayer W, Rotan O, Sokolova V, Wadwa M, Kirschning CJ, Hansen W, Dittmer U, Epple M, Buer J, Westendorf AM. Prophylactic and therapeutic vaccination with a nanoparticle-based peptide vaccine induces efficient protective immunity during acute and chronic retroviral infection. Nanomedicine 2014; 10: 1787-1798. doi: 10.1016/j.nano.2014.06.014.

191) He Q, Mitchell AR, Johnson SL, Wagner-Bartak C, Morcol T, Bell SJ. Calcium phosphate nanoparticle adjuvant. Clin Diagn Lab Immunol 2000; 7: 899-903.

192) Han J, Zhao D, Li D, Wang X, Jin Z, Zhao K. Polymer-based nanomaterials and applications for vaccines and drugs. Polymers (Basel) 2018; 10. pii: E31. doi: 10.3390/polym10010031.

193) Mitchell LA, Gao J, Wal RV, Gigliotti A, Burchiel SW, McDonald JD. Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes. Toxicol Sci 2007; 100: 203-214.

194) Ravi Kumar MNV. A review of chitin and chitosan applications. React Funct Polym 2000; 46: 1-27.

195) Kojima K, Okamoto Y, Kojima K, Miyatake K, Fujise H, Shigemasa Y, Minami S. Effects of chitin and chitosan on collagen synthesis in wound healing. J Vet Med Sci 2004; 66: 1595-1598.

196) Jayakumar R, Nwe N, Tokura S, Tamura H. Sulfated chitin and chitosan as novel biomaterials. Int J Biol Macromol 2007; 40: 175-181.

197) Prego C, Paolicelli P, Díaz B, Vicente S, Sánchez A, González-Fernández A, Alonso MJ. Chitosan-based nanoparticles for improving immunization against hepatitis B infection. Vaccine 2010; 28: 2607-2614. doi: 10.1016/j.vaccine.2010.01.011.

198) Li D, Fu D, Kang H, Rong G, Jin Z, Wang X, Zhao K. Advances and potential applications of chitosan nanoparticles as a delivery carrier for the mucosal immunity of vaccine. Curr Drug Deliv 2017; 14: 27-35. doi: 10.2174/1567201813666160804121123.

199) Liao ZX, Peng SF, Ho YC, Mi FL, Maiti B, Sung HW. Mechanistic study of transfection of chitosan/DNA complexes coated by anionic poly(gamma-glutamic acid). Biomaterials 2012; 33: 3306-3315. doi: 10.1016/j.biomaterials.2012.01.013.

200) Li J, Yu J, Xu S, Shi J, Xu S, Wu X, Fu F, Peng Z, Zhang L, Zheng S, Yuan X, Cong X, Sun W, Cheng K, Du Y, Wu J, Wang J. Immunogenicity of porcine circovirus type 2 nucleic acid vaccine containing CpG motif for mice. Virol J 2016; 13: 185.

201) Wang H, Han Q, Zhao H, Xu D, Zhang J. Single dose HBsAg CS-γ-PGA nanogels induce potent protective immune responses against HBV infection. Eur J Pharm Biopharm 2018; 124: 82-88. doi: 10.1016/j.ejpb.2017.12.003.

202) Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. Control Release 2012; 161: 505-522. doi: 10.1016/j.jconrel.2012.01.043.

203) Facciolà A, Riso R, Avventuroso E, Visalli G, Delia SA, Laganà P. Campylobacter: from microbiology to prevention. J Prev Med Hyg 2017; 58: E79-E92.

204) Taha-Abdelaziz K, Hodgins DC, Alkie TN, Quinteiro-Filho W, Yitbarek A, Astill J, Sharif S. Oral administration of PLGA-encapsulated CpG ODN and Campylobacter jejuni lysate reduces cecal colonization by Campylobacter jejuni in chickens. Vaccine 2018; 36: 388-394. doi: 10.1016/j.vaccine.2017.11.073.