Advances in Nanotechnology for Upper GI, Liver and Pancreatic Cancer

# Summary

Cancers of the upper gastrointestinal tract, liver and pancreas have some of the poorest prognoses of any malignancies. Advances in the diagnosis and treatment are sorely needed to improve patient outcomes. Due to the potential for constructing a tailor made therapy, nanotechnology offers an excellent opportunity for producing nanoparticles capable of targeting specific cancers with multifunctional properties that can be exploited for both diagnosis and treatment. Although development of these therapies is still in the early stages, the use of nanoparticles is becoming more widespread and will likely involve all areas of medicine. Research into nanoparticles is on going for upper GI, liver and pancreatic cancers and their use becoming increasingly popular as contrast media for radiological investigations. A few nanoparticle-based therapies are in clinical use, although more sophisticated technologies capable of active targeting are still in the early stages of assessment for clinical use.

# Keywords

nanoparticles  diagnosis  treatment  targeting  oesophageal cancer  gastric cancer  liver cancer  pancreatic cancer

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# Introduction

Nanotechnology is relatively new area of scientific development that promises advances across all areas of medicine as well as many aspects of our lives. In its basic form, this area of research involves the manufacture of functional systems with more than one dimension ≤100 nm (1 nm = 1 billionth of a metre, or the width of approximately 10 hydrogen atoms) [1]. In the field of oncology, advances in nanotechnology have the potential to provide us with tiny vehicles capable of directly targeting cancer cells and leading to new possibilities for diagnosis and treatment.

Despite major advances during the latter half of the 20th century in our knowledge of cancer pathogenesis and development of new treatments, it remains the third biggest killer worldwide. Worldwide incidence and mortality in 2008 were 12.7 million and 7.6 million respectively and these figures are projected to only increase over the coming decades as the median population age increases [2]. Novel areas of research are therefore urgently needed to improve outcomes for people diagnosed with this disease.

Upper gastrointestinal cancers carry some of the worst prognoses of all cancers. In terms of worldwide cancer mortality, gastric, liver, oesophageal and pancreatic cancers occupy 2nd, 3rd, 5th and 6th places respectively and they have some of the lowest overall five-year survival rates of any cancer, with only gastric cancer above 10% [2]. The incidence of gastric cancer in the United Kingdom has seen a rapid decline over the past 40 years from 21.7 per 100,000 in 1975 to 8.5 in 2008 [3]. With estimates that 60% to 90% of cases are related to *H. pylori* infection, the advent of eradication therapy probably has a large part to play in this reduction [4-7]. However, the remaining upper GI cancers have seen no such improvement in incidence or mortality rates over this period.

Pancreatic cancer has the poorest prognosis of this group, nevertheless recent advances in surgical technique and adjuvant chemotherapy have increased 5-year survival to 29% [8,9]. However, only 20% of patients will have the chance of optimal treatment (surgical resection and adjuvant gemcitabine) and the remainder require improvements in disease prevention and novel techniques that facilitate earlier diagnosis in order to improve their survival.

Nanomedicine has the potential for providing advances in these areas with a myriad of nanomaterials that offer opportunities in drug and gene delivery, molecular recognition and imaging contrast agents [10-17].

# Overview of Nanoparticles

Materials containing nanoparticles have been used for centuries. One of the earliest examples is the use of a luster decoration on ceramics in Mesopotamia as early as the 9th century [18]. However, it was not until the development of the scanning tunnelling microscope (Binnig and Rohrer of IBM in 1981) that it was possible to visualise objects in the atomic detail required to understand and refine our knowledge of this area [19]. Since then developments in nanotechnology have accelerated exponentially, assisted by increasing funding and promotion from both governments and the private sector as the massive potential of this subject has been realised.

As a result of these developments, nanoparticle products for both diagnosis and treatment are now in use throughout oncology. Examples include Abraxane, a modified version of the well-established chemotherapy agent paclitaxel and Combidex, a superparamagnetic iron oxide nanoparticle (SPION) in use as an MRI contrast media [20,21].

Materials

### Metal cores

#### Gold

Gold has been used in medicine for millennia, with its use recorded as early as 2500 BC by the Chinese to increase vital power. In the western world, gold has been used over the past 5 centuries in the treatment of epilepsy (16th century), syphilis (19th century) and tuberculosis in the 1920s [22]. Today, the major use of gold-based agents is in the treatment of rheumatic diseases such as rheumatoid arthritis and psoriasis [23].

