Silver Nanoparticle-Mediated Therapies in the Treatment of Pancreatic Cancer

*Rachel Foulkes, Mohsen Al Asgari, Anthony Curtis, Clare Hoskins**

Institute of Science and Technology in Medicine, Keele University, Keele, Staffordshire, ST5 5BG, UK

Keywords: Pancreatic cancer, silver nanoparticles, cancer therapy, thermal therapy, antimicrobial, drug delivery

Abstract

Pancreatic cancer is an extremely aggressive disease for which patient prognosis is poor and survival is extremely unlikely. It is an extremely under-represented cancer in the research field, due to the dense tumors formed and complexities surrounding treatment requirements. The use of nanomedicines in cancer therapy has been explored over the past few decades. Such nanomedicines are slowly coming to fruition in the clinical setting. One such platform which may possess promise in the fight against pancreatic cancer is the use of colloidal silver. Silver nanoparticles have shown potential in other cancer types over a host of treatment strategies and these are likely to be translatable into pancreatic cancer. This review will focus on the potential use of silver nanoparticles in pancreatic cancer therapy, reporting on a range of different strategies being employed which may be directly applicable in improving the clinical outcomes for pancreatic cancer patients.

Introduction

Cancer is a global problem, with a high incidence and mortality. The increases in occurrence of many types of cancers can be attributed to the increasingly ageing population and an increase in other significant and varied risk factors [1]. Pancreatic cancer involves the uncontrolled growth of malignant cells, often leading to the formation of tumors. Pancreatic cancer occurs most often in people between the ages of 40 and 80 [2]. According to the most recently published edition of Cancer Statistics, the survival rates for pancreatic cancer are the lowest of all cancers, with patients from the United States having only an 8% chance of survival [2]. A study by Bray *et al*. looked at pancreatic cancer on a global scale, determining that there were 458,918 new cases of pancreatic cancer and 432,243 deaths, showing the number of deaths is as high as the incidence [1]. Additionally, the same study determined that pancreatic cancer was the 7th leading cause of cancer-related death worldwide in both men and women, with a proportion of sufferers being men.

There are several different reasons why survival rates of pancreatic cancer are so dire, including lack of early symptoms, making diagnosis very difficult until the cancer has progressed [3,4], minimal response from the immune system, especially with some immune cells being hijacked by tumors [3, 4], metastasis developing early in the disease progression [4, 5], the lack of biomarkers, and resistance to chemotherapeutics [6,7]. Additionally, there are no established causes of pancreatic cancer, but some risk factors have been identified, including smoking [4,8], obesity [5,9,10,11], heavy alcohol consumption [7,8,12,13], long-term diabetes [10,1114,15], and some hereditary factors [12,13,16,17]. There has been some evidence for a genetic basis for pancreatic cancer, due to there being heterogeneity found in tumor tissues and metastasised tissues [14,18]. There is also evidence that there are genetic variations between individual tumors, known as intratumoral heterogenicity [6,15].

Although diagnosis during the early stages can be difficult, there are several techniques that can be utilised to diagnose pancreatic cancer including biopsy, fine needle aspiration and magnetic

resonance imaging [9,16].

Over half of patients with pancreatic cancer have either locally advanced cancer or metastatic cancer [3,17] and over 90% of patients with pancreatic cancer have Pancreatic Ductal Adenocarcinoma (PDAC), developing from pancreatic exocrine cells [19]. PDAC is one of the most aggressive malignant cancers and is projected to become the second greatest cause of cancer-related death by 2030 in the United States [20]. A key characteristic of PDAC is the appearance of precursor lesions, as documented by Matthaei *et al*., Wu *et al*., and Macgregor-Das and Iacobuzio-Donahue [21-23]. These lesions are graded according to the level of potentially cancerous cells present, numbered 1 to 3, where 1 indicates low grade dysplasia and 3 high grade dysplasia [24]. Metastasis is common in pancreatic cancer, especially for patients with PDAC of which 90% present with metastasis at death [25], and common sites of spread including the peritoneum, liver and lungs [3]. Alongside being the most prevalent pancreatic cancer, PDAC has one of the greatest mortality rates of all cancer types, with a consistent 6% 5-year survival rate [26].

Cancer therapies: History and Future Prospects

Although various therapeutics have been trialled for the treatment of for pancreatic cancer tumors, the more standard chemotherapeutics and radiotherapy are known to be ineffective due to resistance [27]. Hence, gemcitabine remains a front-line treatment, though it only prolongs the life of patients rather than being a cure [28]. Gemcitabine (2',2'-difluorodeoxycytidine) (Figure 1) is used to treat multiple cancer types, including pancreatic, lung and breast cancers [29]. However, gemcitabine has been recently faced with resistance in a similar manner to other traditional chemotherapies. To overcome this, there have been several attempts to combine gemcitabine with other cancer therapeutics as summarised in Table 1.

A study by Reni *et al.* combined gemcitabine with 5-fluoruracil (5-FU), cisplatin, and epirubicin for patients with advanced pancreatic cancer [30]. They determined that there was a progression free survival (PFS) of 4 months for 60% of patients taking the combination of drugs

compared to 28% for those taking gemcitabine alone. They also determined that 11.5% of patients on the combination of drugs had an overall 2-year survival compared to 2.1% of patients on gemcitabine alone. However, a key problem identified within this study is the short period of time they used for PFS. The majority of studies find a median value for their PFS, giving a greater indication of the efficacy of their drug or combination of drugs.

Cunningham *et al.* combined gemcitabine with capecitabine, which gave an improved progression free survival (PFS) for patients with advanced pancreatic cancer, with a median survival of 7.1 months compared to a median survival of 6.2 months for patients on gemcitabine monotherapies [31] They also determined that there was an overall PFS of 5.3 and 3.8 months for gemcitabine-capecitabine and gemcitabine alone respectively. Grade 3 (severe) and grade 4 (potentially life threatening) side effects generally decreased except in the case of neutropenia and hand-foot syndrome, which increased by 13% and 4% respectively. This approach is currently used in the UK as a therapy for pancreatic cancer.

Another treatment suggested was FOLFIRINOX, a combination of folinic acid, irinotecan and oxaliplatin [32]. Conroy *et al.* compared gemcitabine to FOLFIRINOX for patients with metastatic pancreatic cancer. They found a greater overall survival and a greater PFS of 11.1 and 6.4 months respectively compared to gemcitabine's 6.8-month overall survival and 3.3-month PFS [33]. However, there were more side effects noted when using FOLFIRINOX, with incidences of Grade 3 and 4 side effects, including neutropenia, thrombocytopenia and sensory neuropathy all increasing, with no statistics to show how much worse these side effects are in patients. This increase in prevalence of side effects indicates that FOLFIRINOX has a much higher toxicity to healthy cells and was also found to only work for around 30-40% of patients [33].

A study by von Hoff *et al.* used a combination of gemcitabine and nab-paclitaxel (Abraxane®) to treat patients with metastatic pancreatic adenocarcinoma and found a 1.8-month greater survival than using gemcitabine alone, but this is only a small improvement overall [34]. They also found a median PFS of 5.5 months compared to 3.7 months for gemcitabine alone. There

was an increase in the percentage of some grade 3 and 4 side effects compared to gemcitabine alone. These included neutropenia (11% increase), fatigue (10% increase) and neuropathy (16% increase).

A follow up to this study by Reni *et al.* used gemcitabine-nab-paclitaxel alongside cisplatin and capecitabine (PAXG) compared to nab-paclitaxel-gemcitabine (AG) [35]. There was an increase in the median PFS of patients taking the PAXG compared to the AG, 12.5 and 9.9 months respectively. As well as this, the median survival was found to be very similar for both treatments, with PAXG patients having 20.7 months and AG patients 19.1 months. They also noted that there was a decrease in grade 3 and 4 side effects, except for neutropenia and cholangitis, which increased by 15% and 1% respectively.

Although there has been some success in increasing the life expectancy of patients with pancreatic cancer, with the studies listed here showing a general improvement in median PFS and overall survival, the majority of these treatments provide no cure**.** They are mainly used to extend the life of patients and attempt to improve their quality of life. There are also issues with current therapeutics as all of these studies reported several side effects, mainly grade 3-4, commonly attributed to damage to healthy cells caused by the drugs, which could be avoided using a more targeted delivery system. The only treatment currently available that provides any form of "cure" is surgical resection. Surgical resection is, however, only possible for around 15% of patients and only in the early stages of pancreatic cancer [36] and is subsequently followed by chemotherapy [37]. However, this is still unsuccessful in most cases as 80% of patients relapse and there is a 5-year patient survival rate, despite undergoing chemotherapy after surgical resection [38,39].

