Nanodiagnostic and Nanotherapeutic Molecular Platforms for Cancer Management

A. Lyberopoulou[1], E.P. Efstathopoulos[2] and M. Gazouli[1,*]

[1]*Department of Basic Medical Science, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece*

[2]*Second Department of Radiology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece*

Abstract: Over the last ten years rapid progress is being made regarding the incorporation of nanoparticles in cancer diagnosis and treatment. Besides the limitations that have to be addressed, there are various research studies suggesting some promising nanodiagnostic and nanotherapeutic platforms for cancer managment. Nanotherapeutic platforms are based on the localized application of nanoparticles using targeting moieties, most usually antibodies, in order to *in vivo* direct nanoparticles to cancer cells. Thereafter, either nanoparticles react to external stimulus, for example under radiofrequency waves nanoparticles generate thermal energy, or they are used for targeted drug-delivery platforms, which allows the augmentation of drug concentration in the cancerous site of the body and thus minimizing side effects and increasing the efficacy of the drug. Regarding nanodiagnostics, particular focus is paid on nanoparticles that can act as contrast agents in cancer imaging for *in vivo* nanodiagnostics and on nanobiochips and nanobiosensor, devices that incorporate the "lab on a chip" notion for *in vitro* nanodiagnostics. In this review, several advanced nanodiagnostic and nanotherapeutic platforms are discussed, on the development of more effective and targeted molecular techniques in the diagnosis and treatment of cancer.

Keywords: Nanotechnology, cancer, nanodiagnostics, nanotherapeutics.

INTRODUCTION

Nanomedicine, the application of nanotechnology to medicine, has been defined as the monitoring, repair, construction and control of human biological systems at the molecular level, using engineered nanodevices and nanostructures. It is an emerging new field, which accounts for about 5% of nanotechnology publications worldwide and includes several distinct application areas, such as drug delivery, *in vivo* imaging, *in vitro* diagnostics, biomaterials and active implants. In the context of *in vitro* nanodiagnostics, nanotechnology allows the construction of novel sensors and *in vitro* assays, in order to improve the sensitivity of existing tests, to develop new diagnostic test platforms and to allow point of care applications [1-3]. Such approaches are the biosensor platforms that consist of nanomaterials and the use of nanoparticles as biomolecule markers. Furthermore, nowadays new multifunctional nanoplatforms can be constructed that have capabilities for *in vivo* disease targeting, using moieties such as antibodies. Such nanoplatforms are endowed with image contrast enhancement capabilities for techniques like MRI and PET, and contain therapeutic payloads that can be released at the disease site. The notion of combining diagnostics and therapeutics for the management of several diseases is now being referred to as theranostics. Regarding cancer management, the combination of *in vitro* diagnostics techniques, *in vivo* nano-imaging and nanobased drug delivery could lead to targeted tumor disruption or removal. Up to now, cancer still remains the leading cause of death worldwide according to the World Health Organization, besides significant advances in cancer treatment the last decades. Current diagnostic and therapeutic approaches for cancer management are mostly based on invasive (i.e. random biopsies, surgery) and non-specific techniques (irradiation, chemotherapeutic agents). Thus, the use of molecular nanoplatforms for the development of molecular diagnostic tests and targeted therapeutics will allow cancer management based on the goals of individualizing treatment by targeting therapy to an individual's' specific cancer type and genetic profile. This strategy will enable optimization of drug efficacy and safety and will assist in streamlining the drug development process [3].

This review provides a comprehensive summary of recent progress in nanomedicine as it relates specifically to cancer nanodiagnostic nanoparticles, to cancer theragnostic nanoparticles and drug delivery and advanced nanotherapeutic strategies for cancer management.

IN VITRO NANODIAGNOSTICS

An *in vitro* diagnostic tool can be a single chemosensor or biosensor, or an integrated device

*Address correspondence to this author at the Laboratory of Biology, Medical School, National and Kapodistrian University of Athens, Athens, Greece; Tel: +302107462231; E-mail: mgazouli@med.uoa.gr

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containing many sensors. Nanobiosensors are devices on the nanoscale, which are consisted of an element that is able to identify a biochemical change, activity or concentration of a specific molecule of biological importance in solution. A transducer is used to convert the biochemical signal into a quantifiable signal. Thus far, many types of nanomaterials, such as AuNPs, semiconductor II-VI quantum dots (QDs), silicon nanowires (SNWs), carbon nanotubes (CNTs) and graphene, have been utilized for constructing high-performance nanoscale biosensors for the detection of cancer markers (proteins/peptides or DNA/RNA) in a sensitive and specific manner [1-3].

Nanowires and Nanotubes

Nanowires are currently believed to be unique and advantageous for the construction of a nanobiosensing device. Nanowires are nanoscales channels through which current is passed and can be constructed from carbon nanotubes, metal oxides or silicon. Antibodies are usually used as detectors in the surface of the nanowire. When antibodies interact with the biological target of interest, the conformational change results in a change in the current that passes through the nanowire allowing the extremely sensitive and specific detection. Because of the nanoscale size of a nanowire, with a diameter of about 10 nm, even a small change, like bonding an additional molecule to the nanowire, can make a noticeable change in its electrical properties. Using several nanowires in an array with each nanowire functionalized with a different antibody, allows each nanowire to be used to detect a different type of disease or cancer type [4]. Sensors based on silicon nanowires (SiNWs) operate as field effect transistors (FETs) and their nano-dimensions allow the sensitive detection of biomolecules [5].

