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A New Perspective for Pancreatic Ductal Adenocarcinoma Management: Diagnosis and Therapy Using Nanoparticle-Based Technology

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ABSTRACT

Pancreatic cancer is the 4th most common cause of cancer-related mortality in the Western world. Pancreatic ductal adenocarcinoma (PDAC) is usually diagnosed at a later stage and mechanisms of growth and progression have yet to be clarified. Treatment fails mostly due to chemo- and radiation resistance. In order to overcome this issue, nanomedicine has become an opportunity for new diagnostic and therapeutic approaches, providing a tool to enhance drug delivery to treat PDAC. It allows the development of a nanoparticle-based drug delivery system (DDS) that combines several chemotherapeutic agents with nanocarriers for PDAC management. In this review, we describe the latest therapies and advances in pharmaceutical nanoformulations for PDAC management.

BACKGROUND

Advances in nanotechnology over the last years provide a new pathway for diagnosis and treatment of cancer disease. Current pancreatic cancer diagnosis and treatment options are suboptimal and it is urgent to look for new strategies. Pharmaceutical nanoformulations could have better site-specificity and diagnostic and therapeutic efficiency. This efficiency could also have an impact on the number of patients rescued for curative surgery, which currently amounts to barely 15% of the patients diagnosed with the resectable disease.

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List of abbreviations PDAC Pancreatic Ductal Adenocarcinoma; DDS Drug Delivery System; CAFs Cancer Associated Fibroblasts; PSCs Pancreatic Stellate Cells; MDSCs Myeloid Derived Suppressor Cells; TAMs Tumour Associated Macrophages; CSCs Cancer Stem Cells; PCSc Pancreatic Cancer Stems Cells; Ca Carbohydrate Antigen; CT Computed Tomography; PEG Polyethylene Glycol; IONPs Iron Oxide Nanoparticles; MRI Magnetic Resonance Imaging; PAI Photoacoustic imaging; PLGA Poly (Lactic–Co Glycolic Acid); HER-2 Human Epidermal Growth Factor Receptor 2; MMP-2 Matrix Metalloproteinase-2; siRNA Small Interfering Ribonucleic Acid; SLNs Solid Lipid Nanoparticles; NLCs Nanostructured Lipid Carriers; SMEDDS/SNEDDS Self-Micro/Nano-Emulsifying Drug Delivery Systems; Nab-Paclitaxel Nanoparticle Albumin-Bound Paclitaxel; PHAs Polyhydroxyalkanoates; PCL Poly (Caprolactone); CNTs Carbon Nanotubes; QDs Quantum Dots; NIR Near Infrared;
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In our manuscript, we described the characteristics of pancreatic cancer biology, focusing on the latest therapies and advances in pharmaceutical nanoformulations available in pancreatic ductal adenocarcinoma management. We include different types of nanoformulations tested for diagnosis and treatment and explore the latest clinical trials evaluation of nanoparticle-based drug delivery Systems in PDAC.

Introduction

PDAC is the most frequent malignant tumour in the pancreas and the fourth cause of cancer death in Europe [1].

The characteristics of PDAC, such as tumour stroma and cancer stem cells, play an important role in the physiopathology of pancreatic cancer, and they are strongly related to late diagnosis, bad prognosis and chemoresistance [2].

The PDAC stroma forms the tumour microenvironment and about 80% of the tumour's mass. The proliferation of cellular components of the tumour stroma, such as Cancer Associated Fibroblasts (CAFs) and pancreatic stellate cells (PSCs), leads to a desmoplastic reaction, producing extracellular matrix proteins contributing to tumour growth and migration. Other cellular components, such as Myeloid Derived Suppressor Cells (MDSCs) and Tumour Associated Macrophages (TAMs), may promote tumour progression by

creating an immunosuppressive and fibrotic environment. The acellular component is formed by extracellular matrix proteins (in which hyaluronan is a major component), and extracellular matrix metalloproteinases, both of these promoting tumour proliferation, growth, migration and dissemination. The role of the tumour stroma and the desmoplastic reaction in PDAC has become increasingly important because it has been shown to be responsible for the hypoxic and hypoperfused microenvironment in pancreatic cancer, hindering the effect of permeability and retention of passive targeting, thus limiting the delivery of antitumour drugs, finally leading to chemoresistance. Therefore, components of the stroma are being studied as potential targets for antitumour drug delivery [2, 3, 4].

On the other hand, cancer stem cells (CSCs), components of the tumour cell population, have the capacity for self-renewal and produce heterogeneous clones of cancer cells, thus maintaining tumour growth. They have cell plasticity (predominantly involving transition from the epithelial to the mesenchymal form and metabolic transformations), phenotypic potential, tumorigenicity and metabolic, autophagy, invasive and chemoresistant capacities, allowing self-renewal and contributing to chemoresistance. In fact, pancreatic cancer stems cells (PCSc) are known to be the critical hurdle limiting delivery of antineoplastic drugs to pancreatic tumour cells. In this regard, research focused on targeting the crosstalk between cancer stem cells and the tumour microenvironment seems to be promising in pancreatic cancer treatment. Also, taking into consideration the expression of different protein markers in CSCs, such as CD44, CD24, CD133, CXCR4 or c-Met, these are being used to target new therapies exclusive to the CSC population [2, 3, 4].

PDAC is often asymptomatic or with vague symptoms in the early stages, so the first diagnosis occurs when local invasion or distant metastases are present, with severe symptoms such as intense pain or jaundice that led to the medical consult, contributing to the poor prognosis that affects the majority of PDAC patients [5].

Regarding PDAC diagnosis, there is a lack of economic and non-invasive tools for early detection of PDAC, and even though the carbohydrate antigen (Ca) 19.9 has been approved for use in daily clinical practice, it is not recommended in the diagnostic phase due to its poor sensitivity and specificity. Different biomarkers have been identified and proven efficient, but most of them require complex and expensive technology, not reproducible in a daily practice [6].

Surgery combined with chemotherapy is the only potentially curative treatment for PDAC, but this option can only be offered for resectable disease in approximately 15-20% of patients diagnosed with pancreatic cancer. Even after curative surgery, the oncological results of surgery alone are disappointing, with a median survival of 15–20 months and a 5-year survival of 8–15%, owing to the high frequency of local and distant relapses. Therefore, surgery must be combined with neoadjuvant and adjuvant therapy into a complete treatment sequence, also known as multimodal treatment [7, 8].