Pancreatic Tumors, the Tumor Microenvironment and Resistance to Chemotherapeutics

One of the initial problems with tumors and chemotherapeutic resistance is the entry of chemotherapeutics into tumor cells. The dense stroma formed by pancreatic cancers often hinder drug or particulate penetration into the tumor microenvironment where the rapidly proliferating

cells are located (Figure 2) [40]. There are other components that effect resistance to chemotherapeutics, including healthy cells in the tumour environment. There is substantial evidence that healthy cells in the tumor microenvironment also increase drug resistance to anticancer agents [41-43]. In addition to this, it has been observed that some fibroblasts have the ability to confer resistance to anticancer agents, as in Gellar *et al*., who discovered that the combination of human dermal fibroblasts and colorectal and pancreatic cancer cell lines gave the cancer cells greater resistance to gemcitabine [29]. As well as these, Li *et al*. discovered the presence of cancer stem cells in human pancreatic adenocarcinoma [44]. They identified the pancreatic cancer stem cells using the marker combination $CD44^+$ $CD24^+$ ESA and saw that these cells were able to create other differentiated cells or self-renew in mice. Another study by Hermann *et al*. identified cancer stem cells in pancreas tissues using the surface marker CD133 [45]. They noted that $CD133^+$ expressing cells had high tumorgenicity in contrast to the majority of $CD133^-$ expressing cells, which did not cause tumor formation.

Several components of the tumor microenvironment have been associated with cancer resistance, participating in the initiation, progression and metastasis of cancer [46]. Tumor associated macrophages (TAM's) play a role in pro-tumor activity. TAM's are commonly recruited by a few different types of cytokines, including chemokines and vascular endothelial growth factor [47]. They are also known to have decreased tumoricidal activity and are able to produce several factors that promote angiogenesis, including cyclo-oxygenase-2 (COX-2)-derived prostaglandin E2 and vascular endothelial growth factor [48]. More recently, TAM's have been implicated in producing Sema4D, which promotes angiogenesis [49], and Gas6, which promotes the proliferation of cancer cells [50]. Tumor cell products can promote the pro-tumor abilities of TAM, some of which include CSF-1, IL-10 [51] and certain components of the extra-cellular matrix [52].

Hwang *et al.* determined that human pancreatic stellate cells increased tumor proliferation, invasion, and colony formation [53]. As well as this, they also determined that the COX-2 gene is altered in cancer cells and fibroblasts and highlighted the potential role of pancreatic stellate cells in

causing stroma. Xu *et al.* completed a study to determine the role of pancreatic stellate cells using both *in vitro* and *in vivo* models [53]. They confirmed that these cells facilitate the metastasis and local growth of tumors, as well as their ability to travel to other sites with cancer cells.

Cancer associated fibroblasts (CAFs) have been identified to play two different roles in tumorigenesis. Erez *et al.* identified that CAFs can recruit macrophages to mediate inflammation and stimulate angiogenesis [54]. They were also able to identify a gene signature from CAF that cause their ability to promote tumor growth. CAFs have also been implicated in promoting the growth of tumors in multiple ways, including angiogenesis and extracellular matrix remodelling.

Another cause of tumor progression is chronic inflammation. A study by de Visser, Korets and Coussens looked into chronic inflammation, how it is mediated, and whether this led to initiation of cancer using a HPV16 mouse model [55]. They initially determined that hyperproliferation of cells occurs with inflammation and that B-lymphocytes played a key role in regulation of inflammation that could lead to the formation of malignant cells. These B-lymphocytes were not found to enter the premalignant skin, hence were deemed to act an immunomodulatory manner.

Many different proteoglycans have also been implicated in cancer biology. Iozzo determined that heparin sulphate proteoglycan contributes to pro-angiogenic activity [56], and later determined that C-terminal fragments of other proteoglycans could act in an anti-angiogenic manner in two other studies [57,58]. Sanderson and Yang determined that glypicans have also been involved in various functions in tumors, including promotion/inhibition of cancer initiation and progression [59]. Filmus and Selleck have determined that syndecans participate in the same processes [60]. Iozzo and Schaefer found that small leucine-rich proteoglycans block the activity of receptor tyrosine kinases in tumors [61].

The MYC oncogene has also been implicated in the formation of tumors, due to alterations in copy number variations, where genome sections repeat [62]. This gene has the ability to initiate and progress the formation of tumors alone, which has been seen in several studies [63,64]. A study

by Mazur *et al.* also determined that the Notch2 protein is a modulator of MYC in PDAC as observed in several cell lines [65]. Their study also noted the increased expression of MYC in pancreatic precursor tumors. The study by Witkiewicz *et al*. determined that activity of MYC was amplified and confirmed that there was increased expression of MYC in precursor pancreatic lesions [62].

PDAC in particular has several different characteristics including dense desmoplastic infiltration, common to solid tumors, along with stroma, associated with chemoresistance and furthering tumor progression [46]. In PDAC specifically, stroma makes up over 80% of the mass of tumors [66]. Pancreatic stellate cells have been proposed to be responsible for stroma formation [67], with these cells composing 4% of pancreas tissue [68]. In fact, an index for stroma activation is determined using a marker for the activated form of the pancreatic stellate cells in relation to collagen expression, where a larger value indicates a poorer patient prognosis, a marker commonly used for PDAC patients [69]. With multiple factors causing resistance to chemotherapy and other anti-cancer drug regiments, treating patients with pancreatic cancer has become much more difficult.

Intratumoral bacteria and how they affect anti-cancer therapies

One of the most recently identified causes of resistance to cancer therapeutics involves intratumoral bacteria. Hypoxia and necrosis are common in tumour tissues, providing the perfect environment in which bacteria can easily enter and successfully colonise [70]. The oxygen pressure in tumors has been found much lower than in normal tissues, often being in the range of 0-20 mmHg compared to the normal 20-100 mmHg, indicating hypoxia [71]. Hypoxia and necrosis occur due to fast tumor growth and poor oxygen supply due to poor vascularisation, creating an anaerobic environment in which bacteria can enter and colonise [72].

The clinical use of gemcitabine has been impacted by the current levels of tumor resistance, especially by the presence of intratumoral bacteria. Lehouritis *et al.* looked into the effects of

bacteria against several different chemotherapeutics, including gemcitabine [73]. They used CT26 tumors which were then injected with *E. coli* and treated with gemcitabine. Those tumors showed an increased tumor volume and there was a significant decrease in survival compared to a control (17 days versus 28 days). Hence, these results indicated that gemcitabine was less effective in controlling tumor growth when bacteria were present. In a similar study by Geller *et al.*, they filtered their fibroblast-conditioned medium and resistance to gemcitabine was lost, indicating the presence of something else which may mediate resistance [29]. They discovered the presence of *Mycoplasma* DNA in the human dermal fibroblasts they co-cultured with the cancer cell lines, with almost all of the bacteria present being *Mycoplasma hyorhinis*. Using a mouse model, they also determined that *M. hyorhinis* was able to infiltrate tumor cells and cause resistance to gemcitabine and can convert gemcitabine to 2'2-difluorodeoxyuridine by deamination. They tested 27 species of bacteria for gemcitabine resistance, finding 13 of these bacteria were able to stop gemcitabine from inhibiting the growth of RKO human colorectal carcinoma cells. Using human PDAC tissue samples, they were also able to detect the presence of bacterial rDNA (ribosomal DNA) and compared the number of bacteria found in PDAC tumors to those in a normal pancreas. 76% of PDAC samples contained bacteria compared to 15% in normal pancreas samples. The most commonly found bacterial species were Gammaproteobacteria.

One possible solution to this would be to co-administer antibiotics with cancer therapeutics. This was investigated by Geller *et al.* in which they utilised a colon carcinoma mouse model and determined that there was an improved efficacy of gemcitabine when used alongside antibiotics (Figure 3) [29]. However, over-use of antibiotics has caused several species of bacteria to become multi-drug resistant (MDR) and this resistance is transferable via the super-resistance gene NMD-1 [74]. One of the possible solutions for this is to administer a bactericidal agent that faces minimal to no resistance from bacteria which also have the ability to form the reactive oxygen species that are also known to have a bactericidal effect.

Silver nanoparticles

Colloidal silver is not new. It has been used in multiple sectors for many years. Medically, silver nanoparticles (AgNPs) have been impregnated into wound dressings and other topical treatments where antimicrobial action is required, as well as being used for surgical coatings, dietary supplements, cosmetics, textiles, food packaging, medical implants, and water sterilized applications [75]. In 2011, AgNPs accounted for 55.4% of the total nanomaterial-based products on the market [76].

With a few exceptions, borohydride-mediated reduction has been employed in the majority of syntheses of dispersible AgNPs reported [77]. Sodium borohydride ($NaBH_4$) is one of the strongest reducing agents for making small size nanoparticles, which facilitates rapid nuclei generation, resulting in the formation of monodispersed and uniform sized silver colloids [78]. However, $NaBH_4$ is not desirable for making large AgNPs where a slower reaction rate is required [79], hence the weaker reducing agent trisodium citrate (TSC) is used. It contributes to the creation of relatively large AgNPs, having a wider size distribution [80] and can also result in morphological variations of the colloids forming cubes, triangles, rods and spherical nanoparticles [81,82]. Another method of AgNP fabrication is via the co-reduction method. Here, the combination of two different reductants ($NaBH_4$ and TSC) is employed which results in a more tuneable reaction with a high control over the growth of nucleation and nanoparticle formation [83,84]. Using this method, it is possible to produce stabilized AgNPs over a wide size range (5–100 nm) [85].

The characterisation of these materials is relatively simple, with most techniques being both well-known and well used in a variety of sectors. Some of the most commonly used techniques include UV-vis, dynamic light scattering (DLS), scanning electron microscopy and transmission electron microscopy. UV-vis, DLS and both types of electron microscopy give insight into the size of the nanoparticles, with electron microscopy also giving insight into the shape of the nanoparticles and DLS being able to determine the surface charge and the aggregation of nanoparticles in the solution, known as the polydispersity index.