For instance, FET-SiNWs have been used for the detection of several prostate cancer biomarkers, such as PSA (Prostate-specific antigen) at the level of fg/ml of PSA for monitoring prostate cancer and predicting the risk of early biochemical relapse [6, 7] and the prostate biomarker 8-hydroxydeoxyguanosine (8-OHdG) by using a SiNW functionalized with antibodies against 8-OHdG [8]. PSA, prostate-specific membrane antigen, platelet factor-4 and interleukin-6 prostate cancer biomarkers have also been detected by electrochemical nanotubes [9]. Furthermore, using a nanowire technology (nCounter Analysis System) ribonucleic acid (RNA) expression levels of CTAs (cancer-testis antigens) have been measured, as biomarkers for aggressive prostate cancer [10]. This nanowire technology offers the possibility of a sensor chip, able to simultaneously detect more than one of cancer marker and measure a panel of biomarkers related to a specific cancer type or/and individual, thus contributig to the personalization of cancer diagnosis. Chen *et al*. measured the urinary of apolipoprotein A II protein (APOA2 protein), a biomarker for the diagnosis of bladder cancer, using an n-type polycrystalline silicon nanowire field-effect transistor (poly-SiNW-FET) [11]. SiNWs functionalized with specific antibodies were also used to detect lung cancer biomarker, interleukin-10, using a nanostructure approach which leads to enhanced optical response, in comparison with the bare silica substrate [12]. Abiri *et al*. constructed a silicon nanowire-based electrical cell impedance sensor (SiNW-ECIS) as a diagnostic tool for cancerous cultured living lung cells by monitoring their spreading state compared to the normal ones [13], while Lee *et al*. developed a nanowire substrate-enabled laser scanning imaging combined with flow cytometry for the isolation and quantitation of circulating tumor cells from a human lung carcinoma sample mixture of tumor cells and leukocytes [14]. Interestingly, carbon nanotubes and SiNWs have been utilized for detection of various volatile organic compounds (VOCs) in breath samples of lung and gastric cancer patients respectively [15, 16]. Similar to other cancer types, FET-SiNWs were used in order to detect ALCAM biomarker, which is an early diagnostic biomarker for breast cancer. Thus, Tran *et al*. constructed a portable readout NWs on-a-chip device, by the addition of a metal oxide semiconductor (CMOS) on FET-SiNWs, creating a nano-platform that could detect ALCAM in serum at a detection limit of 15.5 pg/ml, in less than 30 minutes [17]. In addition, FET-nanotubes functionalized with antibodies against IGFIR and HER2 breast cancer biomarkers, were utilized to detect circulating breast cancer cells in blood [18], while carbon nanotube based electrical cell impedance sensing biosensor (CNT-ECIS) has been indicated as a rapid, sensitive and specific device for the detection of colon cancer cells [19].

Besides the cancer biomarker detection, FET-SiNWs and zinc oxide nanowires (ZnONWs) have been used to detect ssDNA, as the binding of this negatively charged polyanionic macromolecule to p-type NW surfaces leads to an increase in conductance [20, 21]. These DNA biosensors have been used to detect cancer mutations related with cancer types. For example, a nanowire platform functionalized with ssDNA detected the BRAF mutation for breast cancer,

while a ZnONWs conjugated with a specific ssDNA oligonucleotide detected the specific for the breast cancer 1 (BRCA1) gene [22, 23]. Furthermore, FET-SiNW biosensors have been fabricated in order to detect mi-RNAs (related to the initiation and progression of various cancer types. For example, Lu *et al*. have detected miRNAs (of miR-21 and miR-205), at a limit of detection of 1 zeptomole in total RNA extracted from lung cancer cells, as well as human serum samples using CMOS on FET-SiNW [24].

Nanocantilevers

Nanocantilevers, are based on the mechanical detection of the three dimensional properties of an object, with a precision of about 10nm. As force sensors they can allow measurements at the level of single molecules, thus cantilever nanobiosensing provides an alternative PCR with possibilities to replace microarray detection methodologies for the identification of SNPs, oncogenes, viruses, bacteria and other pathogens. For instance, Huber *et al*. used microcantilever arrays to detect BRAFV600E mutation nanomechanically without amplification, from total RNA samples isolated from malignant melanoma cells [25]. Wang *et al*. fabricated a new cantilever array-based bio-sensor based on micro-electromechanical systems (MEMS) for the detection of Alpha-Fetoprotein (AFP), a liver cancer biomarker, with high accuracy [26], while Liu *et al.* detected the same biomarker for hepatocellular carcinoma at the level of ng/ml, using a resonant microcantilever electromagnetic resonance-exciting and piezoresistive readout elements on-chip integrated, in order to measure frequency-shift versus specific-adsorbed mass [27]. Just like other nano-biosensors, nanocantilevers were demonstrated to be able to detect PSA at low levels (0.2 ng/ml to 60 μg/ml) for the detection of prostate cancer [28]. PSA was also detected in a liquid environment using piezoeclectric nanomechanical cantilevers, where resonance frequency shift of the cantilever was proportional to the PSA antigen concentration of analyte solution [29].

Nanoparticles

The use of nanoparticles promises to help promote *in vitro* diagnostics to the next level of performance. Quantum dots (QDs), gold nanoparticles (AuNPs), and superparamagnetic nanoparticles are the most promising nanostructures for *in vitro* diagnostic applications. These nanoparticles can be conjugated to recognition moieties such as antibodies or oligonucleotides for detection of target biomolecules. In the field of *in vitro* diagnostics, nanoparticles are mainly used as markers for biomolecules. On the other hand conventional fluorescent dyes already used in medical laboratory tests, in PCR assays and in biochips are not photostable or suitable for multiplexing. Jokerst *et al*. used semiconductor nanoparticle quantum dots (QDs) combined with a microfluidic biosensor for the multiplex quantitation of cancer biomarkers like, carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), and Her-2/Neu (C-erbB-2) in serum and whole saliva specimens [30]. This QD nano-bio-chip assay system resulted in a 30 times signal amplification, compared with standard molecular fluorophores. Moreover, supermagnetic iron oxide nanoparticles are used, integrated with applications like cell sorting, nucleic acid extraction and purification for the detection and isolation of circulating tumor cells of several types of cancer such as colorectal, lung and breast cancer [31-33].

IN VIVO NANODIAGNOSTICS AND THERAGNO-STICS

Nanostructures are incorporated in *in vivo* nanodiagnostics *via* their use as contrast agents in a variety of *in vivo* imaging modalities. Nowadays, multiple imagine techniques hold a substantial role in all stages of cancer management: prognosis, screening, biopsy guidance, staging and detection of metastasis, therapy, surgical guidance and recurrence. In current clinical practice, cancer imaging includes non-invasive imaging modalities, such as Computed Tomography (CT) Scans, Magnetic Resonance (MR) Imaging Scans, Positron Emission Tomography (PET) Scans, Single Photon emission CT (SPECT), Ultrasound (US) Scans and optical imaging for macroscopically visualising tumours [34]. All of these imaging modalities provide different information about the patient: CT scan provides information about structure and is most sensitive to electron-dense elements, such as those found in bones and on the other hand MRI scan provides information about soft tissues, and is most sensitive to tissues and organs, while PET scan can provide information about metabolic parameters, and can be particularly sensitive to detection of high-metabolism tumors and infections. The resolution of both CT and MRI scans can be significantly improved by introduction of contrast agents that will further increase the electron density. However, molecular imaging offers new insights to fight cancer in microscopic level, for the detection of cancer- specific biomolecules and signalling pathways in order to diagnose cancer metastasis at early stages and to

design drug systems focused on cancerous tissues towards an era of personalized medicine [35].