Gold is, however, highly toxic due to its accumulation in the kidneys resulting in nephrotoxicity. Other side effects, occurring in up to one-third of patients, include: dermatitis, stomatitis, thrombocytopenia and liver toxicity [24]. Modifying the surface of gold nanoparticles with a silica shell can reduce its toxicity and it remains an attractive candidate for biological applications due to its affinity for binding thiols, disulfides, phosphine and amines as this allows easy conjugation with peptides, proteins and antibodies [25].

##### Manufacture:

There are three main methods for gold nanoparticles synthesis: Physical and chemical methods generally involve the reduction of a soluble gold salt, such as chlorauric acic (HAuCl4), by electromagnetic radiation or using chemicals like sodium citrate or hydroquinone [26,27]. Biological techniques have been developed more recently and are emerging as ‘green’ methods for gold nanoparticle production by using bacteria or fungi to perform the reduction process [28-31].

#### Iron oxide

Widder and Senyi first proposed iron oxide (IO) based nanoparticles for use in biomedical applications in the late 1970s [32]. At the nanoscale, IO particles possess the unique property of superparamagnetism, in that they are magnetized only in the presence of an external magnetic field and are termed SPIONs (superparagmagnetic iron oxide nanoparticles). This property can be used for targeting (accumulation at an external magnetic field focused at the desired site), diagnosis (MRI contrast) and treatment as an alternating magnetic field causes the particles to heat up resulting in thermal ablation of adjacent cells [33-35].

##### Manufacture:

The simplest method of iron oxide nanoparticle production is through a pH dependent co-precipitation from an aqueous solution of iron(II) or iron(III) ions [36]. Although this method is simple it can be difficult to simultaneously add a protective coating needed to prevent decomposition and reduce cytotoxicity, so alternative procedures such as using microemulsions, polysaccharide template synthesis or high-temperature decomposition have been developed [37,38].

### Quantum Dots

Quantum dots (QDs) are semiconductors that possess the ability to fluoresce upon excitation by a broad range of electromagnetic wavelengths (spanning from UV to near-infrared). They are typically composed of a cadium or indium based crystal core that is stabilised by a zinc sulphide shell to improve their optical and physical properties. The colour of their fluorescence can be tuned by altering the size and composition of the core. The most commonly used cores are cadmium-selenium (CdSe) and cadmium-tellurium (CdTe) and often these are coated in a polymer layer to improve their biocompatibility.

Because of their fluorescent properties, they have many potential uses in either diagnosis or as a treatment when combined with photosensiting drugs, such as porphyrin analogues [39]. There are obvious concerns over their toxicity due to their heavy metal composition and more work is required to ascertain their pharmacokinetic and toxicological properties [40].

##### Manufacture:

Historically, quantum dots were synthesized using a proves involving organic solvents, which impeded their use in biological systems due to their hydrophobicity and was not scalable to allow commercial application. New, more scalable, techniques have recently been developed using a molecular cluster compound upon which the crystal growth occurs; nevertheless bulk-manufactured QDs remain scarce at present.

### Polymers

For use in biological systems, metal-based nanoparticles generally require a coating in order to prevent decomposition, reduce toxicity to the target organism and avoid uptake by the RES (in full) – so called stealth polymers. Polymers are by far the most common materials used for this purpose. These can either be naturally occurring (dextran, albumin and lipids) or synthesized (polyethylene glycol (PEG), polyvinyl alcohol (PVA) or polyoxazolines (POZ)) [41,42]. Alternatively polymers can also be used independently of a metal core, either directly bound to a drug (albumin-bound paclitaxel) or as encapsulating micelles (liposomes or amphiphilic copolymers) designed to break down and release their cargo after undergoing endocytosis at their target. [FIGxxx]

Diagram of coated nanoparticle

### Carbon

Carbon NPs can take several forms, the most commonly used and established of which are the fullerenes. Like graphite, fullerenes are composed entirely of carbon and form either hollow spheres of 60 carbon atoms called ‘buckyballs’, or are graphene sheets folded into cylindrical nanotubes. They are lightweight, chemically and thermally stable and have high tensile strength and conductivity [43]. Their potential uses in medicine include tissue scaffolding for osteoblast proliferation, drug delivery and thermal ablation agents [41,44-46].

##### Manufacture:

Arc discharge was the first technique used for the production of carbon nanoparticles. Two graphite electrodes are placed in an inert gas and an electric current is passed between the two causing the cathode to be vaporised into a mixture of carbon nanotubes and other carbon allotropes that can the be separated.