Generally, nanoparticles have several properties that make them useful in imaging, drug delivery, and creating cancer biomarker profiles of cancerous tumors [86]. AgNPs have several applications for cancer, they have been associated with anticancer properties and have been used against several cancer types including leukaemia [87], breast cancer [88], lung carcinoma [89], hepatocellular carcinoma [90] and glioblastoma [86]. However, there are only limited number of studies reported concerning the use of AgNPs in pancreatic cancer therapy. Here, we will discuss the progress made to date and possible avenues which could be explored based on the existing scientific literature. Table 2 summarised the use of AgNPs for cancer therapy and their stage within preclinical testing.

Exploiting inherent cytotoxicity

AgNPs themselves can be used as a potent anti-cancer agent when administered within a certain size range. Hence, there is potential that administration of AgNPs alone into pancreatic tumors may result in tumor reduction or retardation. A size dependent cytotoxicity is often experienced using AgNPs, where particles below 10 nm exhibit an increased cytotoxic profile compared to their larger counterparts. This phenomenon has been observed in a study on pancreatic cancer. Zielinska *et al.* reported the effects of AgNPs on both PANC-1 cancer cells and hTERT non-cancerous cell lines, using 2.6 and 18 nm AgNPs. They determined that there was a greater cytotoxic effect on the PANC-1 cells than the non-cancerous cells [91]. Additionally, they determined that the 2.6 nm AgNPs had a much higher cytotoxicity, around 16 times greater than that of the 18 nm AgNPs. The same study also compared using AgNPs to gemcitabine alone, determining that gemcitabine was unable to decrease the viability of the PANC-1 cells to the same extent as AgNPs. AgNPs were also found to prevent cell proliferation, induce apoptosis and necrosis, and cause some changes in the ultrastructure of PANC-1 cells to induce cell death (Figure 4).

There are different methods by which AgNPs act in an anti-cancer manner. Guo *et al.*

determined that AgNPs produce reactive oxygen species, reducing the viability of the cells, causing DNA damage and inducing apoptosis [87]. This confirmed results from Lim *et al.* and Arora *et al.* [92,93]. Lim, Gurung and Hande determined that AgNPs have antineoplastic activity, appearing to act in a similar manner to many current chemotherapeutics and have been seen to cause cytoskeletal deformities [86]. Another study completed by Sriram *et al*. looked into the effects of AgNPs against Dalton's lymphoma, using a xenografted mouse model [94]. They found that there was a significant decrease in tumor volume of 68.5% compared to the control (7.3 mL in the control versus 2.3 mL). They also confirmed that AgNPs administered via intraperitoneal injection were able to extend the lifespan of the mice to 32 days compared to the control (18 days).

One explanation for this size dependant cytotoxicity is related to the amount of reactive oxygen species generation which is greater with smaller nanoparticles which possess a higher surface to volume ratio. Barcińska *et al.* explored this concept utilising the same cell lines as in Zielinska *et al*., determining that the production of reactive oxygen species in the cancer cell lines was double that of the non-cancerous cell lines [95]. Their results in terms of the cytotoxic effects of AgNPs on PANC-1 cells confirm those presented by Zielinska *et al*. As well as this, they determined that there was a much higher concentration of nitric oxide present in the PANC-1 cells treated with 2.6 nm and 18 nm AgNPs. Both sizes of nanoparticles were able to cause an increase in the concentration of nitric oxide present in PANC-1 cells, compared to a control, with the 2.6 nm AgNPs causing the greatest increase. The same study also determined that there were significant effects on the cell cycle and changes in mitochondrial ultrastructure when utilising AgNPs.

Another approach reported is to co-administer the AgNPs with a chemotherapy to induce synergistic toxicity. Yuan, Peng and Gurunathan utilised AgNPs with gemcitabine to determine if combining the two increased the anti-cancer effects against ovarian cancer cells [96]. They initially tested AgNPs and gemcitabine individually and determined that a concentration of 25 nM of gemcitabine produced a cytotoxic effect and that there was an increase in cytotoxicity at 25 nm of AgNPs, both after a 24-hour incubation period. They determined that by combining AgNPs and

gemcitabine, there was a much more efficient decrease in cell viability compared to the two alone. Their results also showed that a lower concentration of AgNPs alongside gemcitabine was able to cause the death of cancer cells.

AgNPs have also been trialled alongside the plant-derived Berberine against human squamous carcinoma cells, in this case using the cell line SCC-25 [97]. Dziedzic *et al.* determined that there was a dose-dependent and time-dependent effect on these cells over a 24- and 48-hour period using AgNPs alone. These were also shown to cause increase the expression of pro-apoptotic Bax at a concentration of 10 $\mu g\ mL^{-1}$, as well as noting significant morphological changes between the control and treated cells. For the combination treatment, it was found that there was lower cytotoxicity than each component alone. There was mainly Bcl-2 gene expression, with the anti-apoptotic pathway activated. This would be useful for cells that were not cancerous, as they would not be as damaged by the combination treatment as they would the AgNPs alone.

DNA-dependent protein kinases have been used alongside AgNPs, modifying their anticancer ability against glioblastoma (U257) and breast cancer (MCF-7 and MDA-MB-231) cells [86]. Lim, Gurung and Hande found that after 48 hours, the shape of the cancerous cells had altered, compared to their control counterparts, much like other studies. Further, their report found that AgNPs were able to reduce cancerous cell proliferation, cause cell death, a reduction in the expression of c-Myc and caused DNA damage.

There are, as of yet, no clinical trials that have looked into the use of AgNPs as a potential treatment for pancreatic cancer, and the majority of the studies listed here are in very early stages. There is a clear future for these technologies with their success in these studies, but there is likely to be a significant delay before any come into fruition. However, one thing is certain, given their known cytotoxicity issues at elevated doses, any new potential therapies will need to be heavily scrutinised in terms of accumulation site, residence time, clearance and all associated risks which come with these in terms of breakdown and safety before clinical translation could be realised.

Exploiting inherent antimicrobial nature of silver nanoparticles

As greater understanding of the tumor microenvironment is elucidated, these environments are being increasingly implicated in the clinical failure of current chemotherapeutics. For instance, the recent study by Geller *et al.* published in *Science* described how colorectal tumors were becoming resistant to gemcitabine due to the presence of *Gammaproteobacteria* and subsequent expression of cytidine deaminase within the tumor site, which converts gemcitabine into an inactive form [29]. With this knowledge, it may be possible to improve the efficacy of existing therapies by addressing the bacteria residing in the microenvironment. One of the most well-known materials with antibacterial properties is silver. Silver itself has been used in therapies since ancient times, and is used in various applications, including films and dressings [98].

Silver ions inhibit bacteria using several mechanisms, including inhibition of respiratory chain enzymes, interfering with membrane permeability [78] and affecting DNA replication [100] (Figure 5). The way in which silver ions are distributed differs, with 60% entering the cell and 40% binding to the surface of the bacteria [98]. It has been suggested by Marini *et al.* that AgNPs have a similar mode of action to silver nitrate, releasing silver ions [100]. A study by Shrivastava *et al.* determined that the main pathway by which AgNPs exhibit antibacterial activity was by attaching to and then passing through the bacterium's cell wall [101].

AgNPs possess excellent antimicrobial properties as well as being able to affect other microbes, including protozoa and fungi [102,103]. Several studies have looked into the use of AgNPs on various viruses including hepatitis B [104], influenza virus [105] and HIV-1 [106, 107], all of which found that AgNPs had some effect. As well as these, there have been studies into the use of AgNPs against fungi. A study by Kim *et al.* looked into the effects of AgNPs on several different types of fungi that were pathogenic towards plants [108]. These included *A. alternata, B. cinereal* and *F. oxysporum*. They found that when AgNPs with a relatively low concentration were effective against the fungi tested, but these were only tested *in vitro.*

The physical properties of AgNPs have an impact on their antimicrobial activity. One of

these important properties is their size. Studies by both Martinéz-Castañón *et al.* and Morones *et al.* found that smaller AgNPs possess greater bactericidal activity [109,110].

There have been several studies that have examined and utilised the antibacterial properties of AgNPs. Lok *et al.* synthesised both reduced and oxidised AgNPs and tested them for their antimicrobial activity against *E. coli* [99]. They noted that oxidised AgNPs had better antibacterial properties, linked to their size and shape. Shrivastava *et al*. tested the antibacterial effects of AgNPs against *E. coli*, *S. aureus*, ampicillin-resistant *E. coli* and an MDR strain of *S. typhus* [101]. Their study determined that using increased concentrations of AgNPs enhanced their effect, with a concentration of 25 $\mu g\ mL^{-1}$ inhibiting the growth of all of the bacterial strains listed. Martinéz-Castañón *et al.* completed a study looking into how different sizes of AgNPs impacted their antimicrobial effect [109]. They generated AgNPs that were 7, 29 and 89 nm. The largest size of AgNPs also exhibited a slightly different shape, being less spherical than the two smaller counterparts. Overall, they determined that the smaller size of AgNPs presented with the greatest antibacterial effect against both *E. coli* and *S. aureus*.