Several NPs have been successfully combined with imaging modalities, because of their beneficial properties as fluorescent probes (controllable emission wavelenghts, sharp emission profiles, robust signal strength and the use of a single excitation source) and their potential for functionalization with peptides, antibodies and various drugs such as chemotherapeutics [36]. Most studies suggest that NPs systems based on passive targeting of tumor sites, can be more effective for targeting solid, primary tumors with fairly large size (at least 2mm) and well developed vasculatory system. However, early stage primary tumors and micro-metastases do not demand robust blood supply and are not detectable *via* passive targeting. Therefore, tumor-specific detection *via* active targeting is still a challenge of great significance [37]. Recently, there is a growing interest for theragnostic NPs, which combine therapy and diagnosis in a single biocompatible and biodegradable nanosystem. However, none of the so far described nanosystems are incorporated in clinical practice, except for iron oxide NPs (IONPs), particularly due to the lack of reproducibility, suitable biodistribution and pharmacokinetics [35]. The combination of the existing imaging technology with theragnostic NPs, gives a great advantage for high-resolution *in vivo* cancer imaging, drug monitoring and drug delivery in a specific mode of action. So far, FDA has approved 35 imaging or/ and therapeutic NPs for clinical trials among them, IONPs, gold nanocages and nanoshells, biodegraded polymeric NPs, silica and silica-gold NPs. However, the incorporation of NPs in molecular imaging still needs a lot of progress since such nanomaterials are characterized by pharmacokinetic properties that cannot be easily controlled [36].

Targeted Delivery

Targeted delivery strategies offer the opportunity for targeted and specific delivery of drugs to cancer sites. There are two targeting strategies for drug delivery, the active and the passive targeting, based on the EPR effect (enhanced permeability and retention) [37]. In active targeting, the NPs have to be functionalized with a antibody, aptamer, peptide, oligonucleotide that are cancer-specific. In the case of passive targeting, the NPs are not specifically targeted by ligants, but intermediate NPs (10-500nm in size) have the ability to enter the tumorous permeable vasculature and basement membrane, allowing the accumulation of NPs in tumor sites. There have also been developed several strategies for controlled drug release, such as thermo-responsive release, enzyme responsive release and pH responsive release [38]. For example, biodegradable polyelectrolyte microcapsules (PMCs) are supramolecular assemblies of enzyme responsive release into viable cells that are suggested to be very effective as drug-carriers that maximize drug's exposure enhancing antitumor activity of neoplastic drug in cancer cells [39]. Vergara *et al.* constructed stable nanocolloids of paclitaxel and lapatinib *via* sonication-assisted layer-by-layer (SLBL) technology and treated a multidrug-resistant ovarian cancer cell line. These multidrug nanocolloids inhibited cancer cell growth indicating a strong potential for *in vivo* application, especially in resistant or relapsed patients [40]. The use of SLBL technology, offers the advantage of reducing drug-dose escalation in clinical settings. Pattekari *et al.* created nanocapsules with controllable paclitaxel release rates, by assembling multilayer shells in the surface of the nanocapsules [41], while Zhang *et al*. proposed polyelectrolyte layer-by-layer encapsulation for sustained drug release from nanoparticles over the range of 10-20 h [42]. Researchers have constructed various nanosystems for targeted drug delivery such as, liposomes and micelles, natural and synthetic polymer nanoparticles, metal, gold and magnetic nanoparticles, hydrogels, Polymersomes, Nanotubes, Dendrimers, Nanocells, XPclad® NPs. The choice of carrier system is a difficult task, since there are many factors to be taken into consideration like the type of drug delivered (biodistribution and pharmacokinetics) and the carriers' properties like its stability, size, rigidity, charge, solubility and surface modifications. So far, the most important nanocarrier systems in current drug delivery are liposome-based delivery systems and polymer nanoparticles [38, 43].

Theragnostic Nanoparticles: Image-Guided Cancer Management

NPs can be easily combined with MRI, optical imaging and photoacoustic imaging. When appropriately functionalized with imaging probes they can be incorporated in nearly all imaging modalities. SPECT, PET and even more multimodal imaging techniques like PET/SPECT, MRI, CT, NIRF combined with NPs, allow high sensitivity with minimized background noise, measurement quantification and non-invasive procedures, creating an indispensable tool for targeted *in vivo* molecular imaging [44, 45]. There are various theragnostic NPs and delivery

strategies used, depending on the imaging modality combined with. Their size has a range of 10 to 100nm, manufactured from soluble or colloidal polymeric materials and functiolalized with an imaging probe (encapsulated in the core or conjugated on the surface) and another probe on the surface that recognizes the tumor in a specific way [45, 46] (e.g targeting the folate receptor, integrin αvβ3, VEGF, PSMA that are upregulated in different cancer types) [47-50]. The desired nanosystem should be non-toxic, non-immunogenic and active only in the tissue of interest and not in bloodstream. PEG polymer (polyethylene glycol) is approved by the FDA and conjugated to several drugs such as, Oncaspar (asparaginase), Neulasta (granulocyte colony stimulating factor), Peg-Intron (a-interferon 2b). Monoclonal antibodies and recombinant DNA are used to reduce immunogenic reaction, enhance the stability of the nanosystem and thus prolong half-life in bloodstream, while conjugated linkers responsive to specific stimuli release the encapsulated drug only in specific environments (eg. pH sensitive, thermosensitive) [35, 36].

Polymeric micelles, liposomes and dendrimers are usually combined with molecular imaging technology in order to study pharmacokinetics, targeted drug delivery, drug release and therapeutic efficacy. Xiao *et al*. [51], demonstrated that multifunctional unimolecular micelles showed passive and active tumor-targeting abilities *via* c-RGD peptides for integrin αvβ3 targeting, with pH-controlled drug release and PET imaging capabilities for cancer-targeted drug delivery. The anti-cancer drug, doxorubicin (DOX) was covalently conjugated to the arms of a hyperbranched amphiphilic block copolymer, in order to study its release and target efficacy to the tumor. Tagami *et al*. [52], created a liposomal nanosystem encapsulated with an MRI gadolinium-based agent (Gd-DTPA) and DOX, which is simultaneously released in a locally heated tumor (HaT: Hyperthermia-activated-cytoToxic), to predict the anti-tumor efficacy and release of DOX in a standard pharmacological response model. In two other studies [53, 54], theragnostic nanoparticles were developed, to follow up tumor size in real time in relation to drug uptake. Kaida *et al.* [53], used a mouse model with human pancreatic tumor to evaluate the effect of platinum anticancer drugs, in a polymeric micelle conjugated with gadolinium-based agent, while Phillips *et al.* [54], developed a radionuclide (rhenium 186) liposome for enhanced contrast MRI scan, to study the efficacy of brachytherapy in a glioma rat model, with outsanding results regarding the tumor size. Another interesting approach is the binding of siRNA anti-tumor small drugs in solid lipid NPs or iron oxide NPs (IOs), with the possibility of co-encapsulation of other chemotherapeutic drugs like paclitaxel for synergistic chemotherapy and imaging at the same time [55-58].