In terms of advances in PDAC treatment, over the past five years, the 5-year survival rate for PDAC has increased from 6 to 10%, basically due to the improvement in neoadjuvant and adjuvant therapies. In addition, rapid metastatic dissemination, frequent sudden adverse events and degradation of the performance status score makes many patients unfit for chemotherapy, so new strategies are needed for resectable and unresectable disease. But PDAC responsivity to treatments such as chemotherapy, alone or in association with radiotherapy, remains poor [9, 10]. In this setting, different and individualized therapies are been investigated in order to target the stroma desmoplastic reaction by using immune checkpoint inhibitors, cancer vaccines, adoptive T-cell transfer or cancer stem inhibitors, enhancing immune system activity against cancer cells [11].

Taking these previous issues into account, new strategies for screening high-risk patients to detect pancreatic tumours at earlier stages, and new therapeutic approaches are required to make a clinically significant impact as regards overall survival, comparing the current regimens.

With this in mind, nanotechnology is emerging as a promising avenue to enhance drug delivery to PDAC, and research in this field has grown extensively over the past decade. Its unique characteristics allow the development of nanoparticle-based drug delivery systems, combining several contrast and chemotherapy agents with nanocarriers for PDAC management [12, 13, 14, 15].

So, this review provides a summary of the recent advances in pharmaceutical nanoformulations for PDAC diagnosis and treatment, aiming to overcome the limitations of actual tools for diagnostics and therapies, also focusing on the future implications in PDAC management.

Nanomedicine in PDAC

Systemic chemotherapy requires high dosages due to loss of effectiveness until the tumour cells are reached, inducing a significant number of adverse effects. Over the last twenty years, progress in nanobiotechnology and nanomedicine allowed the experimental investigation of nanoscale components (materials with at least one dimension less than 100 nanometres), increasing the number of nanotherapeutics and nanodiagnostics available, which are trying to solve the limitations of systemic chemotherapy and improve diagnosis in oncology [12, 15].

Nanobiotechnology allows us to modify the physiochemical properties of nanocarriers, improving solubility and stability, and multiple active pharmaceutical compounds can be encapsulated in a single nanoparticle, which could potentially offer synergistic effects to promote therapeutic efficacy, while limiting drug resistance [15].

Some nanocarriers, such as nanoparticle-based DDS, achieve controlled drug release and site-specific delivery

of therapeutic agents, also reducing toxicity and enhancing drug delivery to the pancreas via both passive and active targeting mechanisms [14].

Some of these nanoparticle-based DDS include **(Figure 1)** [13]

-Lipid nanotransporters such as liposomes, solid-lipid nanoparticles, carriers of nanostructured lipids, pre-concentrates of micro / nanoemulsions [lipid conjugated drugs].

-Polymeric nanotransporters such as polymeric nanoparticles (nanospheres, nanocapsules), polymeric micelles, and dendrimers (transport of conjugated or encapsulated drugs) [conjugated drugs in polymers].

-Inorganic nanotransporters metallic / non-metallic and optionally armoured cover, ligand, drug or diagnostic test.

These nanoformulations seem to overcome specific drug delivery challenges in PDAC, such as low drug sensitivity and high resistance, stromal barrier, high toxicity and poor pharmacokinetic performance [15].

Diagnosis in PDAC based on nano-DDS

Nanomaterials possess unique features regarding optical, magnetic and physicochemical properties, allowing the development of nanoparticle-based imaging agents. These nanoparticles can improve contrast enhancement, detection sensitivity, targeted biodistribution and multimodal imaging capacity [15].

Nanoparticles can be used for multimodal imaging. Using radioisotopes and contrast agents, multifunctional nanoparticles can be recognized in computed tomography (CT), ultrasound and magnetic resonance, improving sensitivity and diagnostic precision [16].

For example, Zou et al., described a nanocomposite combining antibodies conjugated to the surface of polyethylene glycol (PEG) modified magnetic iron oxide nanoparticles (IONPs). In vivo studies in nude mice by magnetic resonance imaging (MRI) showed an anti-pancreatic cancer effect. Nanoparticles were bound specifically and internalized by MUC4/CEACAM6/CD44v6-expressing PDAC cells, shortening the MRI T2-signal intensity both in vitro and in vivo, demonstrating an anti-pancreatic cancer effect [17].

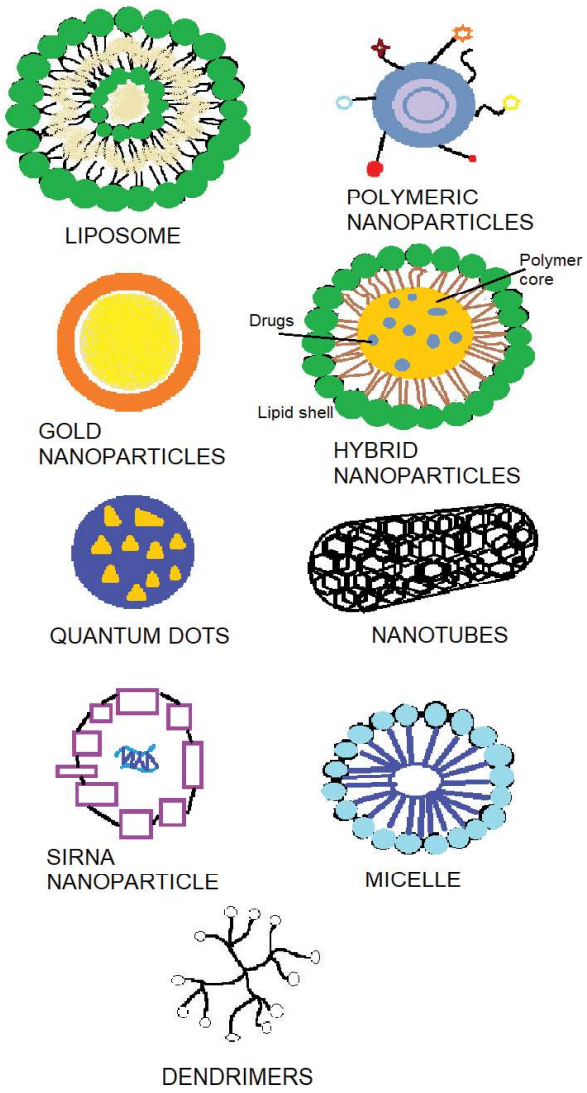

Figure 1. Principal nanoformulations applied for PDAC management.