As well as nanoparticles possessing their own antimicrobial activity, several studies, including those by Fayaz *et al.* and Shahverdi *et al.* have investigated the use of antibiotics alongside AgNPs [111], including attaching antibiotics to the surface of AgNPs [112]. Fayaz *et al*. tested biogenically synthesised AgNPs with antibiotics against four different species of bacteria, including two strains of gram-negative (*E. coli and S. typhi*) and two strains of gram-positive (*S. aureus* and *M. luteus*) bacteria. They discovered that the antimicrobial properties of all the antibiotics they utilised were increased when co-administered with AgNPs. Shahverdi *et al.* determined that the nanoparticles were more effective against *S. aureus* than against *E. coli*. Brown *et al.* used AgNPs to carry ampicillin, aiming to improve the efficacy of the drug [113]. The data showed that the AgNPs which were functionalised with ampicillin were effective against a broad-spectrum of bacteria (both Gram-negative and Gram-positive) and excitingly, they possessed the ability to subvert the antibiotic resistance mechanisms in multiple-drug-resistant bacteria.

There have also been studies looking into the combination of two different antimicrobial agents, one of which being AgNPs, and determining if this produces an increased or synergistic antibacterial effect. Chen *et al.* developed polymer colloids which were coated with AgNPs [114]. These were tested against *E. coli* and were found to completely inhibit its growth, similar to the results found in the study of Lok *et al.* [99]. They also determined that these effects were dependent on both the size and dose used, with the lower concentration range of 1 to 10 μg mL^{-1} having little effect [114].

Shankar, Wang and Rhim tested the release of silver ions from a variety of materials including AgNPs, within alginate-based composite films in order to allow for a steady release of silver over time [115]. They synthesised AgNPs using citrate as the reducing agent and also used laser ablated AgNPs and tested them against *E. coli* and *L. monocytogenes*. It was determined that the reduced AgNPs had potent antibacterial activity whereas the laser ablated AgNPs had little to no effect on bacteria. There was also a greater effect on the gram-negative *E. coli* than the gram-positive *L. monocytogenes*. Similar to the study by Shankar, Wang and Rhim, Acharya *et al*. developed AgNPs alongside alginate and gelatin hydrogel films [116]. They were tested against several bacterial strains, including *S. typhi*, *P. aeruginosa*, *E. coli* and *S. aureus* and found that the Gram-negative bacteria strains were more affected by the composites than the Gram-positive bacteria.

Kong and Jang synthesised AgNP-containing poly(methyl methacrylate) nanofibers for antibacterial purposes, in order to allow for a steady release of silver over time, alike the work reported by Shankar, Wang and Rhim [115,117]. They tested their nanofibers against *E. coli* and *S. aureus* and determined that the antibacterial activity of the nanofibers was superior to those of both silver nitrate and silver sulfadiazine alone.

Overall, the antibacterial properties of AgNPs have been well tested, with various papers using AgNPs alone and others using AgNPs alongside other materials in order to allow for controlled release. The majority of studies that utilised a different species of bacteria determined

that there was a difference in the efficacy of AgNPs on Gram-positive and Gram-negative bacteria, with Gram-negative bacteria generally being better affected. Therefore, we propose that this is one major area for consideration in improving drug efficacy for gemcitabine treatment. The AgNPs will selectively reduce bacterial presence, hence allowing the drug to carry out its well-established function. Additionally, the AgNPs may act as a drug carrier to bring the gemcitabine to its site of action. This concept will be further addressed in the drug delivery section below.

Drug Targeting and Delivery

The surface of AgNPs is well suited to modification both electrostatically and covalently. A net negative zeta potential renders cationic charge-charge van der Waals interactions as one possibility for attachment of drugs or other molecules of interest. Additionally, covalent conjugation of molecules onto the surface of AgNPs is easily achieved using dative covalent bonding with thiol or disulphide residues.

There is great debate within the nanomedicine community regarding the passive targeting ability of nano-carriers *via* enhanced permeability and retention (EPR) effect in pancreatic tumors. Multiple studies have shown increased drug efficacy after either conjugation onto the surface of, or encapsulation within the core of, nanoparticles [118]. However, this increase in efficacy may solely be down to the prolonged circulation times of these nanoparticles and not due to EPR effect given the highly dense stroma surrounding these problematic tumors. To compensate for this lack of clarity, the use of targeting moieties is routinely used in nanomedicine design for pancreatic cancer therapy [119]. These include the use of folic acid [120], peptides [121] and specific antibodies [122]. These moieties undergo active transport internalisation processes (Figure 6) which help to preferentially traffic the therapies into the cancerous cells which allow for deeper tumor penetration whilst evading healthy tissue and indeed macrophage clearance in the bloodstream.

A wealth of data has been generated using gold nanoparticles (AuNPs) for pancreatic cancer drug delivery [123-125]. Gold coated iron oxide have been shown to enhance activity of gemcitabine through controlling its release with laser initiated heat resulting in tumour reduction in

pancreatic xenografts compared with the free drug (Figure 7) [124]. It is postulated that these could be replicated using AgNPs with very similar mechanism of action, with the added benefit of antimicrobial activity. As long as any inherent toxicity is addressed in terms of appropriate particle size and surface modification with polymers or other biocompatible moieties, issues such as toxicity on administration and accumulation can be addressed. The use of AgNPs for targeted drug delivery has already been shown in other cancers. Folic acid conjugated AgNPs were fabricated and anticancer drug doxorubicin was electrostatically attached onto their surface [126]. The study showed that drug release occurred into the cytoplasm after 4 hours with significant cell death observed after 8 hours.

Another study reported that galactomannan conjugated AgNPs possess enhanced anticancer action in adenocarcinoma alveolar basal (A549), colorectal carcinoma (HCT116) and hepatocellular carcinoma (HepG2) cancer cell lines, compared against a control of mouse fibroblast cell lines (3T3-L1), displaying a lower level of toxicity towards normal cells [127].

It is envisaged that data reported to date in these other cancer types will spark the interest of pancreatic cancer researchers and subsequent studies will commence in this area.

Thermal therapy

Tumor ablation has been tested for use against several different types of cancer, including prostate [128] kidney, bone and lung cancers [129]. There are several types of ablation that have been tested for use as therapeutics, including radiofrequency ablation [130], cryoablation [131] and focal laser ablation [132]. Like AuNPs, AgNPs possess a surface plasmon resonance wavelength at which irradiated light becomes scattered and absorbed. The absorption rapidly transfers light into thermal energy allowing the nanoparticles to act as localised heaters which can be used for both ablation and heat mediated drug release. Although the absorption is reduced in AgNPs compared with AuNPs, theory suggests that the heating potential of AgNPs should be sufficient to generate thermal increases required for irreversible cell damage. As of yet no studies have been reported in

the pancreatic field, however, with all the benefits of using AgNPs outlined above, it is postulated that this will be a further area of research interest with a high likelihood of success. Additionally, these platforms may be tailored, as AuNPs have been in order to act as a triggered stage for drug release using light and/or thermal energy [133].

Radiation enhancers

Radiation therapy is common in patients with pancreatic cancer. AuNPs have already been shown to work as radiation sensitizers in breast cancer therapy [134] [135], hence this application may be another opportunity to harness the power of AgNPs. Swanner *et al.* reported that AgNPs were selectively cytotoxic towards triple negative cells at doses which had minimal effect on non-tumor cells [136]. Here the study confirmed that AgNPs induced more DNA and oxidative damage in the cancerous cells which is responsible for their ultimate mortality. They used these AgNPs in combination with infrared radiation at doses of 0–4 Gy (dose rate of 2.39 Gy/min) both *in vitro* and *in vivo*. Excitingly, the combination therapy resulted in a dose-enhancement effect in the triple negative breast cancer and was found to be nontoxic to healthy tissue. Similarly, Lu *et al.* demonstrated that protein coated AgNPs enhanced X-ray radiation dosages to a different strain of breast cancer cells (231) *in vitro* compared to the radiation alone [137]. Liu *et al.* found that AgNPs actually outperformed AuNPs in their radiosensitizing properties in U251 cells and in an intracranial mouse glioma model [138]. This exciting finding gives much promise to the future of AgNPs within this arena. Currently, the use of AuNPs for cancer therapy is a huge area of investigation across multiple cancer types. Liu postulated that the increased enhancement ability possessed by the AgNPs compared to that of the AuNPs was due to the increased pro-apoptotic effects. Another example of how the many attributes of silver, if harnessed correctly, may lead to an upsurge in scientific investigation over the coming decade, particularly for difficult diseases such as pancreatic cancer.

Conclusion

The number of articles on silver nanoparticles in pancreatic cancer therapy is growing. The recent review chapter written by Skonieczna and Hudy shows the increasing popularity of silver nanoparticles for their various properties, including antimicrobial and anticancer properties [139]. Increased interest in these systems has been realised due to their unique nanoscale properties. The combination of ease of manufacture, tailoring ability, relative cost, antimicrobial properties tuneable optical and toxicological profiles renders these particles interesting platforms for pancreatic cancer therapy. Since these nanoparticles are currently at the early stages of testing and fabrication, there would need to be careful regulation of AgNPs before administering any of the listed treatments to patients, especially due to the fact that there is evidence of AgNPs causing DNA damage to normal human cell lines [140]. Possible "interfering factors" include the shape, size, stability, pharmacokinetics and accumulation of these nanoparticles. As well as this, there would need to be an investigation into any possible effects that this could have on the environment, as silver is known to be toxic to aquatic life.