Consequently, drug delivery and imaging nanosystems exhibit many advantages regarding the access in cancerous sites and the vasculatory system of the tumor, the prolonged circulation, the encapsulation of various drugs and imaging agents. Thus, theragnostic NPs are suitable for imaging liver, spleen, lymph nodes, organs that simultaneously take up NPs and interesting candidates for the development of a drug delivery nanosystem for imaging-guided interventions regarding cancer management [58, 59].

ADVANCED NANO-THERAPEUTICS

Drug-Delivery Nanoplatforms for Cancer Vaccines

Cancer cells carry molecules in their membrane that are not recognized as harmful from the immune system or have the ability to evade the recognition procedure by downregulation of MHC class I expression or mutations on MHC class I gene. Even if the immune system recognizes the cancer antigen, a strong attack against cancer cells is not triggered, since cancer cells suppress immune response by turning off activated T-cells. But at the same time, it not fully understood how cancer cells escape recognition and attack the immune system, thus cancer vaccines are difficult to be successfully fabricated. Treatment cancer vaccines are mainly based on immunotherapy and consist of tumor-associated antigens (TAAs), adjuvants or DNA/RNA that could trigger the immune system to recognize those antigens and orchestrate a response. In addition, treatment vaccines can act based on targeted therapy and aim to boost the immune system against cancer. NPs have many properties that make them appropriate for carriers of encapsulated or conjugated vaccine molecules. They are proven to be stable and have prolonged stay in circulation and to enter deep in the tumor mass for the targeted release of the payload. For instance, NPs of 10-100nm in diameter, have the ability to passively enter lymphoid tissues and stay there long enough, in order to allow APCs (antigen-presenting cells) to present the vaccine [60]. Actually, it has been demonstrated that nanobeads of 40nm conjugated with an antigen (protein or peptide), trigger a specific T-cell immune response that is boosted compared to currently used cancer vaccines such as Alum (aluminum salts), MPLA (monophosphoryl lipid A) and

Quil-A (saponins' triterpene aglycone) [61]. Recently, it has been shown that tumor-draining lymph node is a great target of nano-vaccines (TAA-NPs and CpG-NPs) in comparison to non tumor-draining lymph node, since nano-vaccines can initiate there a strong cytotoxic CD8+ T-cell response, locally and systemically [62]. To the same end, Thomas *et al*. used 30 nm polymeric NPs, designed to target dendritic cells (DCs, CD11c+) in the lymph node, which are the most potent APCs. CpG motifs (when unmethylated they act as immunostimulants) and paclitaxel where encapsulated in the NPs to activate DCs, which in turn caused the redistribution of CD4(+) T cells, increased the frequency of antigen-specific CD8(+) T cells in the tumor and thus, decreased tumor growth [63]. NPs have been also used as multifunctional carriers combined with MRI [64] or NIRF [65], in order to simultaneously track the nano-vaccine *in vivo*. A pH controlled delivery strategy has been used to fabricate nano-vaccines that promote cross-presentation, since the presentation of an antigen *via* MHC-I to activate cytotoxic T cells (CD8+ T cells), presupposes the involvement of endoplasmic reticulum and endosomes, subcellular compartments with acidic environment. This strategy offers a safe and efficient cancer immunotherapy [66].

DNA vaccination therapy is proposed to be an appropriate type of cancer vaccine formulation, since they it can trigger humoral and cellular immune responses and is the safest vaccine up to now. DNA/ mRNA sequences that encode for tumor-related proteins/peptides or immunostimulatory molecules, are antigens of great interest, since they are easy to fabricate. Polymers, ceramics, metals and biological materials of various shapes, spherical, branched, shell structures, are some of the nanoparticles used for DNA delivery [67, 68]. Researchers are trying to deliver naked DNA with no satisfying response rates and to incorporate viral vectors and electroporation in order to successfully transform the vaccine DNA, but safety issues are still needed to be addressed. Synthetic delivery systems seem to be a non toxic solution for DNA vaccine in comparison to viral vectors. For example, LPDs (liposome-polycation-DNA nanoparticles), are a combination of DOTAP (1,2-dioleoyl-3-trimethylammonium propane) modified cationic liposome and a cationic polymer (protamine) condensed DNA [69]. Cui *et al.* used an LPD (cationic liposome-polycation-plasmid DNA) nanoparticle platform to trigger immunostimulation. LPD-NPs entered the tumor lymphoid tissues and managed to activate APCs [70].

On the other hand, whole-cell cancer vaccines are also an interesting strategy due to the broad immune response they can trigger towards a more personalized therapy approach. Fang *et al.* used polymeric nanoparticles with a shell-layer of membrane from cancer cells that could promote a tumor-specific immune response for use in vaccine applications [71]. Gross *et al.* proposed a PLGA microparticle vaccine formulation composed of a cocktail of Toll-like receptor (TLR)-stimulating adjuvants, in order manage metastatic breast cancer. The vaccine formulation entered the lymph nodes and was shown to be successfully phagocytosed by dendritic cells (DCs) within 48 h and to reduce metastatic lung tumors by 42%, without inducing autoimmunity [72]. Wang *et al*. used a TLR3 agonist, polyribocytidylic acid (PIC), combined with DOTAP cationic liposome to show that PIC-DOTAP nanoformulation leaded to an enhanced uptake of PIC compared to DOTAP or PIC alone. They showed that PIC-DOTAP is a potent nanoformulation that is able to increase antitumor effect of cancer vaccines, *via* the promotion of DCs maturation, the production of type I IFN and the triggering of TLR3 signaling in BMDCs (bone marrow DCs) [73].

DNA Nanostructures for Drug Delivery

Currently, one field that meets great interest is DNA nanotechnology and the use of this technology in cancer treatment. The breakthrough came in 2006 with DNA origami, a method for folding long, single-stranded DNA molecules into arbitrary two-dimensional shapes, such as squares, disks and five-pointed stars with a spatial resolution of 6 nm and a total diameter of 100nm [74]. Such DNA nanostructures can be very useful in drug delivery or/and image guided therapy of cancer. They have several advantages over conventional delivery nanoparticles (liposomes, polymeric systems) that are heterogeneous in size, in composition and in their surface chemistry, leading to decreased tissue specificity and toxicity. On the other hand, ssDNA structures can be more easily be controlled, thus cancer-specific ligands on the nanoparticle surface can be successfully controlled [75]. For example, Zhang *et al*. constructed a triangle-shaped DNA origami loaded with the anticancer drug doxorubicin, which passively accumulated in tumor sites. These nanostructures are proposed to be a safe approach of drug delivery of cancer therapeutics *in vivo*, since no systemic toxicity has been observed in mouse models [76]. Cho *et al*. created a DNA structured multilayer nano-film, to achieve biodegradable drug (doxorubicin)-delivery in a