More recently, chemical vapour deposition (CVD) has been developed and is the most promising high-yielding technique for industrial manufacture of carbon nanoparticles. Carbon nanotubes are grown on top of a metal catalyst by heating a hydrocarbon gas in temperatures up to 1150°C. Other methods include laser ablation and electrolysis.

### Viruses

Viruses have been used in medicine since Edward Jenner (1796) postulated that milkmaids did not generally contract smallpox as a result of their exposure to the less virulent cowpox, these experiments famously led to the development of immunology.

Viruses can be likened to bundles of genetic material coated in a protein capsid and occasionally encapsulated in a lipid envelope derived from the host cell membrane. At sizes of about 20 to 300 nm viruses fulfil the criteria for nanoparticles and, given their pathogenic function, have evolved to specifically target cells and gain easy entry to facilitate their replication [47]. These properties make them ideal candidates for use in nanomedicine as either cancer vaccines, for example HPV vaccine for cervical cancer, or as cancer treatments, such as the use of the oncolytic poxvirus [48,49]. Both of these examples are very recent developments and demonstrate the progress that has been made in this field over the past 5 years despite early concerns regarding the safety of virus-based treatments. Adverse events that have resulted in the cessation of clinical trials include the development of multi-organ failure as a result of a massive inflammatory response in a phase I dose escalation study and a second study where two participants developed induced lymphoproliferative disorder [50-52]. The immune response induced by early virus treatments was overcome by developing viral vectors that are stripped of all viral genes and has demonstrated that obstacles encountered during the development of novel treatments are not insurmountable [53].

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| --- | --- | --- | --- |
| Type | Properties | Examples | References |
| Polymer-drug conjugates | 1. Passive targeting
2. Antigen/receptor-ligand targeting
3. Tumour microenvironment-dependent drug release
 | Albumin-bound paclitaxel (Abraxane)Stearoyl gemcitabine | [54][55] |
| Polymer-coated metal cores | 1. RES evasion (‘stealth’)
2. Multimodal targeting:- Passive- Antigen/receptor-ligand- Magnetic targeting (Fe2O3)
3. Multimodal imaging:- MRI contrast- Fluorescence
4. Multiple treatment opportunities
 | PEGylated goldPEGylated iron-oxide | [56][57] |
| Quantum dots | 1. Passive and active targeting
2. Imaging through tuneable auto-fluorescence
3. Treatment when combined with photosensitising agent
 | PEG coated CdSe-ZnS | [58] |
| Carbon  | 1. Passive and active targeting
2. Treatment:- Therapeutic cargo delivery- Radio-frequency Thermal ablatio
 | Mesoporous carbon nanoparticleCarbon nanotube | [59][60] |
| Viruses | 1. Active targeting
2. Treatment opportunities:- Cancer vaccines- Gene therapy
 | Cowpea mosaic virus with surface vimentin | [61] |

*Table 1*: Summary of types of nanoparticle and their properties with current examples.

# Toxicity

The potential health hazards posed to researchers, manufacturers and patients from nanomaterials are largely unknown at present; however, no adverse effects have been reported from the use of these materials to date. Concerns about iron sequestration and gold-related nephropathy have been raised and the potential effect of these materials needs to be anticipated and assessed in order for them to be accepted in clinical practice. Parallels can be drawn from ultrafine particles (UFPs), about which much more is known. UFPs such as diesel exhaust particles, quartz and asbestos, are all associated with medical conditions that result in significant morbidity and even mortality [17,62]. The principle mechanism of toxicity of these particles arises from the generation of reactive oxygen species and subsequent oxidative injury as a result of their large surface area, hence the comparison with manufactured nanoparticles [63]. From our knowledge of UFPs it is therefore possible to predict, assess and modify the manufacturing of nanoparticles so as to prevent or minimise any adverse effects and, consequently, research into nanotoxicology is a growing area of interest.

UFPs are technically nanoparticles by size criteria, however, as they are naturally occurring, they differ from their manufactured counterparts in several important ways:

* Manufactured nanoparticles are carefully engineered, allowing high uniformity in their chemical composition.
* UFPs are generally inhaled and cause the majority of their adverse effects in the respiratory tract (e.g. fibrosis), whereas pharmaceutical nanoparticles are generally designed to be injected and therefore bypass the respiratory system [43].

Finally, there are stringent requirements for new agents to reach clinical use. These include *in vitro* toxicity studies, animal models to assess pharmacokinetics and efficacy, progressing through three phases of clinical trial. This work is crucial to understanding toxicological pathways specific to engineered nanomaterials and preventing any unnecessary harm that could occur as a result of exposure to them.