As more investment in this under-represented cancer is realised, so too will the full potential of colloidal silver in its treatment. They say that every cloud has a silver lining, in this case we hope that the silver lining of tumors will lead to increased patient survival and long-term quality of life.

Corresponding Author

*Clare Hoskins, Tel: +441782 734799; email: c.hoskins@keele.ac.uk

Author Contributions

RF, MA and CH wrote the manuscript. AC provided the figures. CH supervised and approved the content. All authors have given approval to the final version of the manuscript.

References

1. F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, A. Jemal, Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, *CA Cancer J. Clin*., 2018, **0**, 1-31
2. R. L. Siegel, K. D. Miller and A. Jemal, Cancer statistics, 2018. *CA Cancer J. Clin*., 2018, **68**, 7-30
3. J. Kleef, M. Korc, M. Apte, C. La Vecchia, C. D. Johnson, A. V. Biankin, R. E. Neale, M. Tempero, D. A. Tuveson, R. H. Hruban and J. P. Neoptolemos., Pancreatic Cancer., *Nat. Rev. Dis. Primers*, 2016, **2**, 16022
4. L. Zou, R. Zhong, N. Shen, W. Chen, B, Zhu, J. Ke, X. Lu, T. Zhang, J. Lou, Z. Wang, L. Liu, L. Qi and X. Miao, Non-Linear Dose–Response Relationship Between Cigarette Smoking and Pancreatic Cancer Risk: Evidence from a Meta-Analysis of 42 Observational Studies *Eur. J. Cancer*, 2014**, 50,** 193–203
5. S. C. Larsson, N. Orsini and A. Wolk, Body Mass Index and Pancreatic Cancer Risk: A Meta-Analysis of Prospective Studies. *Int. J. Cancer*, 2007, **120**, 1993–98
6. S. Chand, K O'Hayer, F. F. Blanco, J. M. Winter and J. R. Brody, The Landscape of Pancreatic Cancer Therapeutic Mechanisms, *Int. J. Biol. Sci.*, 2016, **12**, 237-282
7. I. Tramacere, L. Scotti, M. Jenab, V. Bagnardi, R. Bellocco, M. Rota, G. Corrao, F. Bravi, P. Boffetta, and C. La Vecchia. Alcohol Drinking and Pancreatic Cancer Risk: A Meta-Analysis of the Dose-Risk Relation. *Int. J. Cancer*, 2010, **126**, 1474–86.
8. D. S. Michaud, A. Vrieling, L. Jiao, J. B. Mendelsohn, E. Steplowski, S. M. Lynch, J. Wactawski-Wende, A. A. Arslan, H. B. Bueno-de-Mesquita, C. S. Fuchs, M. Gross, K. Helzlsouer, E. J. Jacobs, A. LaCroix, G. Petersen, W. Zheng, N. Allen, L. Ammundadottir, M. M. Bergmann, P. Boffetta, J. E. Buring, F. Canzian, S. J. Chanock, F. Clavel-Chapelon, S. Clipp, M. S. Freiberg, J. M. Gaziano, E. L. Giovannucci, S. Hankinson, P. Hartge, R. N. Hoover, F. A. Hubbell, D. J. Hunter, A Hutchinson, K. Jacobs, C. Kooperberg, P. Kraft, J. Manjer, C. Navarro, P. H. M. Peeters, X. Shu, V. Stevens, G. Thomas, A. Tjønneland, G. S.

Tobias, D. Trichopoulos, R. Tumino, P. Vineis, J. Virtamo, R. Wallace, B. M. Wolpin, K. Yu, A. Zeleniuch-Jacquotte, and R. Z. Stolzenberg-Solomon, Alcohol Intake and Pancreatic Cancer: A Pooled Analysis from the Pancreatic Cancer Cohort Consortium (PanScan), *Cancer Causes Control*, 2010, **21**, 1213-–25.

9. L. Jiao, A. Berrington de Gonzalez, P. Hartge, R. M. Pfeiffer, Y. Park, D. M. Freedman, M. H. Gail, M. C. R. Alavanja, D. Albanes, L. E. Beane Freeman, W. Chow, W. Huang, R. B. Hayes, J. A. Hoppin, B. Ji, M. F. Leitzmann, M. S. Linet, C. L. Meinhold, C. Schairer, A. Schatzkin, J. Virtamo, S. J. Weinstein, W. Zheng, and R. Z. Stolzenberg-Solomon. Body Mass Index, Effect Modifiers, and Risk of Pancreatic Cancer: A Pooled Study of Seven Prospective Cohorts, *Cancer Causes Control*, 2010, **21,** 1305–1314
10. Q. Ben, M. Xu, X. Ning. J. Liu, S. Hong. W. Huang, H. Zhang, and Z. Li, Diabetes Mellitus and Risk of Pancreatic Cancer: A meta-analysis of cohort studies. Eur J Cancer, 2011, **47**, 1928–37.
11. J. Starup-Linde, Ø. Karlstad, S. Aistrup Eriksen, P. Vestergaard, H. K. Bronsveld, F. de Vries, M. Andersen, A. Auvinen, J. Haukka, V. Hjellvik, M. T. Bazelier, A. de Boer, K. Furu, and M. L. De Bruin, CARING (CAncer Risk and INsulin analoGues): The Association of Diabetes Mellitus and Cancer Risk with Focus on Possible Determinants - A Systematic Review and a Meta-Analysis. Curr. Drug Saf., 2013, **8**, 296–332.
12. J. Permuth-Wey and K. M. Egan, Family History is a Significant Risk Factor for Pancreatic Cancer: Results from a Systematic Review and Meta-Analysis. Fam. Cancer, 2009, **8**, 109–117
13. E. J. Jacobs S. J Chanock, C. S. Fuchs, A. Lacroix, R. R. McWilliams, E. Steplowski, R. Z. Stolzenberg-Solomon, A. A. Arslan, H. B. Bueno-de-Mesquita, M. Gross, K. Helzlsouer, G. Petersen, W. Zheng, I. Agalliu, N. E. Allen, L. Amundadottir, M. C. Boutron-Ruault, J. E. Buring, F. Canzian, S. Clipp, M. Dorronsoro, J. M. Gaziano, E. L. Giovannucci, S. E. Hankinson, P. Hartge, R. N. Hoover, D. J. Hunter, K. B. Jacobs, M. Jenab, P. Kraft, C. Kooperberg, S. M. Lynch, M. Sund, J. B. Mendelsohn, T. Mouw, C. C. Newton, K. Overvad, D. Palli, P. H. Peeters, A. Rajkovic, X. O. Shu, G. Thomas, G. S. Tobias, D. Trichopoulos, J.

Virtamo, J. Wactawski-Wende, B. M. Wolpin, K. Yu, and A. Zeleniuch-Jacquotte. Family History of Cancer and Risk of Pancreatic Cancer: A Pooled Analysis from the Pancreatic Cancer Cohort Consortium (PanScan). *Int. J. Cancer*, 2010, **127**, 1421–1428

14. S. Yachida, S. Jones, I. Bozic, T. Antal, R. Leary, B. Fu, M. Kamiyama, R. H. Hruban, J. R. Eshleman, M. A. Nowak, V. E. Velculescu, K. W. Kinzler, B. Vogelstein & C. A. Iacobuzio-Donahue, Distant Metastasis Occurs Late During the Genetic Evolution of Pancreatic Cancer, *Nature*, 2010, **467**, 1114-1119
15. R. A. Burrell, N. McGranahan, J. Bartek and C. Swanton, The Causes and Consequences of Genetic Heterogeneity in Cancer Evolution, *Nature*, 2013, **501**, 338-345
16. E. S. Lee and J. M. Lee, Imaging Diagnosis of Pancreatic Cancer: a state-of-the-art review. *World J. Gastroenterol*., 2014, **20**, 7864-7877
17. M. Chiaravalli, M. Reni and E. M. O'Reilly, Pancreatic Ductal Adenocarcinoma: State-of-the-Art 2017 and New Therapeutic Strategies, *Cancer Treatment Reviews*, 2017, **60**, 32-43
18. M. T Saung and L. Zhang, Current Standards of Chemotherapy for Pancreatic Cancer, *Clin. Therap.*, 2017, **39**, 2125-2134
19. M. Hidalgo, S. Cascinu, J. Kleef, R. Labianca, J. M. Löhr, J. Neoptolemos, F. X. real, J. L. Van Laethem, and V. Heinemann. Addressing the Challenges of Pancreatic Cancer: Future Directions for Improving Outcomes. *Pancreatology*, 2015, **15**, 8-18
20. L. Rahib, B. D. Smith, R. Aizenberg, A.B. Rosenzweig, J. M. Fleshman, and L. M. Matrisian. Projecting Cancer Incidence and Deaths to 2030: The Unexpected Burden of Thyroid, Liver, and Pancreas Cancers in the United States. *Cancer Res.*, 2014, **74**, 2913-2921
21. H. Matthaei, R. D. Schulick, R. H. Hruban and A. Maitra, Cystic Precursors to Invasive Pancreatic Cancer. *Nat. Rev. Gastroenterol. Hepatol.*, 2011, **8**, 141-150
22. J. Wu, Y. Jiao, M. Dal Molin, A. Maitra, R. F. de Wilde, L. D. Wood, J. R. Eshleman, M. G. Goggins, C. L. Wolfgang, M. I. Canto, R. D. Schulick, B. H. Edil, M. A. Choti, V. Adsay, D. S. Klimstra, G. Johan A. Offerhaus, A. P. Klein, L. Kopelovich, H. Carter, R. Karchin, P. J. Allen,