controlled manner [77]. Wang *et al*. showed that DNA origami nanostructures can act as nano-carriers for anticancer-drugs that can effectively circumvent the drug resistance of cultured cancer cells [78]. In addition, DNA nanostructures can be combined with metallic or polymer nanomaterials appropriate to be used as electronic nanodevices, while they will be able to be loaded with various molecules and cell-targeting agents simultaneously, in ordered to achieve targeted therapy and combined administration of drugs. For instance, Mou *et al.* proposed a DNA-protein hybrid co-assembling nanowires, where proteins allow to the nanostructure to perform a variety of functions on biomolecules, while self-assembly DNA nanostructures are limited in affecting other molecules. DNA nanostructures are also good candidates for image guided therapy [79]. Bhatia *et al*. assembled an icosahedral DNA nanostructure loaded with a fluorescent biopolymer that functions as a pH reporter, an appropriate strategy for controlled drug delivery in cancer targeted therapy that offers the opportunity of *in vivo* imaging [80]. More recently, Zhang *et al.* suggested an aptamer-based self-assembled DNA dendrimer, as a potential multifunctional candidate for image guided, targeted and controllable drug-delivery in cancer cells [81]. Besides drugs, DNA nanostructures could serve as nanocarriers for gene therapy. For example, Lee *et al.* proposed a self-assembled DNA tetrahedral nanoparticle for siRNA delivery in cancer cells, in order to silence cancer related genes in tumors [82]. The future in the use of DNA nanostructures in nanomedicine and cancer management are DNA nanorobots, which will be able to interact with living animals [83] and with computational regulated function [84].

DISCUSSION

The first critical step in cancer management is to diagnose and stage the disease. Cancer diagnosis is based on pathological examination, *in vitro* assays and in the use of several imaging modalities. CAT scan is able to show the presence, size and location of tumors, while MRI the difference between normal and diseased soft tissues of the body, like breasts, bones and joints, organs in the pelvis, chest and abdomen (heart, liver, kidney, spleen). The combination of PET with CT imaging offers precision and detailed computerized pictures of sites in the body that can aid with cancer diagnosis and follow-up regarding the effectiveness of treatments. Currently, PET-CT is used as the number one tool in the delineation of tumor volumes, stratification and treatment selection. This combination has proven to be beneficial for active treatment decisions, disease recurrence monitoring and patient outcomes, such as disease-free progression. Cancer imaging is subtended by *in vitro* assays, based on the identification of cancer biomarkers, like a cancer related protein, peptide and mutation. For example, PSA detection in bloodstream for prostate cancer diagnosis, the detection of the amplification of *HER-2/neu* gene for herceptin treatment selection in breast cancer, the detection of mutations in several cancer related genes such as *Ras, Braf, Brca* that are all assays approved by FDA and are already in clinical practice. Regarding therapy, there are three major approaches surgery, radiotherapy and systemic therapy, but usually there is a combination of the three. Surgery alone or radiation alone can only succeed in localized and small sized tumors, while chemotherapy alone can only be effective in leukaemias and lymphomas. Besides the outstanding development in the management of cancer disease, so far it is based on invasive therapy techniques and late diagnosis, when at the same time approaches towards a more personalized way of disease management are not yet used in every day clinical practice.

The incorporation of nanotechnology in cancer management has so far beneficial effects, since several nanoplatforms have been successfully combined with all imaging modalities, have been used for the construction of nanodiagnostic chips that can *in vivo* recognize cancer biomarkers in the tumor site or/and in CTCs (circulating tumor cells) in blood and have been used for nanotherapeutic approaches, since they are a great choice for targeted drug delivery. The currently approved nanoplatforms have improved drug efficacy and reduced drug toxicity. Nanoplatforms have the advantage of multifunctionality regarding, the ligands that can be conjugated, or/and the molecules that can be encapsulated and the engeeniring capabilities, like DNA origami, that will lead to the next generation of theragnostic nanoplatforms, which will be able to diagnose, stage the disease and treat at the same time. Current research is now working on fabricating such theragnostic nanoplatforms, incorporating safer nanomaterials with novel engineering approaches, so that an increasing number of multifunctional nanoparticles enter the clinic in the future.

CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

REFERENCES

[1] Peng F, Su Y, Zhong Y, Fan C, Lee S-T, He Y. Silicon nanomaterials platform for bioimaging, biosensing, and cancer therapy. Acc Chem Res 2014; 47(2): 612-623. http://dx.doi.org/10.1021/ar400221g

[2] Li Z, Barnes JC, Bosoy A, Stoddart JF, Zink JI. Mesoporous silica nanoparticles in biomedical applications. Chem Soc Rev 2012; 41(7): 2590-2605. http://dx.doi.org/10.1039/c1cs15246g

[3] Lee DE, Koo H, Sun IC, Ryu JH, Kim K, Kwon IC. Multifunctional nanoparticles for multimodal imaging and theragnosis. Chem Soc Rev 2012; 41(7): 2656-2672. http://dx.doi.org/10.1039/C2CS15261D

[4] Reimhult E, Höök F. Design of surface modifications for nanoscale sensor applications. Sensors (Basel) 2015; 15(1): 1635-75. http://dx.doi.org/10.3390/s150101635

[5] Shen MY, Li BR, Li YK. Silicon nanowire field-effect-transistor based biosensors: from sensitive to ultra-sensitive. Biosens Bioelectron 2014; 60: 101-11. http://dx.doi.org/10.1016/j.bios.2014.03.057

[6] Zheng G, Patolsky F, Cui Y, Wang WU, Lieber CM. Multiplexed electrical detection of cancer markers with nanowire sensor arrays. Nat Biotechnol 2005; 23(10): 1294-1301. http://dx.doi.org/10.1038/nbt1138

[7] Gao A, Lu N, Dai P, Fan C, Wang Y, Li T. Direct ultrasensitive electrical detection of prostate cancer biomarkers with CMOS-compatible n- and p-type silicon nanowire sensor arrays. Nanoscale 2014; 6(21): 13036-42. http://dx.doi.org/10.1039/C4NR03210A

[8] Mohd Azmi MA, Tehrani Z, Lewis RP, *et al.* Highly sensitive covalently functionalised integrated silicon nanowire biosensor devices for detection of cancer risk biomarker. Biosens Bioelectron 2014; 52: 216-24. http://dx.doi.org/10.1016/j.bios.2013.08.030

[9] Chikkaveeraiah BV, Bhirde A, Malhotra R, Patel V, Gutkind JS, Rusling JF. Single-wall carbon nanotube forest arrays for immunoelectrochemical measurement of four protein biomarkers for prostate cancer. Anal Chem 2009; 81(21): 9129-34. http://dx.doi.org/10.1021/ac9018022

[10] Takahashi S, Shiraishi T, Miles N, Trock BJ, Kulkarni P, Getzenberg RH. Nanowire analysis of cancer-testis antigens as biomarkers of aggressive prostate cancer. Urology 2015; 85(3): 704.e1-7. http://dx.doi.org/10.1016/j.urology.2014.12.004