Zhao et al. published another example regarding use of nanoparticles in PDAC diagnosis. They studied nanoparticles composed of gold nanorod, mesoporous silica, and a gadolinium oxide carbonate, for using in MRI, X-ray, CT and photoacoustic imaging (PAI). In this experiment, a negative contrast within the tumour area in CT/PAI and a positive contrast in MRI were observed in genetically engineered mouse models. As this publication shows, these hybrid nanoparticles may improve early diagnosis and accuracy in PDAC [18].

In addition, interaction between nanoparticles and plasma leads to the formation of a shell of biomolecules, forming the biomolecular corona, often called protein corona because of its high protein content The physiochemical properties of nanoparticles (such as materials, size, electric charge and hydrophobicity), environmental factors, and experimental source characteristics determine the protein corona's shape. Nanoparticles are able to concentrate at the cancer's alteration in the human proteome, so, based on this, they have been studied for early cancer detection. For instance, Caputo et al., described DOPG [1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)] liposomes that allow us to distinguish patients affected with T1 and T2 PDAC from T3 and metastatic pancreatic cancer [19]. Another example published by Caracciolo et al., consists in the formation of liposomes after the interaction between nanoparticles and plasma of PDACs and non-oncological subjects, thus allowing us to distinguish between the two groups by differences in electric charge and size of the protein corona of nanoparticles [20].

These tests do not identify cancer biomarkers, but allow detection of global changes in protein corona patterns at early stages of disease progression or after chemotherapy or surgery. But even these blood tests based on the interaction between nanoparticles and plasma seem to have superior sensitivity and specificity than actual biomarkers, more studies will be required to further validate the clinical application of nanoparticles to early diagnosis in PDAC [21].

Nanotheranostics in PDAC

Nanotheranostics, by using molecular testing and genetic profile, have the capacity to identify imaging agents and simultaneously targeting therapies at the tumour site [12, 13, 14, 15, 16].

Multifunctional nano DDSs are designed to support and deliver at the same time chemotherapeutics and contrast agents. When used combined with imaging modalities, they increase their effectiveness to identify tumours and assess therapeutic efficacy of targeted nanoparticles. Interventional treatments allow local drug delivery, targeted vascular embolization, direct tumour ablation and disruption of the tumour stroma in PDAC [12, 13, 14, 15, 16].

Some of these multifunctional nano DDS are nanoparticles, nanowires, nanotubes and nanocantilevers. These can be loaded with biosensors, targeting delivery of imaging dyes and photosensitive compounds, thus improving biodistribution, sensitivity and contrast in imaging [12, 13, 14, 15, 16].

For example, pegylated iron-oxide-gold core-shell nanoparticles have been investigated in the MRI imaging of in vivo PDAC models, and they have shown increased uptake of targeted treatments, as well as limited toxic effects to adjacent organs. Gold and iron oxide nanoparticles may distinguish the hypoperfusion in PDAC stroma, increasing the staging capacity of the images [22].

IONPs may also generate heat after external irradiation, so some publications have shown that poly (lactic–co glycolic acid) [PLGA]-based nanoparticles encapsulating fluorescent IONPs and Gemcitabine, conjugated with anti-human epidermal growth factor receptor 2 (HER-2) antibody, have potential for contrast enhancement and tumour regression in in vivo models of pancreatic cancer [23]. Zhou et al., also described the effect of IGF1R-targeted therapy, using the theranostic IGF1-IONP-Doxorubicin, inhibiting the growth of patient derived xenograft pancreatic tumours, which could be detected by MRI with IONP-induced contrasts [24].

Chen et al., reported supramolecular aggregation-induced emission nanodots for image-guided drug delivery, combining matrix-metalloproteinase-2 sensitive PEG-peptide and functional cyclodextrins conjugated with Gemcitabine and a near infrared luminogen. These nanoparticles accumulated in tumour tissues due to the enhanced permeability and retention effect, and responded to the tumour's overexpressed matrix metalloproteinase-2 (MMP-2) and intracellular reductive microenvironment, achieving enhanced cancer cell uptake and selective Gemcitabine release within cancer cells, and exhibiting tumour inhibition capacity in subcutaneous and orthotopic pancreatic cancer models [25].

In addition, nanotheranostics in PDAC seem to be important for developing screening modalities to identify pancreatic cancer cells and PanIN lesions. Minimally invasive nanosystems capable of detecting PanIN cells in the earliest stages, and used in combination with molecular and genetic data, may improve early diagnosis and treatments to increase the surgical approach. For instance, miRNA can be engineered in a nano DDS to be tumour and site specific, and has been shown to be effective in the identification of DNA, and in cytological analysis for early diagnosis of PDAC [16].

Nanotherapy in PDAC

Regarding PDAC management, there have been some recent advances in pharmaceutical nanoformulations. Nanoparticle–drug combined formulations for PDAC therapy, tested both in in vitro and in vivo experiments, animal models and some in clinical practice in individualized patients, include liposomes, polymeric nanoparticles, small interfering ribonucleic acid (siRNA) nanoparticles, amphiphilic polymer nanoparticles, dendrimers, carbon nanotubes, hybrid nanoparticles, quantum dots and magnetic and gold nanoparticles [26].

Lipid nanocarriers

Lipid nanocarriers can be classified as lipid vesicular nanocarriers and lipid particulate nanocarriers [27].

Lipid vesicular nanocarriers are liposomes and exosomes that facilitate the incorporation of both lipophilic and hydrophilic drugs. For example, conventional liposomes, which enclose an aqueous core, are being studied in PDAC for the delivery of therapeutic agents such as Irinotecan, 5-Fluorouracil, Gemcitabine, Paclitaxel, ellagic acid, curcumin, pirfenidone and MMP-2 responsive peptide (a pancreatic stellate cell regulator) [14, 27]. Chirio et al., described a lipid nanoparticle anchored to the lipophilic polymer stearoyl chitosan, using curcumin as the model drug. In vitro studies have shown a cytotoxic effect in cultures of PANC-1 cells [28].