Methods used to predict the effects an agent may have on a biological system involve *in vitro* cell-culture studies and *in vivo* animal models. Example of *in vitro* cytotoxicity assays include trypan blue and tetrazolium salt (e.g. MTT) assays that measure membrane integrity and mitochondrial activity respectively [64-66]. However, the use of *in vitro* experiments to predict *in vivo* toxicity is not always reliable as a result of the different conditions found in a biological system [65]. *In vivo* studies undertaken in mouse or rat models are consequently required to assess the impact of nanoparticle exposure on a living organism. As well as basic lethal dose experiments, more advanced methods for measuring changes in serum chemistry, cell morphology and particle biodistribution can be used to glean as much toxicity information as possible before progression to clinical trials.

Toxicity studies to date have shown that cytotoxic affects are dose- and time-dependent and probably the result of the generation of reactive oxygen species and internalization of the nanoparticles [43]. In all types of nanoparticles, modification of their surface coating can result in improving their cytotoxicity profile. Functional groups, surface charge and the presence of hydrophilic molecules play an important role in determining the toxicity of the resulting nanoparticle.

# Targeting and Drug Delivery

Nanotechnology offers many possibilities for targeting cancer afforded by the variety of materials available and the opportunity to construct tailor made particles to a specific problem. In theory, specific targeting should allow a higher concentration of the nanoparticle to accumulate within the vicinity of malignant tissue. When coupled with a drug release mechanism, this could increase the efficacy of established chemotherapy agents whilst simultaneously reducing the systemic side effects almost universal to current chemotherapy agents.

Targeting strategies can be divided into passive and active:

## Passive

Passive targeting of nanoparticles can be achieved by engineering a nanoparticle that is capable of prolonged circulation times and thus natural accumulation at the site of the tumour will occur. This monopolizes on the relatively leaky tumour endothelia, but does not restrict particle delivery to these tissues exclusively.

### Circulation time

Nanoparticles are susceptible to rapid clearance via macrophage phagocytosis in the organs of the reticuloendothelial system (RES) [67]. This property is useful for targeted delivery to tumours in these organs, including hepatic metastases, but leads to subtherapeutic concentrations of the conjugated drug at other tumour sites [68]. Surface chemistry modification with hydrophilic ‘stealth’ polymers, such as polyethylene glycol (PEG) or polyethylene imine (PEI), can prevent opsonisation and subsequent phagocytosis [69,70]. This increases the blood circulation time allowing a greater number of nanoparticles to come into contact with the tumour.

### Accumulation

Leaky vasculature is a feature of the tumour environment that leads to enhanced permeation and retention of nanoparticles [71]. It is possible to take advantage of these properties by tuning the size of the nanoparticle. The maximum pore size of endothelial cells in leaky tumour vasculature has been shown to be about 500 nm compared to pore diameters up to 20 nm in normal capillaries [72,73]. However, large molecules are susceptible to capture by the sinusoids of the RES, whose pores only allow particles of diameter up to 100 nm to pass through freely [74]. Studies have shown that nanoparticles engineered with diameters up to 100nm will pass out of the RES back into the circulation and leak into tumour tissues where impaired lymphatic drainage results in their accumulation [75-77].

### Tissue microenvironment

Once accumulation has occurred, the abnormal physiology of the tumour microenvironment can be used to initiate release of a chemotherapeutic cargo. A state of hypoxia and an inadequate supply nutrients to proliferating cancer cells with a high metabolic rate, results in activation of catabolic processes. Glycolysis leads to the production of acid and a pH in the surrounding tissue below normal physiological values. This acidic environment can be exploited by designing nanoparticles, such as liposomes, that are stable under normal physiological conditions, yet break down at lower pH levels, releasing their cytotoxic payload [78,79].

An alternative method of release is to make use of the proteins secreted by cancer cells, such as secretin protein acidic and rich in cysteine (SPARC) or matrix metalloproteinases (MMPs) [20,80]. SPARC is an albumin binding protein that is overexpressed in some cancers and has been taken advantage of by an albumin-bound variant of paclitaxel (Abraxane) that uses this binding protein to preferentially accumulate at the site of the tumour [20,81].

## Active

Active targeting of a tumour by nanoparticles is facilitated by conjugation of the particle with either a peptide ligand or tumour specific antibody-antigen interaction, or through the use of an external stimulus, such as a magnetic field, used to guide SPIONs to the desired location.