[11] Chen HC, Chen YT, Tsai RY, *et al.* A sensitive and selective magnetic graphene composite-modified polycrystalline-silicon nanowire field-effect transistor for bladder cancer diagnosis. Biosens Bioelectron 2015; 66: 198-207. http://dx.doi.org/10.1016/j.bios.2014.11.019

[12] Sekhar PK, Ramgir NS, Joshi RK, Bhansali S. Selective growth of silica nanowires using an Au catalyst for optical recognition of interleukin-10. Nanotechnology 2008; 19(24): 245502. http://dx.doi.org/10.1088/0957-4484/19/24/245502

[13] Abiri H, Abdolahad M, Gharooni M, *et al.* Monitoring the spreading stage of lung cells by silicon nanowire electrical cell impedance sensor for cancer detection purposes. Biosens Bioelectron 2015; 68: 577-85. http://dx.doi.org/10.1016/j.bios.2015.01.057

[14] Lee SK, Kim GS, Wu Y, *et al.* Nanowire substrate-based laser scanning cytometry for quantitation of circulating tumor cells. Nano Lett 2012; 12(6): 2697-704. http://dx.doi.org/10.1021/nl2041707

[15] Shehada N, Brönstrup G, Funka K, Christiansen S, Leja M, Haick H. Ultrasensitive silicon nanowire for real-world gas sensing: noninvasive diagnosis of cancer from breath volatolome. Nano Lett 2015; 15(2): 1288-95. http://dx.doi.org/10.1021/nl504482t

[16] Peng G, Trock E, Haick H. Detecting simulated patterns of lung cancer biomarkers by random network of single-walled carbon nanotubes coated with nonpolymeric organic materials. Nano Lett 2008; 8(11): 3631-5. http://dx.doi.org/10.1021/nl801577u

[17] Tran DP, Wolfrum B, Stockmann R, *et al.* Complementary metal oxide semiconductor compatible silicon nanowires-on-a-chip: fabrication and preclinical validation for the detection of a cancer prognostic protein marker in serum. Anal Chem 2015; 87(3): 1662-8. http://dx.doi.org/10.1021/ac503374j

[18] Shao N, Wickstrom E, Panchapakesan B. Nanotube-antibody biosensor arrays for the detection of circulating breast cancer cells. Nanotechnology 2008; 19(46): 465101. http://dx.doi.org/10.1088/0957-4484/19/46/465101

[19] Abdolahad M, Taghinejad M, Taghinejad H, Janmaleki M, Mohajerzadeh S. A vertically aligned carbon nanotube-based impedance sensing biosensor for rapid and high sensitive detection of cancer cells. Lab Chip 2012; 12(6): 1183-90. http://dx.doi.org/10.1039/c2lc21028b

[20] Patolsky F, Zheng G, Lieber CM. Nanowire sensors for medicine and the life sciences. Nanomedicine (Lond) 2006; 1(1): 51-65. http://dx.doi.org/10.2217/17435889.1.1.51

[21] Patolsky F, Zheng G, Lieber CM. Fabrication of silicon nanowire devices for ultrasensitive, label-free, real-time detection of biological and chemical species. Nat Protoc 2006; 1(4): 1711-24. http://dx.doi.org/10.1038/nprot.2006.227

[22] Wu CC, Ko FH, Yang YS, Hsia DL, Lee BS, Su TS. Label-free biosensing of a gene mutation using a silicon nanowire field-effect transistor. Biosens Bioelectron 2009; 25(4): 820-5. http://dx.doi.org/10.1016/j.bios.2009.08.031

[23] Wu CC, Liu FK, Lin LH, *et al.* Surface cleaning of the nanowire field-effect transistor for gene detection. J Nanosci Nanotechnol 2011; 11(12): 10639-43. http://dx.doi.org/10.1166/jnn.2011.3952

[24] Lu N, Gao A, Dai P, *et al.* CMOS-compatible silicon nanowire field-effect transistors for ultrasensitive and label-free microRNAs sensing. Small 2014; 10(10): 2022-8. http://dx.doi.org/10.1002/smll.201302990

[25] Huber F, Lang HP, Backmann N, Rimoldi D, Gerber Ch. Direct detection of a BRAF mutation in total RNA from melanoma cells using cantilever arrays. Nat Nanotechnol 2013; 8(2): 125-9. http://dx.doi.org/10.1038/nnano.2012.263

[26] Wang J, Wang S, Wang X, Zhu Y, Yang J. Cantilever array sensor for multiple liver cancer biomarkers detection. Sensors 2014; 2014: 343-346. http://dx.doi.org/10.1109/icsens.2014.6985004

[27] Liu Y, Li X, Zhang Z, Zuo G, Cheng Z, Yu H. Nanogram per milliliter-level immunologic detection of alpha-fetoprotein with integrated rotating-resonance microcantilevers for early-stage diagnosis of heptocellular carcinoma. Biomed Microdevices 2009; 11(1): 183-91. http://dx.doi.org/10.1007/s10544-008-9223-2

[28] Wu G, Datar RH, Hansen KM, Thundat T, Cote RJ, Majumdar A. Bioassay of prostate-specific antigen (PSA) using microcantilevers. Nat Biotechnol 2001; 19(9): 856-60. http://dx.doi.org/10.1038/nbt0901-856

[29] Hwang KS, Lee JH, Park J, Yoon DS, Park JH, Kim TS. In-situ quantitative analysis of a prostate-specific antigen (PSA) using a nanomechanical PZT cantilever. Lab Chip 2004; 4(6): 547-552. http://dx.doi.org/10.1039/b410905h

[30] Jokerst JV, Raamanathan A, Christodoulides N, *et al.* Nano-bio-chips for high performance multiplexed protein detection: determinations of cancer biomarkers in serum and saliva using quantum dot bioconjugate labels. Biosens Bioelectron 2009; 24(12): 3622-9. http://dx.doi.org/10.1016/j.bios.2009.05.026

[31] Gazouli M, Lyberopoulou A, Pericleous P, *et al.* Development of a quantum-dot-labelled magnetic immunoassay method for circulating colorectal cancer cell detection. World J Gastroenterol 2012; 18(32): 4419-260. http://dx.doi.org/10.3748/wjg.v18.i32.4419

[32] Wang Y, Zhang Y, Du Z, Wu M, Zhang G. Detection of micrometastases in lung cancer with magnetic nanoparticles and quantum dots. Int J Nanomedicine 2012; 7: 2315-24.