For the purpose of reducing protein adsorption and enhancing circulation time, pegylated liposomes have been developed. Pegylation consists in PEG polymer chains attachment to molecules such as drugs and therapeutic proteins, which can have the capacity of reducing immunogenicity and prolonging its circulatory time. Pegylation can also provide water solubility to hydrophobic drugs and proteins [27]. For example, Liposomal irinotecan (Onivyde®) is a pegylated liposome, approved by the FDA in 2015, in combination with 5-Fluorouracil and Leucovorin for PDAC previously treated with Gemcitabine as first-line treatment. A randomised trial showed that patients treated with Onivyde® and 5-Fluorouracil (5-FU)/Leucovorin increase their overall and disease-free survival comparing with those who didn't received Onivyde® [29]. Also, drugs such as Cromolyn, Gemcitabine and Mitomycin-C have been studied for delivery by pegylated liposomes in PDAC [14]. For instance, Chen et al., generated Paclitaxel loaded pegylated-liposomes with good penetrating capacity in patient-derived orthotopic xenograft nude mouse models of PDAC [30].

Moreover, targeted liposomes and immunoliposomes (antibody-conjugated liposomes) for pancreatic tumours have been developed and studied for the delivery of drugs such as Gemcitabine [14, 27, 31]. Urey et al., developed a Gemcitabine-loaded MUC4-targeted immunoliposome, where an anti-proliferative effect was seen in a MUC4-positive pancreatic cancer cell line, Capan-1, showing promising results for targeted treatment and improved retention of Gemcitabine for treatment of PDAC [32].

Lipid vesicular nanocarriers, such as exosomes, are extracellular vesicles originated in cells that can contain proteins, mRNA and miRNA, and genetically engineered exosomes are being considered for PDAC therapy because of their increased blood retention and their superior cell transfection potential [33, 34]. An example is fibroblast-derived exosomes engineered to incorporate oncogenic KrasG12D (PDAC mutation), siRNA or short hairpin RNA (iExosomes). These iExosomes have a higher circulation time compared with conventional liposomes, related to CD47-assisted protection against phagocytosis [35].

On the other hand, liposomes can be lipid particulate nanocarriers, aimed for the delivery of lipophilic and poorly bioavailable drugs. Some of them are Solid Lipid Nanoparticles (SLNs), Nanostructured Lipid Carriers (NLCs), Micro/Nanoemulsions, and Self-Micro/Nano-Emulsifying Drug Delivery Systems (SMEDDS/SNEDDS) [36].

One example of SLN is the nanoparticle albumin-bound Paclitaxel (Nab-Paclitaxel, Abraxane®), produced by homogenization of Paclitaxel with serum albumin into a nanoparticle colloidal suspension, approved in combination with Gemcitabine for local advanced and metastatic pancreatic cancer [37].

Other SLNs are being investigated. For instance, Affram et al., recently published the cytotoxic effects of Gemcitabine-loaded SLN on the primary pancreatic cancer cell lines Mia PaCa-2 [38]. In this field, Thakkar et al., published that SLN of ibuprofen alone or in combination with sulforaphane may produce anti-inflammatory and antioxidative properties in transgenic animal models of pancreatic cancer, thus enhancing systemic anticarcinogenicity in PDAC [39].

However, these liposomal formulations, due to the exposure to blood proteins, increase their toxic effect and lose their selectivity, so other nanocarriers are being studied in order to overcome this limitation [27].

Polymeric nanocarriers

Polymeric nanocarriers include polymeric nanoparticles, polymeric micelles and dendrimers [40].

Polymeric nanoparticles allow conjugation of highly lipophilic drugs onto the hydrophilic polymer resulting in the formation of core-shell aggregates, therefore improving the aqueous solubility of anticancer agents, prolonging circulation time and reducing the required drug dosage, and also enhancing antitumor efficacy [40, 41]. Materials of polymeric nanoparticles range from natural sources, including gelatine, chitosan, alginate, dextran, heparin, collagen, albumin and polyhydroxyalkanoates (PHAs), to synthetic sources, including PLGA, poly-n (cyanoacrylate), poly (caprolactone) (PCL), PEG and cyclodextrins [13, 14].

PLGA-based nanocarriers incorporating Gemcitabine, 5-Flurouracil, curcumin, anthothecol or ormeloxifene have been studied for PDAC management because this type of polymer has sustained releasing properties, biodegradability and biocompatibility. These nanocarriers have exhibited apoptotic potential against pancreatic cell cultures and PSCs [14, 40]. Fu et al., studied the therapeutic effects of Paclitaxel loaded PEG-PLGA copolymer nanoparticles on MIA-PaCa2 cells in rats. These nanoparticles have shown great drug loading capacity, targeting of pancreatic cancer MIA PaCa-2 cells and apoptosis [42].

Chitosan-based polymeric nanoparticles of Gemcitabine, quercetin, 5-Fluorouracil and metformin can be used for targeted drug delivery, and they have been

shown to enhance antitumour efficacy compared with the drug alone, so they have also been investigated in the PDAC field [43]. Xiao et al., developed new nanobioconjugates based on chitosan for site specific delivery of Gemcitabine and anti-EGFR antibody for pancreatic cancer treatment. The synthesised nanobioconjugates were shown to reduce pancreatic cancer cell growth, colony formation and inhibit migration and invasion of human SW1990 pancreatic cancer cells [44].

Polymeric micelles are polymeric nanocarriers consisting of a core–shell structure formed in aqueous solvents and, when combined with pegylated nanoparticles, their high biocompatibility allows targeted treatment, tumour surveillance and contrast imaging in PDAC tumors [41]. Daman et al., generated polymeric micelles of PEG-PLGA copolymer as a carrier for Salinomycin (an inhibitor of CSC) against Gemcitabine-resistant pancreatic cancer. In vitro and in vivo experiments showed increased cell mortality and apoptosis, and significant tumour eradication [45].

Moreover, dendrimers are the smallest polymeric nanocarriers and they can be adjusted to manage solubility and act as selective gates to control entry of small molecules, such as drugs, antibodies or polymers. They can mediate Gemcitabine, difluorobenzylidene, curcumin, Paclitaxel, TR3 siRNA, genes, and fluorescent dyes such as Alexa Fluor 555 delivery, enhancing drug rate dosage in the tumour [14]. Wang et al., recently demonstrated a polyamidoamine dendrimer-camptothecin conjugate that penetrates PDAC tumours *via* γ-glutamyl transpeptidase, triggering cell endocytosis and transcytosis. This dendrimer exhibited high antitumour activity in multiple mouse tumour models of PDAC, including patient-derived xenograft and cell line derived xenograft, compared with Gemcitabine for advanced pancreatic cancer [46].