### Antigen

Certain tumours have been demonstrated to posses certain surface proteins or receptors. Targeting is achieved through conjugation of a nanoparticle with an antibody against these antigens. Ideally, the antigen selected should exist homogenously on all tumour cells and not be expressed in normal tissue. However, most of the proteins that have been demonstrated on the surface of tumour cells to date are merely overexpressed varieties of normally occurring proteins.

Despite this, the quantity in which these proteins are expressed by tumour cells, combined with the passive targeting processes described above, mean that active targeting can further promote aggregation of nanoparticles at the site of tumour. In addition to the accumulation in the interstitial space of the tumour, receptor-mediated endocytosis results in internalisation of the nanoparticles and provides further opportunities for targeted drug release.

Examples proteins that are overexpressed in upper GI malignancies include: eGFR, CEA, CA125, CA19-9, uPAR, EpCAM, VEGF and galactosamine (see Table 2).

### Receptor-ligand

A similar form of targeting can be achieved by conjugating nanoparticles with a ligand to a receptor known to be overexpressed on the target tumour cells. As for antigen targeting, the receptor would ideally only be present on the target cancer cells. Conjugation of the ligand onto the surface of nanoparticles leads to their capture by the target cell surface receptor and, if activated, subsequent receptor-mediated endocytosis. Examples of receptors that have been targeted can be seen in Table 2.

|  |  |  |  |
| --- | --- | --- | --- |
| Targeting Method | Target | Type of cancer | Reference |
| Antibody | HIF-1α and HIF-2αBRCAA1CD44v6CEAGlypican-3VEGFGalactosamine (PK2)CA19-9CA125BombesinEpCAM | OesophagealGastricGastricGastric, PancreaticLiverLiverLiverPancreaticPancreaticPancreaticPancreatic, Liver, Gastric | [82][83][84][85-88][89][90][91][86][92][93,94][95] |
| Receptor-ligand | uPAReGFRFolate receptor | PancreaticPancreaticGastric | [57][96,97][98] |

*Table 2*: Potential targets for upper GI malignancies.

### Magnetic

As described above, iron oxide nanoparticles exhibit superparamagnetic properties. This opens up the possibility of using an external magnetic field to focus a magnetic gradient at the site of interest and attract magnetic nanoparticles to the tumour site [99]. The magnitude of a magnetic field follows an inverse square law making this technique very suitable for superficial tumours such squamous cell carcinoma of the skin, but more problematic for tumours of deeper viscera [33].

In addition to attracting magnetic nanoparticles to the site of a tumour, using an alternating magnetic field results in the nanoparticles generating heat via effects such as hysteresis and friction caused by Brownian rotation [100]. This property can be exploited once the particles have reached their target to generate local heating of a tumour and results in thermal ablation of the tissue and producing rise in temperature to 42-45 °C is sufficient to cause necrosis of the tumour without significant damage to surrounding normal tissues [101]. Pilot studies in animals have shown near complete regression of tumours when using this treatment technique [101,102]. As the alternating magnetic field does not require the precision required for targeting the particles, penetration into deeper tissues is possible. This could allow thermal ablation to develop into a local treatment for cancer with minimal systemic effects.

Diagram of targeting and drug delivery techniques.