[33] Guo S, Chen YQ, Lu NN, Wang XY, Xie M, Sui WP. Ultrasonication-assisted one-step self-assembly preparation of biocompatible fluorescent-magnetic nanobeads for rare cancer cell detection. Nanotechnology 2014; 25(50): 505603. http://dx.doi.org/10.1088/0957-4484/25/50/505603

[34] Pericleous P, Gazouli M, Lyberopoulou A, Rizos S, Nikiteas N, Efstathopoulos EP. Quantum dots hold promise for early cancer imaging and detection. Int J Cancer 2012; 131(3): 519-28. http://dx.doi.org/10.1002/ijc.27528

[35] Chen F, Ehlerding EB, Cai W. Theranostic nanoparticles. J Nucl Med 2014; 55(12): 1919-22. http://dx.doi.org/10.2967/jnumed.114.146019

[36] Torchilin VP. Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery. Nat Rev Drug Discov 2014; 13(11): 813-27. http://dx.doi.org/10.1038/nrd4333

[37] Maeda H, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging *in vivo*. Adv Drug Deliv Rev 2013; 65(1): 71-9. http://dx.doi.org/10.1016/j.addr.2012.10.002

[38] Jain KK. Current status and future prospects of drug delivery systems. Methods Mol Biol 2014; 1141: 1-56. http://dx.doi.org/10.1007/978-1-4939-0363-4_1

[39] Vergaro V, Scarlino F, Bellomo C, *et al.* Drug-loaded polyelectrolyte microcapsules for sustained targeting of cancer cells. Adv Drug Deliv Rev 2011; 63(9): 847-64. http://dx.doi.org/10.1016/j.addr.2011.05.007

[40] Vergara D, Bellomo C, Zhang X, *et al.* Lapatinib/Paclitaxel polyelectrolyte nanocapsules for overcoming multidrug resistance in ovarian cancer. Nanomedicine 2012; 8(6): 891-9. http://dx.doi.org/10.1016/j.nano.2011.10.014

[41] Pattekari P, Zheng Z, Zhang X, Levchenko T, Torchilin V, Lvov Y. Top-down and bottom-up approaches in production of aqueous nanocolloids of low solubility drug paclitaxel. Phys Chem Chem Phys 2011; 13(19): 9014-9. http://dx.doi.org/10.1039/c0cp02549f

[42] Zheng Z, Zhang X, Carbo D, Clark C, Nathan C, Lvov Y. Sonication-assisted synthesis of polyelectrolyte-coated curcumin nanoparticles. Langmuir 2010; 26(11): 7679-81. http://dx.doi.org/10.1021/la101246a

[43] Wagner E. Programmed drug delivery: nanosystems for tumor targeting. Expert Opin Bio The 2007; 7: 587-5. http://dx.doi.org/10.1517/14712598.7.5.587

[44] Bae KH, Chung HJ, Park TG. Nanomaterials for cancer therapy and imaging. Mol Cells 2011; 31(4): 295-302. http://dx.doi.org/10.1007/s10059-011-0051-5

[45] Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. Nat Rev Clin Oncol 2010; 7(11): 653-64. http://dx.doi.org/10.1038/nrclinonc.2010.139

[46] Lvov YM, Pattekari P, Zhang X, Torchilin V. Converting poorly soluble materials into stable aqueous nanocolloids. Langmuir 2011; 27(3): 1212-7. http://dx.doi.org/10.1021/la1041635

[47] Sudimack J, Lee RJ. Targeted drug delivery *via* the folate receptor. Adv Drug Deliv Rev 2000; 41(2): 147-62. http://dx.doi.org/10.1016/S0169-409X(99)00062-9

[48] Zhen Z, Tang W, Guo C, *et al.* Ferritin nanocages to encapsulate and deliver photosensitizers for efficient photodynamic therapy against cancer. ACS Nano 2013; 7(8): 6988-96. http://dx.doi.org/10.1021/nn402199g

[49] Gazouli M, Bouziotis P, Lyberopoulou A, *et al.* Quantum dots-bevacizumab complexes for *in vivo* imaging of tumors. In Vivo 2014; 28(6): 1091-5.

[50] Chen Z, Penet MF, Nimmagadda S, *et al.* PSMA-targeted theranostic nanoplex for prostate cancer therapy. ACS Nano 2012; 6(9): 7752-62. http://dx.doi.org/10.1021/nn301725w

[51] Xiao Y, Hong H, Javadi A, *et al.* Multifunctional unimolecular micelles for cancer-targeted drug delivery and positron emission tomography imaging. Biomaterials 2012; 33(11): 3071-82. http://dx.doi.org/10.1016/j.biomaterials.2011.12.030

[52] Tagami T, Foltz WD, Ernsting MJ, *et al.* MRI monitoring of intratumoral drug delivery and prediction of the therapeutic effect with a multifunctional thermosensitive liposome. Biomaterials 2011; 32(27): 6570-8. http://dx.doi.org/10.1016/j.biomaterials.2011.05.029

[53] Kaida S, Cabral H, Kumagai M, *et al.* Visible drug delivery by supramolecular nanocarriers directing to single-platformed diagnosis and therapy of pancreatic tumor model. Cancer Res 2010; 70(18): 7031-41. http://dx.doi.org/10.1158/0008-5472.CAN-10-0303

[54] Phillips WT, Goins B, Bao A, *et al.* Rhenium-186 liposomes as convection-enhanced nanoparticle brachytherapy for treatment of glioblastoma. Neuro Oncol 2012; 14(4): 416-25. http://dx.doi.org/10.1093/neuonc/nos060

[55] Liu G, Xie J, Zhang F, *et al.* N-Alkyl-PEI-functionalized iron oxide nanoclusters for efficient siRNA delivery. Small 2011; 7(19): 2742-9. http://dx.doi.org/10.1002/smll.201100825

[56] Bae KH, Lee JY, Lee SH, Park TG, Nam YS. Optically traceable solid lipid nanoparticles loaded with siRNA and paclitaxel for synergistic chemotherapy with in situ imaging. Adv Healthc Mater 2013; 2(4): 576-84. http://dx.doi.org/10.1002/adhm.201200338

[57] Wang Z, Liu G, Zheng H, Chen X. Rigid nanoparticle-based delivery of anti-cancer siRNA: challenges and opportunities. Biotechnol Adv 2014; 32(4): 831-43. http://dx.doi.org/10.1016/j.biotechadv.2013.08.020

[58] Kumagai M, Sarma TK, Cabral H, *et al.* Enhanced *in vivo* Magnetic Resonance Imaging of Tumors by PEGylated Iron-Oxide-Gold Core-Shell Nanoparticles with Prolonged Blood Circulation Properties. Macromol Rapid Commun 2010; 31(17): 1521-8. http://dx.doi.org/10.1002/marc.201000341

[59] Ryu JH, Koo H, Sun IC, *et al.* Tumor-targeting multi-functional nanoparticles for theragnosis: new paradigm for cancer therapy. Adv Drug Deliv Rev 2012; 64(13): 1447-58. http://dx.doi.org/10.1016/j.addr.2012.06.012