Stimuli responsive or "smart polymers" undergo active responses to environmental changes or external signals. The reported stimuli may be physical (temperature, ultrasound and light), chemical (pH, ionic strength) or biological (biomolecules). The major application is the delivery of anticancer therapeutics when precision control and site specificity are needed. Multiple stimuli sensitive polymers have been used as co-delivery systems in different forms (micelles, nanoparticles mixed micelles, hydrogels, etc.) for in vitro experiments in pancreatic cancer cells lines [47, 48]. For example, Karandish et al., prepared smart polymers from the dexamethasone-PEG-PLA conjugate along with a synthesised stimuli-responsive polymer PEG-S-S-PLA. The dexamethasone group dilates the nuclear pore complexes and transports the vesicles to the nuclei. They observed that the nucleus-targeted, stimuli-responsive polymersomes released 70% of the encapsulated cancer stemness inhibitor BBI608, reducing the viability of human BxPC3 pancreatic cancer cells. Also, Fan et al., proposed long-circulating, pH-sensitive nanoparticles composed of cellular membrane-disruptive molecules that permeabilize the tumour stroma inhibiting tumour growth and promotion of metastasis in xenograft murine models [49].

Inorganic nanoparticles

Some examples of inorganic nanoparticles studied for PDAC treatment are carbon nanotubes (CNTs), quantum dots (QDs), IONP's, ferritin nanoparticles, gold nanoparticles and hybrid iron oxide-gold nanoparticles [13, 14, 15]. Although they possess interesting magnetic, electrochemical and optical properties for their use in cancer therapy, they can become trapped in the reticuloendothelial system of the liver due to protein adhesion to the surface. This fact leads to accumulation in normal tissues, increasing their toxicity, so limiting their use in cancer therapy [13, 14, 15].

Magnetic IONP's have been studied in PDAC for the delivery of Gemcitabine, Doxorubicin, carbachol, atropine and metalloproteinases, resulting in specific targeting of pancreatic tissue [13, 50]. For example, Khan et al., developed a superparamagnetic ION formulation of curcumin, which delivers bioactive curcumin to pancreatic tumours, simultaneously enhancing Gemcitabine uptake and its efficacy [51].

Recently, Guo et al., reported the in vitro effects of the chemotherapeutic drug formulated by silver nanoparticles containing Berberis thunbergii leaf against human pancreatic cancer culture cells PANC-1, AsPC-1 and MIA PaCa-2 [52].

Other nanocarriers, such as mesoporous silica nanoparticles, are being studied for PDAC treatment. Meng et al., demonstrated that coated bilayer silica mesoporous nanoparticles can improve the synergistic delivery of Gemcitabine and Paclitaxel in human PDACs, based on their results in animal models where they observed that these nanocarriers may overcome abnormal perfusion of the stromal tumour [53].

Furthermore, research efforts in nanobiotechnology are focusing on developing more biocompatible nanoparticles, such as gold nanoparticles or gold nanosuspension. Gold nanocarrier absorption of near infrared (NIR) radiation can generate an electromagnetic wave which can be used for targeting a specific area and producing cancer cell death via a photothermal effect. Because of their site specificity, damage to nearby tissues is prevented [54, 55]. Gemcitabine, photothermally activated prodrugs such as cathepsin E, and gene therapeutics such as siRNA have been described for delivery via gold nanocarriers, and have been investigated for inhibition of tumour growth in PDAC models by reducing matrix deposition in the tumour microenvironment [55, 56]. For instance, in a review by Tomsa et al., they described that gold nanoparticles appear to be the most efficient, with scant systemic toxicity and promising results in apoptosis of pancreatic cancer cells, although with limited data, so these gold nanoparticles require further studies before they can be used in clinical practice [57].

Hybrid nanocarriers

Hybrid nanoparticles combine different types of lipid, polymer or inorganic nanoparticles, adding new properties or improving them. For instance, biopolymer coating of metal nanoparticles improves biocompatibility, stability and systemic circulation time, and these are capable of targeting tumour cells and releasing drugs in response to specific stimuli [14, 15, 16].

Joubert et al., reported the formulation of hybrid nanoparticles consisting of aggregated gold nanoparticles impregnated into a Gemcitabine-polymer conjugate matrix that exhibit synergistic photo-chemo-therapeutic activity against pancreatic cancer [58].

For example, Kumar Thapa et al., developed bortezomib and IR-820-loaded hybrid-lipid mesoporous silica nanoparticles conjugated with the hydrophobic-binding peptide, cyclosporine A. Upon reaching the tumour site, cyclosporine A interacted hydrophobically with the cancer cell membranes, and the high cellular uptake of the nanoparticles was evident, with pronounced apoptotic effects in PANC-1 and MIA PaCa-2 and enhanced antitumour effects in PANC-1 xenograft models [59].

Oluwasanmi et al., trying to improve the effectiveness of Gemcitabine, developed a thermally activated system by introducing a linker between this drug and hybrid nanoparticles. Heat generation resulting from laser irradiation of the hybrid nanoparticles promoted linker breakdown, resulting in prodrug liberation in in vitro and in vivo evaluation in xenograft models, showing less cytotoxicity than Gemcitabine alone and improving cellular uptake [60].

Also, Xu et al., generated gold nanoshell coated rod-like mesoporous silica nanoparticles incorporating Gemcitabine. These hybrid inorganic nanocarriers may penetrate the fibrotic stroma in PDAC, having a photothermal effect [61].

Other examples, reported by authors such as Liu et al., are pegylated lipid (long circulating)-coated gold nanoshells loaded with bortezomib and Gemcitabine, which released these drugs at a specific site upon NIR laser treatment [62]. In addition, anticancer efficacy against the MIA PaCa-2 PDAC cell line was seen with a combination of polymeric and inorganic nanoparticles, such as magnetic nanoparticles with Hsp90 and the drug 17-N-allylamino-17-demethoxygeldanamycin coated with PLGA [63].