# Nanotechnology Advances in Upper GI Malignancies

## Oesophageal Cancer

|  |  |  |  |
| --- | --- | --- | --- |
| Use | Nanoparticle type and intended use | Description | Reference |
| Diagnostic | Ferumoxide (SPION)*Use:* MR contrast | SPIONs were injected endoscopically at the site of the tumour and subsequent MR lymphography was performed. Results demonstrated that the SPIONs were able to enhance delineation of lymphatic flow and in the future*Potential use:* could provide a method for directing lymphadenectomy performed at the time of resection. | Clinical study[103] |
| Diagnostic | Ferumoxtran-10 (dextran-coated SPION)*Use:* MR contrast | Patients underwent MR imaging following an IV infusion of ferumoxtram-10. Enhancement patterns of lymph nodes were characterised and defined as non-metastic or metastatic. Histological evaluation of the nodes was undertaken following resection to confirm the pre-operative findings.*Potential use:* could be used to enhance characterisation of metastatic lymph nodes on pre-operative MR imaging and direct lymphadenectomy performed at the time of surgical resection. | Clinical study[104] |
| Diagnostic | Ultra-small dextran and sodium citrate coated SPION*Use:* MR contrast | Ultra-small SPIONs were inject intravenously and MR imaging performed 24-36 after administration. At resection the lymph nodes were placed on an anatomical grid and rescanned. Histological assessment was then undertaken.*Potential use:* may have high potential value as a new non-invasive staging modality instead of or in addition to EUS and PET CT. | Clinical study[105] |
| Therapeutic | PEI with NGR targeting molecule*Use:* hTERT targeted ASODN gene therapy. | *In vitro* and *in vivo* studies showed an increase in the efficacy of transfection with PEI/ASODN nanoparticles resulting in a decrease of hTERT expression. NGR/PEI/ASODN nanoparticles showed targeting of tumour cells *in vivo*.*Potential use:* targeted induction of apoptosis in tumour cells through gene therapy. | *In vitro* and *in vivo* animal study[106] |
| Therapeutic | Near-infrared (NIR) quantum dots and HSA800 (albumin-conjugated CW800 fluorophore) *Use:* lymph node identification | A submucosal injection of two novel lymph tracers was performed in the oesophagus of pigs was performed. NIR-fluorescent imaging was used to identify and precisely resect the sentinel lymph node.*Potential use:* intra-operative identification of sentinel lymph nodes | *In vivo* animal study[107] |
| Therapeutic | Nab-paclitaxel (Albumin-bound paclitaxel) | Patients who had undergone three previous chemotherapy regimens for thoracic tumours were investigated to determine the maximum tolerated does (MTD) of nab-paclitaxel in combination with gemcitabine.*Potential use:* New combination chemotherapy with higher efficacy and reduced systemic effects. Phase II trial is required to evaluate efficacy further. | Phase I clinical trial[108] |

*Table 3*: Examples of nanoparticles currently under investigation for oesophageal cancer.

# Gastric Cancer

|  |  |  |  |
| --- | --- | --- | --- |
| Use | Nanoparticle type and intended use | Description | Reference |
| Diagnostic | Ferumoxtran-10 (dextran-coated SPION) | 31 patients diagnosed with gastric cancer underwent MR imaging using ferumoxtran-10 contrast agent to assess the presence of regional lymph node metastases. Compared to other imaging modalities such as CT, US and conventional MRI the results of this study demonstrated a far superior sensitivity (96.2%), specificity (92.5%) and accuracy (93.3%).*Potential use:* Pre-operative assessment of regional lymph node metastases using SPION based contrast may enhance appropriate treatment for patients with gastric cancer. | Clinical study[109] |
| Diagnostic | Aminosilane modified fluorescent magnetic nanoparticles (FMNPs, iron-oxide core) conjugated with a BRCAA1 monoclonal antibody | MGC803 gastric cancer cells known to express the BRCAA1 protein were treated with BRCAA1-FMNPs. *In vitro* results showed enhanced labelling of the cells with of the BRCAA1 targeted FMNPs compared to FMNPs with no targeting antibody. *In vivo* studies showed that the BRCAA1-FMNPs were capable of targeting a gastric cancer xenograft in nude mice and could be used for both fluorescent and MR imaging.*Potential use:* Novel targeted imaging agent in gastric cancer. | *In vitro* and *in vivo* animal study[83] |
| Therapeutic | NK105 – micelle incorporating paclitaxel | Phase II clinical trial of 56 patients with advanced gastric cancer who were unresponsive to first-line chemotherapy. NK105 has been shown to have a plasma area under the curve 15-fold greater than conventional paclitaxel despite a 30% lower recommended dose [109]. Overall response rate was 25% with a further 30.4% achieving stable disease and an overall survival of 14.4 months. NK105 was generally well tolerated and high a similar side-effect profile to other formulations of paclitaxel.*Potential use:* NK105 warrants further investigation in as a chemotherapeutic agent for use in treating advanced gastric cancer. | Phase II clinical trial[110] |
| Therapeutic | Calcium phosphate conjugated to a CEA promoter gene coupled with the yCDglyTK suicide gene. (CPNP-CV-yCDglyTK). | uCDglyTK is a thymidine kinase that can convert a non-toxic 5-FC prodrug into the cytotoxic agent 5-FU. SGC7901 gastric cancer cells treated with CPNP-CV-yCDglyTK were compared to the same cells directly transfected with the suicide gene and untreated cells. The study demonstrated efficient delivery of the suicide agent with a drop in cell viability from 99% in the group with no gene therapy to 25.4% in those treated with the nanoparticles. These findings were replicated in an *in vivo* nude mouse model where inhibition of tumour growth in CEA expressing cells was demonstrated.  | *In vitro* and *in vivo* animal study[88] |
| Therapeutic | Chitosan-deoxycholic acid micelle nanoparticle loaded with MAGE-3 peptide vaccine. | MAGE-3 is an antigen expressed by many tumours. The MAGE-3 loaded nanoparticles produced an enhanced immune response in nude mice that resulted in a tumour inhibition rate that was significantly greater than the control goup (p=0.0125).*Potential use:* nanoparticle-based vaccines could enhance immune response and result in a greater efficacy of anti-cancer vaccines. | [111] |

*Table 4*: Examples of nanoparticles currently under investigation for gastric cancer.