[60] Irvine D.J, Swartz M.A, Szeto G.L. Engineering synthetic vaccines using cues from natural immunity. Nat. Mater 2013; 12: 978-990. http://dx.doi.org/10.1038/nmat3775

[61] Fifis T, Gamvrellis A, Crimeen-Irwin B, *et al.* Size-dependent immunogenicity: Therapeutic and protective properties of nano-vaccines against tumors. J Immunol 2004; 173: 3148-3154. http://dx.doi.org/10.4049/jimmunol.173.5.3148

[62] Jeanbart L, Ballester M, de Titta A, *et al.* Enhancing efficacy of anticancer vaccines by targeted delivery to tumor-draining lymph nodes. Cancer Immunol Res 2014; 2: 436-447. http://dx.doi.org/10.1158/2326-6066.CIR-14-0019-T

[63] Thomas SN, Vokali E, Lund AW, Hubbell JA, Swartz MA. Targeting the tumor-draining lymph node with adjuvanted nanoparticles reshapes the anti-tumor immune response. Biomaterials 2014; 35(2): 814-24. http://dx.doi.org/10.1016/j.biomaterials.2013.10.003

[64] Rahimian S, Kleinovink JW, Fransen MF, *et al.* Near-infrared labeled, ovalbumin loaded polymeric nanoparticles based on a hydrophilic polyester as model vaccine: *In vivo* tracking and evaluation of antigen-specific CD8(+) T cell immune response. Biomaterials 2015; 37: 469-477. http://dx.doi.org/10.1016/j.biomaterials.2014.10.043

[65] Cruz LJ, Tacken PJ, Zeelenberg IS, *et al.* Tracking targeted bimodal nanovaccines: Immune responses and routing in cells, tissue, and whole organism. Mol Pharm 2014; 11: 4299-4313. http://dx.doi.org/10.1021/mp400717r

[66] Yoshizaki Y, Yuba E, Sakaguchi N, Koiwai K, Harada A, Kono K. Potentiation of pH-sensitive polymer-modified liposomes with cationic lipid inclusion as antigen delivery carriers for cancer immunotherapy. Biomaterials 2014; 35(28): 8186-96. http://dx.doi.org/10.1016/j.biomaterials.2014.05.077

[67] Rice J, Ottensmeier CH, Stevenson FK. DNA vaccines: Precision tools for activating effective immunity against cancer. Nat Rev Cancer 2008; 8: 108-120. http://dx.doi.org/10.1038/nrc2326

[68] Shah MA, He N, Li Z, Ali Z, Zhang L. Nanoparticles for DNA vaccine delivery. J Biomed Nanotechnol 2014; 10(9): 2332-49. http://dx.doi.org/10.1166/jbn.2014.1981

[69] Zhao L, Seth A, Wibowo N, *et al.* Nanoparticle vaccines. Vaccine 2014; 32(3): 327-37. http://dx.doi.org/10.1016/j.vaccine.2013.11.069

[70] Cui Z, Han SJ, Vangasseri DP, Huang L. Immunostimulation mechanism of LPD nanoparticle as a vaccine carrier. Mol Pharm 2005; 2(1): 22-8. http://dx.doi.org/10.1021/mp049907k

[71] Fang RH, Hu CM, Luk BT, *et al.* Cancer cell membrane-coated nanoparticles for anticancer vaccination and drug delivery. Nano Lett 2014; 14(4): 2181-8. http://dx.doi.org/10.1021/nl500618u

[72] Gross BP, Wongrakpanich A, Francis MB, Salem AK, Norian LA. A therapeutic microparticle-based tumor lysate vaccine reduces spontaneous metastases in murine breast cancer. AAPS J 2014; 16(6): 1194-203. http://dx.doi.org/10.1208/s12248-014-9662-z

[73] Wang C, Zhuang Y, Zhang Y, *et al.* Toll-like receptor 3 agonist complexed with cationic liposome augments vaccine-elicited antitumor immunity by enhancing TLR3-IRF3 signaling and type I interferons in dendritic cells. Vaccine 2012; 30(32): 4790-9. http://dx.doi.org/10.1016/j.vaccine.2012.05.027

[74] Rothemund PW. Folding DNA to create nanoscale shapes and patterns. Nature 2006; 440(7082): 297-302. http://dx.doi.org/10.1038/nature04586

[75] Yan J, Hu C, Wang P, *et al.* Growth and origami folding of DNA on nanoparticles for high-efficiency molecular transport in cellular imaging and drug delivery. Angew Chem Int Ed Engl 2015; 54(8): 2431-5. http://dx.doi.org/10.1002/anie.201408247

[76] Zhang Q, Jiang Q, Li N, *et al.* DNA origami as an *in vivo* drug delivery vehicle for cancer therapy. ACS Nano 2014; 8(7): 6633-43. http://dx.doi.org/10.1021/nn502058j

[77] Cho Y, Lee JB, Hong J. Controlled release of an anti-cancer drug from DNA structured nano-films. Sci Rep 2014; 4: 4078. http://dx.doi.org/10.1038/srep04078

[78] Wang ZG, Ding B. Engineering DNA self-assemblies as templates for functional nanostructures. Acc Chem Res 2014; 47(6): 1654-62. http://dx.doi.org/10.1021/ar400305q

[79] Mou Y, Yu JY, Wannier TM, Guo CL, Mayo SL. Computational design of co-assembling protein-DNA nanowires. Nature 2015; 525(7568): 230-3. http://dx.doi.org/10.1038/nature14874

[80] Bhatia D, Surana S, Chakraborty S, Koushika SP, Krishnan Y. A synthetic icosahedral DNA-based host-cargo complex for functional *in vivo* imaging. Nat Commun 2011; 2: 339. http://dx.doi.org/10.1038/ncomms1337

[81] Zhang H, Ma Y, Xie Y, *et al.* A controllable aptamer-based self-assembled DNA dendrimer for high affinity targeting, bioimaging and drug delivery. Sci Rep 2015; 5: 10099. http://dx.doi.org/10.1038/srep10099

[82] Lee H, Lytton-Jean AK, Chen Y, *et al.* Molecularly self-assembled nucleic acid nanoparticles for targeted *in vivo* siRNA delivery. Nat Nanotechnol 2012; 7(6): 389-93. http://dx.doi.org/10.1038/nnano.2012.73

[83] Amir Y, Ben-Ishay E, Levner D, Ittah S, Abu-Horowitz A, Bachelet I. Universal computing by DNA origami robots in a living animal. Nat Nanotechnol 2014; 9(5): 353-7. http://dx.doi.org/10.1038/nnano.2014.58

[84] Douglas SM, Bachelet I, Church GM. A logic-gated nanorobot for targeted transport of molecular payloads. Science 2012; 335(6070): 831-4. http://dx.doi.org/10.1126/science.1214081

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