Targeted therapy in PDAC

In targeted nanoparticles, specific ligands such as peptides, aptamers or antibodies are attached onto the nanoparticle's surface, resulting in preferential binding to receptors of target cells and conferring highly site-specific targeting, resulting in more efficient treatment [13, 14, 15, 16].

In PDAC treatment, several targeted nanoparticles are under investigation and are summarized in **Table 1**.

Advances in nanobiotechnology have allowed the clinical evaluation of a large number of nanoparticle-based DDS, summarised below **(Table 2)** (clinicaltrials.gov Pancreatic Cancer Action Network; Clinical trials search) [82].

Table 1. Targeted nanoparticles for PDAC treatment

Drug delivery system	Targeting strategy	Active drug	Reference
Lipid nanocarriers			
B- cyclodextrin adapted liposome	RGD peptide, MMP2 responsive liposome	Pirfenidone and gemcitabine	64
NLC	Hyaluronic acid	Gemcitabine-stearic acid prodrug and hyaluronic acid-baicalin conjugate	36
Lipid nanovesicles	Apolipoprotein A-II		65
Lipid-peptide conjugate Nano assembly	Hypoxia-responsive lipid		66
Polymeric nanocarriers			
Poly-lactic acid–PEG micelles	Folate	Curcumin	67
Albumin nanoparticles	TRAIL	Paclitaxel	68
Albumin nanoparticles	Folate	Gemcitabine	69
Dendrimer	Tumor-targeting peptide	Paclitaxel and TR3 small interfering RNA	70
Third-generation poly-L lysine dendrigraft	Cell-penetrating peptide	Gene delivery	71
PEG-cored PAMAM dendrimers	Flt-1 (VEGF receptor) antibody	Gemcitabine	72
PAMAM dendrimer	Hyluronic acid	3, 4-Difluorobenzylidene curcumin	73
Inorganic nanocarriers			
Iron oxide magnetic nanoparticles	Anti-CD47 antibody	Gemcitabine	74
Ferritin nanoparticles	Transferrin receptor 1 targeting with EPR effect	Carbachol (neural activity activator) and atropine (neural activity depressor)	75
Silver-graphene quantum nanocomposites with carboxymethyl inulin	Hyaluronic acid	5-Flurouracil	76
Gold nanocluster	U11 peptide	Cathepsin E	77
Gold nanoparticles	Plectin-1-targeting peptide KTLLPTP	Gemcitabine	78
Hybrid nanocarriers			
Lipid bilayer coated mesoporous silica targeted nanoparticles (silicasomes)	Cyclic tumorpenetrating Peptide iRGD	Irinotecan	79

pH-sensitive PEGylated dendritic hybrid nanocarrier comprising mesoporous silica, graphene oxide and magnetite	Folic acid	Gemcitabine	80
Hybrid nanoparticles	Laser irradiation induced linker breakdown	Gemcitabine	60
Gold nanoshell- coated rodlike mesoporous silica nanoparticles	Gold nanoshell allowing photothermal effect upon mild near infrared irradiation	Gemcitabine	81

Table 2. Etiological studies of RAP over 10 years.

Number of clinical trial (NCT)	Study title	Interventions	Locations	Status
3636308	Nab-paclitaxel Plus S-1(AS) Versus Nab-paclitaxel Plus Gemcitabine(AG) in Patients With Advanced Pancreatic Cancer	Nanoparticle/albumin-bound paclitaxel/ S1/ Gemcitabine	Peking University Cancer Hospital, Beijing, China	Recruiting
2336087	Gemcitabine Hydrochloride, Paclitaxel Albumin-Stabilized Nanoparticle Formulation, Metformin Hydrochloride, and a Standardized Dietary Supplement in Treating Patients With Pancreatic Cancer That Cannot be Removed by Surgery	Gemcitabine Hydrochloride/ Paclitaxel Albumin-Stabilized Nanoparticle Formulation/ Metformin Hydrochloride/ Therapeutic Dietary Intervention	City of Hope Medical Center, California, United States	Active, not recruiting
4789486	Gadolinium-based Nanoparticles With Stereotactic Magnetic Resonance-guided Adaptive Radiation Therapy in Centrally Located Nonsmall Cell Lung Cancer and Locally Advanced Pancreatic Ductal Adenocarcinoma	Activation and Guidance of Irradiation X (AGUIX) and SMART, magnetic resonance imaging (MR)-guided stereotactic body radiation therapy (SBRT)	Dana Farber Cancer Institute, Boston, Massachusetts, United States	Recruiting
2562716	S1505: Combination Chemotherapy or Gemcitabine Hydrochloride and Paclitaxel Albumin-Stabilized Nanoparticle Formulation Before Surgery in Treating Patients With Pancreatic Cancer That Can Be Removed by Surgery	Fluorouracil/ Gemcitabine Hydrochloride/ Irinotecan Hydrochloride/ Oxaliplatin Paclitaxel Albumin-Stabilized Nanoparticle Formulation	University of Alabama at Birmingham Cancer Center, Birmingham, Alabama, United States	Active, not recruiting
2194829	Paclitaxel Albumin-Stabilized Nanoparticle Formulation and Gemcitabine Hydrochloride With or Without WEE1 Inhibitor MK-1775 in Treating Patients With Previously Untreated Pancreatic Cancer That Is Metastatic or Cannot Be Removed by Surgery	Adavosertib/ Gemcitabine Hydrochloride/Nab-paclitaxel	Northwestern University, Chicago, Illinois, United States	Active, not recruiting
3410030	Trial of Ascorbic Acid (AA) + Nanoparticle Paclitaxel Protein Bound + Cisplatin + Gemcitabine (AA NABPLAGEM)	Ascorbic Acid/ Paclitaxel protein-bound/ Cisplatin/ Gemcitabine	HonorHealth Research Institute, Scottsdale, Arizona, United States	Recruiting
2178436	Gemcitabine, Nab-paclitaxel and KPT-330 in Advanced Pancreatic Cancer	Gemcitabine hydrochloride/ Selinexor/ Nab paclitaxel	The University of Kansas Medical Center, Westwood, Kansas, United States	Recruiting
2620865	Phase Ib/II Treatment of Advanced Pancreatic Cancer With Anti-CD3 x Anti-EGFR-Bispecific Antibody Armed Activated T-Cells (BATs) in Combination With Low Dose IL-2 and GM-CSF	Paclitaxel Albumin-Stabilized Nanoparticle Formulation, Aldesleukin, Antibody Therapy, Fluorouracil, Gemcitabine Hydrochloride, Irinotecan Hydrochloride, Leucovorin Calcium, Oxaliplatin, Sargramostim	Wayne State University/ Karmanos Cancer Institute, Detroit, Michigan, United States	Active, not recruiting
4484909	NBTXR3 Activated by Radiation Therapy for the Treatment of Locally Advanced or Borderline-Resectable Pancreatic Cancer	Hafnium Oxide-containing Nanoparticles NBTXR3/ Radiation Therapy	M D Anderson Cancer Center, Houston, Texas, United States	Recruiting
4311047	Preoperative Detection of Lymph Node Metastases in Pancreatic and Periampullary Carcinoma Using USPIO MRI	USPIO (ultra-small superparamagnetic iron oxide)-enhanced MRI	Radboudumc, Nijmegen, Netherlands	Recruiting
3910387	Telotristat Ethyl to Promote Weight Stability in Patients With Advanced Stage Pancreatic Cancer	Gemcitabine/Nab-paclitaxel/ Telotristat Ethyl	Emory University Hospital Midtown, Atlanta, Georgia, United States	Recruiting
4115163	Biologically Optimized Infusion Schedule of Gemcitabine and Nab-Paclitaxel for the Treatment of Metastatic Pancreatic Cancer	Gemcitabine/ Nab-paclitaxel	Ohio State University Comprehensive Cancer Center, Columbus, Ohio, United States	Recruiting
4524702	Paricalcitol and Hydroxychloroquine in Combination With Gemcitabine and Nab-Paclitaxel for the Treatment of Advanced or Metastatic Pancreatic Cancer	Gemcitabine/ Hydroxychloroquine/ Nab-paclitaxel/ Paricalcitol	Emory University Hospital Midtown, Atlanta, Georgia, United States	Recruiting
2394535	Nab-Paclitaxel, Capecitabine, and Radiation Therapy Following Induction Chemotherapy in Treating Patients With Locally Advanced Pancreatic Cancer	Capecitabine/ Nab-paclitaxel Radiation Therapy	M D Anderson Cancer Center, Houston, Texas, United States	Active, not recruiting