# Liver Cancer

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| --- | --- | --- | --- |
| Use | Nanoparticle type and intended use | Description | Reference |
| Diagnostic & Therapeutic | Polyethylene oxide (PEO) coated SPION conjugated with a folate receptor-targeting molecule and loaded with doxorubicin (YCC-DOX). | Rat and rabbit models using Hep3B xenografts were used to demonstrate the efficacy of the YCC-DOX nanoparticles compared with free doxorubicin (FD) and a commercial liposome based drug (DOXIL®). YCC-DOX significantly facilitated the targeted delivery of DOX to FR-positive Hep3B cells without producing any toxic side-effects. YCC-DOX was shown to be superior to the conventional MRI contrast agent Resovist®.*Potential use:* Targeted drug delivery vehicle and diagnostic contrast media. | *In vivo* animal study[98] |
| Therapeutic | Protocell formed of liposome-fused nanoporous silica core conjugated with an SP94 targeting peptide and loaded with doxorubicin or a drug cocktail (doxorubicin, 5-FU and cisplatin). | Hep3B hepatocellular carcinoma cell line and normal hepatocytes were used to evaluate the targeting potential and cargo delivery characteristics of the protocells, compared with free doxorubicin and simple liposome particles. 105 fewer protocells were required to kill 90% of the cell population compared to the standard liposomes. The targeted protocells achieved better targeting with 90% viability of normal hepatocytes compared to no specificity demonstrated by free doxorubicin.*Potential use:* Multicomponent targeted delivery vehicle | *In vitro* study[112][88][87][86][85][84][83][82] |
| Therapeutic | Arsenic trioxide (As2O3, ATON) suspended in lipidol. | Intra-arterial injections of ATON particles were performed in rabbits bearing VX2 liver tumours. An external alternating magnetic field was then used to heat the ATONs, resulting in hepatic arterial embolization. Compared to lipiodol and saline controls, tumour growth was delayed and survival prolonged as a result of tumour necrosis.*Potential use:* Targeted thermal ablation therapy for liver tumours. | *In vivo* animal study[113] |
| Therapeutic | PEGylated mesoporous silica nanoparticles loaded with docetaxel (SN-PEG-Dtxl). | ICR mice with H22 (hepatoma) flank tumours were treated with SN-PEG-Dtxl nanoparticles, taxotere or saline. The average tumour inhibition calculated by tumour weight was 72% for the SN-PEG-Dtxl and 57% for the taxotere groups.*Potential use:* Targeted chemotherapy delivery for liver tumours that could be further enhanced through the conjugation of additional targeting ligands. | *In vitro* and *in vivo* study.[114] |

*Table 5*: Examples of nanoparticles currently under investigation for liver cancer.