C. M. Schmidt, Y. Naito, L. A. Diaz Jr., K. W. Kinzler, N. Papadopoulos, R. H. Hruban, and B. Vogelstein. Whole-Exome Sequencing of Neoplastic Cysts of the Pancreas Reveals Recurrent Mutations in Components of Ubiquitin-Dependent Pathways. *Proc. Natl. Acad. Sci. USA*, 2011, **108**, 21188-21193

23. A. M. Macgregor-Das and C. A. Iacobuzio-Donahue, Molecular Pathways in Pancreatic Carcinogenesis. *J. Surg. Oncol.*, 2013, **107**, 8-14
24. R. H. Hruban, N. Volkan Adsay, J. Albores–Saavedra, C. Compton, E. S. Garrett, S. N. Goodman, S. E. Kern, D. S. Klimstra, G. Klöppel, D. S. Longnecker, J. Lüttges, and G. Johan A. Offerhaus, Pancreatic Intraepithelial Neoplasia: A New Nomenclature and Classification System for Pancreatic Duct Lesion. *Am. J. Surg. Pathol.*, 2001, **25**, 579-586
25. D. X. Nguyen and J. Massagué, Genetic Determinants of Cancer Metastasis, *Nat. Rev. Genet.*, 2007, **8**, 341-352
26. H. Ying, P. Dey, W. Yao, A. C. Kimmelman, G. F. Draetta, A. Maitra, and R. A. DePinho. Genetics and Biology of Pancreatic Ductal Adenocarcinoma. *Genes Dev.*, 2016, **15**, 355-385
27. I. Garrido-Laguna and M. Hidalgo, Pancreatic Cancer: From State-of-the-Art Treatments to Promising Novel Therapies. *Nat. Rev. Clin. Oncol.*, 2015, **12**, 319-34
28. H. A Burris 3rd, M J. Moore, J. Andersen, M. R. Green, M. L. Rothenberg, M. R. Modiano M. C. Cripps, R. K. Portenoy, A. M. Storniolo, P. Tarassoff, R. Nelson, F. A. Dorr, C. D. Stephens, and D. D. Von Hoff, Improvements in Survival and Clinical Benefit with Gemcitabine as First-Line Therapy for Patients with Advanced Pancreas Cancer: A Randomized Trial., *J. Clin. Oncol.*, 1997, **15**, 2403-2413
29. L. T. Geller, M. Barzily-Rokni, T. Danino, O. H. Jonas, N. Shental, D. Nejman, N. Gavert, Y. Zwang, Z. A. Cooper, K. Shee, C. A. Thaiss, A. Reuben, J. Livny, R. Avraham, D. T. Frederick, M. Ligorio, K. Chatman, S. E. Johnston, C. M. Mosher, A. Brandis, G. Fuks, C. Gurbatri, V. Gopalakrishnan, M. Kim, M. W. Hurd, M. Katz, J. Fleming, A. Maitra, D. A. Smith, M. Skalak, J. Bu, M. Michaud, S. A. Trauger, I. Barshack, T. Golan, J. Sandbank, K. T. Flaherty, A.

Mandinova, W. S. Garrett, S. P. Thayer, C. R. Ferrone, C. Huttenhower, S. N. Bhatia, D. Gevers, J. A. Wargo, T. R. Golub, and R. Straussman. Potential Role of Intratumor Bacteria in Mediating Tumor Resistance to the Chemotherapeutic Drug Gemcitabine. *Science*, 2017, **357**, 1156-1160

30. M. Reni, S. Cordio, C. Milandri, P. Passoni, E. Bonetto, C. Oliani, G. Luppi, R. Nicoletti, L. Galli, R. Bordonaro, A. Passardi, A. Zerbi, G. Balzano, L. Aldrighetti, Prof. C. Staudacher, E. Villa and Prof V. Di Carlo. Gemcitabine versus Cisplatin, Epirubicin, Fluorouracil, and Gemcitabine in Advanced Pancreatic Cancer: A Randomised Controlled Multicentre Phase III Trial *Lancet Oncol.*, 2005, **6**, 369-376
31. D. Cunningham, I. Chau, D. D. Stocken, J. W. Valle, D. Smith, W. Steward, P. G. Harper, J. Dunn, C. Tudur-Smith, J. West, S. Falk, A. Crellin, F. Adab, J. Thompson, P. Leonard, J. Ostrowski, M. Eatock, W. Scheithauer, R. Herrmann, J. P. Neoptolemos. Phase III Randomized Comparison of Gemcitabine Versus Gemcitabine Plus Capecitabine in Patients With Advanced Pancreatic Cancer. *J. Clin. Oncol*., 2009, **27**, 5513–5518
32. S. Gourgou-Bourgade, C. Bascoul-Mollevi, F. Desseigne, M. Ychou, O. Bouché, R. Guimbaud, Y. Bécouarn, A. Adenis, J. L. Raoul, V. Boige, J. Bérille and T. Conroy. Impact of FOLFIRINOX Compared with Gemcitabine on Quality of Life in Patients with Metastatic Pancreatic Cancer: Results from the PRODIGE 4/ACCORD 11 Randomized Trial. *J. Clin. Oncol.*, 2013, **31**, 23-29
33. T. Conroy, F. Desseigne, M. Ychou, and O. Bouché, FOLFIRINOX versus Gemcitabine for Metastatic Pancreatic Cancer. *N. Engl. J. Med.*, 2011, **364**, 1817-1825
34. D. D. von Hoff, T. Ervin, F. P. Arena, E. G. Chiorean, J. Infante, M. Moore, T. Seay, S. A. Tjulandin, M. Hidalgo, D. Goldstein, E. Van Cutsem, X. Wei, J. Iglesias and M. F. Renschler, Increased Survival in Pancreatic Cancer with nab-Paclitaxel plus Gemcitabine. *N. Engl. J. Med.*, 2013, **369**, 1691-1703

35. M. Reni, S. Zanon, G. Balzano, P. Passoni, C. Pircher, M. Chiaravalli, C. Fugazza, D. Ceraulo, R. Nicoletti, P. G. Arcidiacono, M. Macchini, U. Peretti, R. Castoldi, C. Doglioni, M. Falconi, S. Partelli and L. Gianni, A Randomised Phase 2 Trial of *nab*-Paclitaxel plus Gemcitabine with or without Capecitabine and Cisplatin in Locally Advanced or Borderline Resectable Pancreatic Adenocarcinoma, *Eur. J. Cancer*, 2018, **102**, 95-102

36. A. Stathis and M. J. Moore, Advanced Pancreatic Carcinoma: Current Treatment and Future Challenges. *Nat. Rev. Clin. Oncol.*, 2010, **7**, 163-72

37. T. Conroy, J. B. Bachet, A. Auav, F. Huguet, A. Lambert, C. Caramella, R. Maréchal, J. L. Van Laethem and M. Ducreux, Current Standards and New Innovative Approaches for Treatment of Pancreatic Cancer. *Eur. J. Cancer*, 2016, **57**, 10-22M

38. Ducreux, E. Mitry, M. Ould-Kaci, V. Boige, J. F. Seitz, R. Bugat, J. L. Breau, O. Bouché, P. L. Etienne, J. M. Tigaud, F. Morvan, E. Cvitkovic and P. Rougier. Randomized phase II study Evaluating Oxaliplatin alone, Oxaliplatin combined with Infusional 5-FU, and Infusional 5-FU alone in Advanced Pancreatic Carcinoma Patients. *Ann Oncol*., 2004, **15**, 467-73

39. J. P. Neoptolemos, D. D. Stocken, H. Friess, C. Bassi, J. A. Dunn, H. Hickey, H. Berger, L. Fernandez-Cruz, C. Dervenis, F. Lacaine, M. Falconi, P. Pederzoli, A. Pap, D. Spooner, D. J. Kerr and M. W. Büchler, A Randomized Trial of Chemoradiotherapy and Chemotherapy after Resection of Pancreatic Cancer. *N. Engl. J. Med.*, 2004, **350**, 1200e10

40. H.Y. Tanaka, M.R. Kano, Stromal Barriers to Nanomedicine Penetration in the Pancreatic Tumor Microenvironment, *Cancer Sci*., 2018, **109**, 2085-2092

41. F. Klemm and J. A. Joyce, Microenvironmental Regulation of Therapeutic Response in Cancer. *Trends Cell Biol*., 2015, **25**(4), 198–213S. L. Topalian *et al*., *N. Engl. J. Med*., 2012, **366**, 2443–2454

42. S. L. Topalian, F. S Hodi, J. R. Brahmer, S. N. Gettinger, D. C. Smith, D. F. McDermott, J. D. Powderly, R. D. Carvajal, J. A. Sosman, M. B. Atkins, P. D. Leming, D. R. Spigel, S. J. Antonia, L. Horn, C. G. Drake, D. M. Pardoll, L.Chen, W. H. Sharfman, R. A. Anders, J. M.