4158635	Gemcitabine, Nab-Paclitaxel, and Bosentan for the Treatment of Unresectable Pancreatic Cancer	Bosentan/ Gemcitabine/ Nab-paclitaxel	City of Hope Medical Center, Duarte, California, United States	Not yet recruiting
2930902	Pembrolizumab and Paricalcitol With or Without Chemotherapy in Patients With Pancreatic Cancer That Can Be Removed by Surgery	Gemcitabine Hydrochloride/ Nab-paclitaxel/ Paricalcitol/ Pembrolizumab	M D Anderson Cancer Center, Houston, Texas, United States	Active, not recruiting
4940286	Gemcitabine, Nab-paclitaxel, Durvalumab, and Oleclumab Before Surgery for the Treatment of in Resectable/Borderline Resectable Primary Pancreatic Cancer	Durvalumab, Gemcitabine, Nab-paclitaxel, Oleclumab	M D Anderson Cancer Center, Houston, Texas, United States	Not yet recruiting
2427841	Nab-paclitaxel and Gemcitabine Hydrochloride Followed by Radiation Therapy Before Surgery in Treating Patients With Pancreatic Cancer That Can Be Removed by Surgery	Fluorouracil/ Gemcitabine Hydrochloride/ Image Guided Radiation Therapy Intensity-Modulated Radiation Therapy/ Nab-paclitaxel	OHSU Knight Cancer Institute, Portland, Oregon, United States	Active, not recruiting
4233866	Comparing Two Treatment Combinations, Gemcitabine and Nab-Paclitaxel With 5-Fluorouracil, Leucovorin, and Liposomal Irinotecan for Older Patients With Pancreatic Cancer That Has Spread	Fluorouracil/ Gemcitabine Gemcitabine Hydrochloride Leucovorin/ Leucovorin Calcium/ Liposomal Irinotecan/ Nab-paclitaxel	Saint Alphonsus Cancer Care Center-Boise, Boise, Idaho, United States	Recruiting
3337087	Liposomal Irinotecan, Fluorouracil, Leucovorin Calcium, and Rucaparib in Treating Patients With Metastatic Pancreatic, Colorectal, Gastroesophageal, or Biliary Cancer	Fluorouracil/ Leucovorin Liposomal Irinotecan/ Rucaparib	Mayo Clinic in Arizona, Scottsdale, Arizona, United States	Recruiting
3736720	Liposomal Irinotecan, Fluorouracil and Leucovorin in Treating Patients With Refractory Advanced High Grade Neuroendocrine Cancer of Gastrointestinal, Unknown, or Pancreatic Origin	Fluorouracil/ Leucovorin/ Liposomal Irinotecan	Roswell Park Cancer Institute, Buffalo, New York, United States	Recruiting
4481204	New and Emerging Therapies for the Treatment of Resectable, Borderline Resectable, or Locally Advanced Pancreatic Cancer, PIONEER-Panc Study	Cisplatin/ Fluorouracil Gemcitabine/ Irinotecan Leucovorin/ Nab-paclitaxel Oxaliplatin/ Radiation Therapy	M D Anderson Cancer Center, Houston, Texas, United States	Not yet recruiting

DISCUSSION

Nanobiotechnology and nanomedicine have afforded important developments to the oncology field, and nanomaterials and nanoformulations are being investigated in order to overcome limitations such as late diagnosis and chemoresistance. The properties of these nanomaterials allow a good uptake of contrast agents and chemotherapy, and a good site specificity and cellular targeting, leading to the generation of nanoparticle DDS, which can target a specific population of the tumour and reduce cytotoxicity in normal tissues.16 Regarding this site specificity and drug delivery, personalised medicine based on individual tumour characteristics (size, staging, biomarkers, metastasis, medical conditions, genetics, etc.,) seems to be getting closer to being the gold standard for medical care in cancer [13, 14, 15, 16].