# Pancreatic Cancer

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| --- | --- | --- | --- |
| Use | Nanoparticle type and intended use | Description | Reference |
| Diagnostic | Indium phosphide-zinc sulphide (InP/ZnS) quantum dots conjugated with anticlaudin-4 or anti-prostate stem cell antigen (anti-PSCA) monoclonal antibodies. | MiaPaCa-2 and PANC-1 pancreatic cancer cell lines were incubated with InP/ZnS quantum dots conjugated with either antibodies. Stronger staining was visualised on confocal microscopy in the pancreatic cancer cell lines compared to that of a receptor-negative cell line (KB).*Potential use:* *in vivo* targeting and detection of pancreatic cancer. | *In vitro* study[115] |
| Therapeutic | Gold nanoparticles conjugated with cetuximab (anti-eGFR-1) or PAM4 (anti-MUC1) monoclonal antibody. | PANC-1 (eGFR +ve) and Capan-2 (MUC-1 +ve) pancreatic cancer xenografts were established in nude mice. Subsequent treatment with gold nanoparticles conjugated with a targeting antibody and radiofrequency induced hyperthermia showed a significant reduction in the tumour size compared to the group with no radiofreqency treatment*Potential use:* Targeting of pancreatic cancer cells with a monoclonal antibody and subsequent thermal destruction using radiofrequency. | *In vitro* study[116]*In vivo* study[117] |
| Therapeutic | Folic acid (FA) conjugated mesoporous silica nanoparticles (MSN) loaded with camptothecin (CPT). | MiaPaCa-2 and PANC-1 xenografts were established in nude and SCID mice. Treatment with FA-MSN/CPT resulted in a significantly increased reduction in tumour size compared to MSNs without the FA conjugation in both pancreatic cancer cell lines.*Potential use:* Targeted drug delivery for the treatment of pancreatic cancer. | *In vivo* study[118] |
| Therapeutic | Magnetic functionalised carbon nanotubes (CNTs) loaded with gemcitabine. | BxPC-3 and SW1990 pancreatic cancer cell xenografts were established in nude mice. The mice were then treated with either standalone gemcitabine or conjugated to magnetic CNTs. There was a higher efficiency in delivering gemcitabine when it was conjugated to the CNTs, especially when a magnetic field was used.*Potential use:* Targeted delivery of a chemotherapeutic using an external magnetic field. | *In vitro* and *in vivo* study[119] |
| Therapeutic | Albumin-bound paclitaxel | 19 patients with pancreatic cancer who had previously progressed on gemcitabine-based therapy were given Abraxane, which delivers its chemotherapy payload in response to SPARC expression by the tumour. The 6-month overall survival (OS) was 63%, with a median OS of 7.3 months. The nanoparticle therapy was well tolerated and resulted in clinical benefit for 37% of patients (stable disease or partial response). *Potential use:* Alternative chemotherapeutic for patients who progress on gemcitabine and have a SPARC expressing tumour. | Phase II clinical trial[54] |

*Table 6*: Examples of nanoparticles currently under investigation for pancreatic cancer.

# Expert commentary & five-year view

Nanotechnology has progressed dramatically over the past 10 years many novel opportunities in tailoring a compound for specific detection and treatment of upper gastrointestinal malignancies have been identified. The potential to tailor nanoparticles to specific types of cancer means that nanoparticles provide an excellent foundation on which to develop theranostic treatments.

Cytotoxicty has been identified as a potential barrier and the development of the nanotoxicology field has led to a better understanding of the cytotoxic affects of these materials resulting in refinement of their surface chemistry and improving or removing potentially harmful properties. As more centres developing this technology move into *in vivo* research this understanding will be refined further allowing for progression into clinical trials.

In the radiological world nanoparticles are already in use as contrast agents that enhance magnetic imaging studies, increasing their sensitivity and specificity. With regard to the use of nanoparticles for the treatment of cancer, many avenues are currently being explored. The properties of the nanoparticles themselves are being exploited through development of techniques such as thermal ablation and through advances in chemical methods that allow transportation of existing therapeutic agents as a payload that is released upon reaching the target tissue. Both of these methods have the potential to reduce the systemic effects of current treatments that are the cause so much morbidity for patients with this devastating diseaseand some, such as Abraxane, are already in clinical use having demonstrated superior properties over the original drug.

Iron oxide-based particles in particular show great potential as a combination diagnostic and therapeutic tool with unique magnetic properties that could allow for very specific targeting of cancerous tissues and permitting simultaneous treatment and monitoring of the tumour.

Over the next five years we will see an increase in the use of nanotechnology throughout medicine. Studies that are currently using animal models to demonstrate the potential efficacy of nanoparticles as novel treatments will advance to clinical trials, although it is unlikely that there will be sufficient data for there to be widespread use of these before the end of the decade.

In summary, it is clear that nanotechnology provides the potential to enhance many aspects of medicine and cancer stands to benefit

# Key issues

* Upper GI, liver and pancreatic cancers have some of the worst prognoses of all cancers.
* Nanotechnology has the potential to enhance diagnosis and treatment of these cancers.
* Early concerns over the toxicity of nanoparticles are being overcome as the field of nanotoxicology and research in this area develops.
* Targeting of nanoparticles to specific tumours can be achieved by active and passive means:
	+ Passive: natural accumulation of nanoparticles at the tumour by exploitation of the unique properties associated with the tumour microenvironment.
	+ Active: antigen and receptor-ligand strategies or through the use of an external stimulus such as a focused magnetic field.
* Therapeutic cargos can be delivered directly to the site of the tumour, reducing the potential for adverse systemic effects of current therapeutics.

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