Taube, T. L. McMiller, H. Xu, A. J. Korman, M. Jure-Kunkel, S. Agrawal, D. McDonald, G. D. Kollia, A. Gupta, J. M. Wigginton, and M. Sznol, Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer, *N. Engl. J. Med*., 2012, **366**, 2443–2454

43. R. Straussman, T. Morikawa, K. Shee, M. Barzily-Rokni, Z. Rong Qian, J. Du, A. Davis, M. M. Mongare, J. Gould, D. T. Frederick, Z. A. Cooper, P. B. Chapman, D. B. Solit, A. Ribas, R. S. Lo, K. T. Flaherty, S. Ogino, J. A. Wargo & T. R. Golub, Tumor Micro-Environment Elicits Innate Resistance to RAF Inhibitors through HGF Secretion. *Nature*., 2012, **487**, 500–504
44. C. Li, D. G. Heidt, P. Dalerba, C. F. Burant, L. Zhang, V. Adsay, M. Wicha, M. F. Clarke and D. M. Simeone, Identification of Pancreatic Cancer Stem Cells, *Cancer Res.*, 2007, **67**, 1030-1037
45. P. C. Hermann, S. L. Huber, T. Herrier, A. Aicher, J. W. Ellwart, M. Guba, C. J. Bruns, and C. Heeschen, Distinct Populations of Cancer Stem Cells Determine Tumor Growth and Metastatic Activity in Human Pancreatic Cancer, *Cell Stem Cell*, 2007, **1**, 313-323
46. R. F. Hwang, T. Moore, T. Arumugam, V. Ramahandran, K. D. Amos, A. Rivera, B. Ji, D. B. Evans, and C. D. Logsdon, Cancer-Associated Stromal Fibroblasts Promote Pancreatic Tumor Progression, *Cancer Res.*, 2008, **68**, 918-926
47. A. Mantovani and A. Sica, Macrophages, Innate Immunity and Cancer: Balance, Tolerance, and Diversity, *Curr. Op. Immunol.*, 2010, **22**, 231-237
48. A. Mantovani, P. Allavena, A. Sica and F. Balkwill, Cancer-Related Inflammation. *Nature*, 2008, **454**, 436-444
49. J. R. Sierra, S. Corso, L. Caione, V. Cepero, P. Conrotto, A. Cignetti, W. Piacibello, A. Kumanogoh, H. Kitutani, P. M. Comoglio, L. Tamagnone and S. Giordano, Tumor Angiogenesis and Progression are Enhanced by Sema4D Produced by Tumor-Associated Macrophages, *J Exp Med*, 2008, **205**, 1673-1685
50. S. Loges, T. Schmidt, M. Tjwa, K. van Geyte, D. Lievens, E. Lutgens, D. Vanhoutte, D. Borgel, S. Paisance, M. Hoylaerts, A. Luttun, M. Dewerchin, B. Jonckx and P. Carmeliet, Malignant

Cells Fuel Tumor Growth by Educating Infiltrating Leukocytes to Produce the Mitogen Gas6. *Blood*, 2010, **115**, 2264-2273

51. T. Hagemann, J. Wilson, F. Burke, H. Kulbe, N. F. Li, A. Plüddemann, K. Charles, S. Gordon and F. R. Balkwill, Ovarian Cancer Cells Polarize Macrophages Toward a Tumor-Associated Phenotype, *J. Immunol*., 2006, **176**, 5023-5032
52. S. Kim, H. Takahashi, W. W. Lin, P. Descargues, S. Grivennikov, Y. Kim, J. L. Luo and M. Karin, Carcinoma-Produced Factors Activate Myeloid Cells through TLR2 to Stimulate Metastasis, *Nature*, 2009, **457**, 102-106
53. Z. Xu, A. Vonlaufen, P. A. Phillips, E. Fiala-Beer, X. Zhang, L. Yang, A. V. Biankin, D. Goldstein, R. C. Pirola, J. S. Wilson and M. V. Apte, Role of Pancreatic Stellate Cells in Pancreatic Cancer Metastasis, *American J. of Pathol*., 2010, **177**, 2585-2596
54. N. Erez, M. Truitt, P. Olson, S. T. Arron and D. Hanahan, Cancer-Associated Fibroblasts Are Activated in Incipient Neoplasia to Orchestrate Tumor-Promoting Inflammation in an NF-kappaB-Dependent Manner, *Cancer Cell*, 2010, **17**, 135-147
55. K. E. de Visser, L. V. Korets and L. M. Coussens, De Novo Carcinogenesis Promoted by Chronic Inflammation is B Lymphocyte Dependent, *Cancer Cell*, 2005, **7**, 411–423
56. R. V. Iozzo, Series Introduction: Heparan Sulfate Proteoglycans: Intricate Molecules with Intriguing Functions, *J. Clin. Invest*., 2001, **108**, 165-167
57. R. V. Iozzo, Basement Membrane Proteoglycans: From Cellar to Ceiling, *Nature Rev. Mol. Cell Biol*., 2005, **6**, 646-656
58. R. V. Iozzo, J. J. Zoeller and A. Nyström, Basement Membrane Proteoglycans: Modulators Par Excellence of Cancer Growth and Angiogenesis, *Mol. Cells*, 2009, **27**, 503-513
59. R. D. Sanderson and Y. Yang, Syndecan-1: A Dynamic Regulator of the Myeloma Microenvironment, *Clin. Exp. Metastasis*, 2008, **25**, 149-159
60. J. Filmus and S. B. Selleck, Glypicans: Proteoglycans with a Surprise, *J. Clin. Invest*., 2001, **108**, 497-501

61. R. V. Iozzo and L. Schaefer, Proteoglycans in Health and Disease: Novel Regulatory Signaling Mechanisms Evoked by the Small Leucine-Rich Proteoglycans, *FEBS J.*, 2010, **277**, 3864-3875

62. A. K. Witkiewicz, E. A. McMillan, U. Balaji, G. Baek, W. C. Lin, J. Mansour, M. Mollaee, K. U. Wagner, P. Koduru, A. Yopp, M. A. Choti, C. J. Yeo, P. McCue, M. A. White & E. S. Knudsen, Whole-Exome Sequencing of Pancreatic Cancer Defines Genetic Diversity and Therapeutic Targets, *Nat Commun*., 2015, **6**, 6744(1-11)

63. P. J. Grippo and E. P. Sandgren, Acinar-to-Ductal Metaplasia Accompanies c-myc-Induced Exocrine Pancreatic Cancer Progression in Transgenic Rodents, *Int J Cancer*, 2012, **131**, 1243-1248

64. W. C. Lin, N. Rajbhandari, C. Liu, K. Sakamoto, Q. Zhang, A. A. Triplet, S. K. Batra, R. Opavsky, D. W. Felsher, D. J. DiMaio, M. A. Hollingsworth, J. P. Morris, M. Hebrok, A. K. Witkiewicz, J. R. Brody, H. Rui and K. U. Wagner, Dormant Cancer Cells Contribute to Residual Disease in a Model of Reversible Pancreatic Cancer, *Cancer Res.*, 2013, **73**, 1821-1830

65. P. K. Mazur, H. Einwächtera, M. Leea, B. Siposb, H. Nakhaic, R. Radd, U. Zimber-Stroble, L. J. Stroble, F. Radtkef, G. Klöppelg, R. M. Schmida, and J. T. Sivekea, Notch2 is Required for Progression of Pancreatic Intraepithelial Neoplasia and Development of Pancreatic Ductal Adenocarcinoma, *Proc. Natl. Acad. Sci. USA*., 2010, **107**, 13438–13443.

66. M. Erkan, S. Hausmann, C. W. Michalski, A. A. Fingerle, M. Dobritz, J. Kleeff, and H. Friess, The Role of Stroma in Pancreatic Cancer: Diagnostic and Therapeutic Implications, *Nat Rev. Gastroenterol. Hepatol.*, 2012, **9**, 454-467

67. M. V. Apte, S. Park, P. A. Phillips, N. Santucci, D. Goldstein, R. K. Kumar, G. A. Ramm, M. Buchler, H. Friess, J. A. McCarroll, G. Keogh, N. Merrett, R. Pirola and J. S. Wilson, Desmoplastic Reaction in Pancreatic Cancer: Role of Pancreatic Stellate Cells, *Pancreas*, 2004, **29**, 179–187

68. M. V. Apte, P. S. Haber, T. L. Applegate, I. D. Norton, G. W. McCaughan, M. A. Korsten, R. C. Pirola, and J. S. Wilson, Periacinar Stellate Shaped Cells in Rat Pancreas: Identification, Isolation, and Culture, *Gut*, 1998, **43**, 128–133

69. M. Erkan, C. W. Michalski, S. Reider, C. Reiser-Erkan, I. Abiatari, A. Kolb, N. A. Giese, I. Esposito, H. Friess, and J. Kleef, The Activated Stroma Index is a Novel and Independent Prognostic Marker in Pancreatic Ductal Adenocarcinoma, *Clin. Gastroenterol. Hepatol.*, 2008, **6**, 1155-1161C.