Taking into account that the identification of new PDAC biomarkers did not seem to lead to important advances in early diagnosis, recent publications have shown that nanobiotechnology could provide new options for early cancer detection, and the concept of the protein corona (the layer of plasma proteins that surrounds nanomaterials in bodily fluids) is emerging as a personalised issue, different between cancer patients and healthy individuals. To this end, Caputo et al., have described a nanoparticle-enabled blood test for "molecular" staging that may provide useful information for PDAC prognosis and therapy, regarding tumour dimension and presence of metastasis [19, 21].

Also, there are countless publications about nanomaterials in PDAC oriented towards improving therapy. For instance, lipid nanocarriers may facilitate incorporation of both hydrophilic and lipophilic drugs, even those poorly available. Furthermore, the utilisation of exosomes can incorporate siRNA, mRNA and miRNA, presenting increased retention in the blood circulation and superior cell transfection. However, due to exposure to blood proteins, liposomal formulations may increase their toxic effect, so polymeric nanocarriers have been generated in order to overcome this cytotoxicity. Polymeric nanoparticles have more biocompatibility and biodegradability, and, as they can be conjugated with lipophilic drugs, some antitumour agents can improve their solubility, reducing the drug dosage and the toxic effect in normal cells. In addition, this biocompatibility has allowed the development of polymeric nanoparticles with high specificity for peptides in tumour cells and components of the tumour stroma, making them a good tool for the active targeting of drugs. This is a very important issue to bear in mind for the development of nanoparticles, which could specifically target biomarkers in cancer cells or CSC, with a minimal effect on normal cells [13, 14, 15, 16].

In comparison with polymeric nanoparticles, inorganic nanoparticles possess some electrochemical properties which can be used to damage tumour cells and inhibit tumour growth, but they can become trapped in the reticuloendothelial system of the liver, leading to increased toxicity in normal tissues. There have been different publications concerning imaging diagnosis and nanotheranostics in PDAC, but there are important limitations for their study and more data are required before applying to clinical practice [13, 14, 15, 16].

The imaging-based approach plays an important role in identifying potentially curable PDAC patients in high-risk groups, and nanotheranostics has emerged as a potential tool for simultaneous diagnosis and treatment even in the early stages. For instance, Chen et al., described a nano-sized ultrasound contrast agent that specifically targets pancreatic cancer cells and evaluated its targeting effect in vitro, which can be used for pancreatic cancer diagnosis [83].

Nanoparticle-based therapies for PDAC have been studied by authors such as Lei et al., and Davis et al., to improve biological availability and selectivity of nucleic acids such as siRNA or microRNA, and reduce immune system activity against these agents, but there are still some limitations regarding non-toxic stability, biocompatibility and biodegradability [84, 85].

It has been shown that tumour stroma and cancer stem cells play an important role in pancreatic cancer physiopathology and might contribute to chemoresistance. Also, immunology affects the tumour microenvironment and progression, so nanoparticles combined with immunotherapy are being investigated to achieve a synergistic effect [3, 4, 9]. For example, Liu et al., developed polyanhydride nanoparticles as viable MUC4β-vaccine carriers tested in mice, showing MUC4β-specific antibody responses, allowing the evaluation of this platform for PDAC immunotherapy [86]. Also, Tansi et al., studied the higher uptake of magnetic nanoparticles in cultured cells with hyaluronidase enzymes, indicating that these (acting in the extracellular matrix of the tumour stroma) improve the infiltration of magnetic nanoparticles and make an impact on thermal treatment and cell depletion in pancreatic cancer models [87]. To this end, Lu et al., tested mesoporous silica nanoparticles loaded with Oxaliplatin and indoximod (an inhibitor of immunosuppressive indoleamine 2, 3-dioxygenase) showing their potential to ameliorate immunity against PDAC in vaccination approaches [88]. Another example is the publication of Kim et al., who developed albumin nanoparticles loaded with Paclitaxel, indocyanine green (a hyperthermal agent) and hyaluronidase. These nanoparticles disintegrate hyaluronan, having hyperthermal effect in response to NIR laser irradiation. These nanoparticles produced cytotoxicity to pancreatic AsPC-1 cells [89]. Sun et al., develop a type of nanoparticle co-loaded with Gemcitabine and an indoleamine-2, 3-dioxygenase inhibitor, to deliver photosensitive, chemotherapeutic and immunomodulating agents with the purpose of inhibiting both primary tumour and distal metastasis in pancreatic cancer model PANC02 [90].

Despite publications about nanoparticles in PDAC, they have serious limitations regarding their use in clinical practice. They may become trapped in the liver by the mononuclear phagocytic system, limiting their clearance rate. Furthermore, their increased surface area results in an augmented chemical reactivity, which may cause oxidative stress, inflammation and damage to DNA, proteins and membranes, leading to toxicity. In addition, changes in shape, size and the surrounding environment can lead to toxicity, an important issue to take into account in the oncology field [13, 14, 15, 16].

Although there are important advances in the nanomedicine field to finally develop the current nanoparticles for PDAC management, efforts towards an accurate understanding of the interactions between nanoparticles, body fluids and human cells is needed before establishing these nanomaterials as a valid tool for diagnosis and treatment in the clinical practice of cancer.

CONCLUSION

PDAC is characterised by a complex genetic and tumour biology, which determine its aggressive behaviour, late diagnosis and bad prognosis. The tumour microenvironment and pancreatic cancer stem cells were shown to be related to tumour progression, dissemination, invasion, metastasis and chemoresistance, features leading to bad prognosis in PDAC. Improved knowledge of pancreatic cancer physiopathology and improvement in systemic therapy did not make a significant impact on disease-free and overall survival. Also, there are no methods for screening and treatment in the early stages, thus there is an urgent need for new diagnostic and therapeutic strategies for PDAC management. Nanoparticle properties such as high stability, strong inclusion capability and simple modification of the parental structure have been developed in order to improve nanomaterials as drug carriers. But properties, such as long blood circulation, highly efficient tumour cell uptake and site-oriented drug release inside tumour cells, are still limited. Despite all the nanoparticles investigated and tested in murine models, most of them have not been approved for clinical application because some unresolved questions remain relating to the bioavailability and clearance of nanovectors in order to increase efficacy and reduce the toxic effect. But multiple studies are being performed regarding diagnosis and treatment in PDAC, and it seems that the improvement of nanoparticle properties and a better understanding of physiopathology and immunology in PDAC may lead to new tools for the management of this devastating disease.

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Conflicts of Interest

The authors declare no competing interest.

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Authors' contributions

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