70. C. Zu and J. Wang, Tumor-Colonizing Bacteria: A Potential Tumor Targeting Therapy, *Crit. Rev. Microbiol.*, 2014, **40**, 225-235

71. M. W. Dewhirst, Y. Cao and B. Moeller, Cycling Hypoxia and Free Radicals Regulate Angiogenesis and Radiotherapy Response, *Nat. Rev. Cancer*, 2008, **8**, 425-437

72. A. T. St Jean, M. Zhang, N. S. Forbes, Bacterial Therapies: Completing the Cancer Treatment Toolbox, *Curr. Opin. Biotechnol.*, 2008, **19**, 511-517

73. P. Lehouritis, J. Cummins, M. Stanton, C.T. Murphy, F.O. McCarthy, G. Reid, C. Urbaniak, W.L. Byrne, M. Tangney, Local Bacteria Affect the Efficacy of Chemotherapeutic Drugs. *Sci. Rep.*, 2015, **5**, 14554

74. P. R. Hsueh, New Delhi Metallo-b-lactamase-1 (NDM-1): An Emerging Threat Among Enterobacteriaceae, *J. Formos. Med. Assoc*., 2010, **109**, 685–687

75. D. R. Monteiro, L. F. Gorup, A. S. Takamia, A. C. Ruvollo- Filho, E. R. De Camargo and D. B. Barbosa, The Growing Importance of Materials that Prevent Microbial Adhesion: Antimicrobial Effect of Medical Devices Containing Silver. *Int. J. Antimicrob. Agents*, 2009, **34**, 103–110.

76. Woodrow Wilson Database, An Inventory of Nanotechnology Based Consumer Products Currently on the Market, 2011, http://www.nanotechproject.org/inventories/consumer/ analysis _draft/.

77. X. Z. Lin, X. Teng and H. Yang, Direct Synthesis of Narrowly Dispersed Silver Nanoparticles Using a Single-Source Precursor, *Langmuir*, 2003, **19**, 10081–10085.

78. Y. Tan, X. Dai, Y. Li and D. Zhu, Preparation of Gold, Platinum, Palladium and Silver Nanoparticles by the Reduction of their Salts with a Weak Reductant–Potassium Bitartrate. *J. Mater. Chem.*, 2003, **13**, 1069–1075.

79. J. Yang, H. Yin, J. Jia and Y. Wei, Controlled Particle Growth of Silver Sols through the Use of Hydroquinone as a Selective Reducing Agent. *Langmuir*, 2011, **27**, 5047– 5053.

80. J. A. Creighton, C. G. Blatchford and M. G. Albrecht, Plasma Resonance Enhancement of Raman Scattering by Pyridine Adsorbed on Silver or Gold Sol Particles of Size Comparable to the Excitation Wavelength. *J. Chem. Soc., Faraday Trans. 2*, 1979, **75**, 790–798.

81. A. Pyatenko, M. Yamaguchi and M. Suzuki, Synthesis of Spherical Silver Nanoparticles with Controllable Sizes in Aqueous Solutions. *J. Phys. Chem. C*, 2007, **111**, 7910–7917.

82. V. K. Sharma, R. A. Yngard and Y. Lin, Silver Nanoparticles: Green Synthesis and their Antimicrobial Activities. *Adv. Colloid Interface Sci.*, 2009, **145**, 83–96.

83. J. Thiel, L. Pakstis, S. Buzby, M. Raffi, C. Ni, D. J. Pochan and S. I. Shah, Antibacterial Properties of Silver-Doped Titania. *Small*, 2007, **3**, 799–803.

84. R. R. Arvizo, S. Bhattacharyya, R. A. Kudgus, K. Giri, R. Bhattacharya and P. Mukherjee, Intrinsic Therapeutic Applications of Noble Metal Nanoparticles: Past, Present and Future. *Chem. Soc. Rev.*, 2012, **41**, 2943–2970.

85. S. Agnihotri, S. Mukherji and S. Mukherji, Size-Controlled Silver Nanoparticles Synthesized over the range 5–100 nm using the Same Protocol and Their Antibacterial Efficacy. *RSC Advances*, 2013, **4**, 3974-3983.

86. H. K. Lim, R. L. Gurung and M. P. Hande, DNA-Dependent Protein Kinase Modulates the Anti-Cancer Properties of Silver Nanoparticles in Human Cancer Cells, *Mutat. Res. Genet. Toxicol. Environ. Mutagen.*, 2017, **824**, 32-41

87. D. Guo, L. Zhu, Z. Huang, H. Zhou, Y. Ge, W. Ma, J. Wu, X. Zhang, X. Zhou, Y. Zhang, Y. Zhao and N. Gu, Anti-Leukemia Activity of PVP-Coated Silver Nanoparticles via Generation of Reactive Oxygen Species and Release of Silver Ions, *Biomater.*, 2013, **34**, 7884-7894

88. M. A. Franco-Molina, E. Mendoza-Gamboa, C. A. Sierra-Rivera, R. A. Gómez-Flores, P. Zapata-Benavides, P. Castillo-Tello, J. M. Alcocer-González, D. F. Miranda-Hernández, R. S. Tamez-Guerra and C. Rodríguez-Padilla, Antitumor Activity of Colloidal Silver on MCF-7 Human Breast Cancer Cells, *J. Exp. Clin. Cancer Res.*, 2010, **29**, 148–154

89. R. Foldbjerg, D. A. Dang and H. Autrup, Cytotoxicity and Genotoxicity of Silver Nanoparticles in the Human Lung Cancer Cell Line, A549, *Arch. Toxicol*., 2011, **85**, 743–750

90. S. Kim, J. E. Choi, J. Choi, K. H. Chung, K. Park, J. Yi and D. Y. Ryu, Oxidative Stress-Dependent Toxicity of Silver Nanoparticles in Human Hepatoma Cells, *Toxicol. In Vitro*, 2009, **23**, 1076-1084 (B)

91. E. Zielinska, A. Zauszkiewicz-Pawlak, M. Wojcik and I. Inkielewicz-Stepniak, Silver Nanoparticles of Different Sizes Induce a Mixed Type of Programmed Cell Death in Human Pancreatic Ductal Adenocarcinoma, *Oncotarget*, 2018, **9**, 4675-4697

92. D. H. Lim, J. Jang, S. Kim, T. Kang, K. Lee and I. H. Choi, The Effects of Sub-Lethal Concentrations of Silver Nanoparticles on Inflammatory and Stress Genes in Human Macrophages using cDNA Microarray Analysis, *Biomater.*, 2012, **33**, 4690-4699

93. S. Arora, J. jain, J. M. Rajwade and K. M. Pakinar, Cellular Responses Induced by Silver Nanoparticles: *In vitro* studies, *Toxicology Letters*, 2008, **179**, 93-100

94. M. I. Sriram, S. B. Kanth, K. Ka.ishwaralal and S. Gurunathan, Antitumor Activity of Silver Nanoparticles in Dalton's Lymphoma Ascites Tumor Model, *Int. J. Nanomedicine*, 2010, **5**, 753-762

95. E. Barcińska, J. Wierzbicka, A. Zauszkiewicz-Pawlak, D. Jacewicz, A. Dabrowska and Iwona Inkielewicz-Stepniak, Role of Oxidative and Nitro-Oxidative Damage in Silver Nanoparticles Cytotoxic Effect against Human Pancreatic Ductal Adenocarcinoma Cells, *Oxid. Med. Cell. Longev.*, 2018, **2018**, 8251961(1-15)

96. Y. G. Yuan, Q. L. Peng and S. Gurunathan, Silver Nanoparticles Enhance the Apoptotic Potential of Gemcitabine in Human Ovarian Cancer Cells: combination therapy for effective cancer treatment, *Int. J. Nanomedicine*, 2017, **12**, 6487-6502

97. A. Dziedzic, R. Kubina, R. J. Bułdak, M. Skonieczna and K. Cholewa, Silver Nanoparticles Exhibit the Dose-Dependent Anti-Proliferative Effect against Human Squamous Carcinoma Cells Attenuated in the Presence of Berbine, *Molecules*, 2016, **21**, 365(1-17)

98. K. B. Holt and A. J. Bard, Interaction of Silver(I) Ions with the Respiratory Chain of Escherichia Coli: An Electrochemical and Scanning Electrochemical Microscopy Study of the Antimicrobial Mechanism of Micromolar Ag+, *Biochemistry,* 2005, **44**, 13214-13223C.

99. C. N. Lok, C. M. Ho, R. Chen, Q. Y. He, W. Y Yu, H. Sun, P. K. Tam, J. F. Chiu, and C. M. Che, Silver Nanoparticles: Partial Oxidation and Antibacterial Activities, *J. Biol. Inorg. Chem*., 2007, **12**, 527-534

100. M. Marini, S. De Niederhausern, R. Iseppi, M. Bondi, C. Sabia, M. Toselli and F. Pilati, Antibacterial Activity of Plastics Coated with Silver-Doped Organic-Inorganic Hybrid Coatings Prepared by Sol-Gel Processes, *Biomacromolecules*, 2007, **8**, 1246–1254

101. S. Shrivastava, T. Bera, A. Roy, G. Singh, P. Ramachandrarao, and D. Dash, Characterization of Enhanced Antibacterial Effects of Novel Silver Nanoparticles, *Nanotechnology*, 2007, **18**, 225103 (9pp)

102. M. Jeyaraj, G. Sathishkumar, G. Sivanandhan, D. MubarakAli, M. Rajesha R. Arun, G. Kapildev, M. Manickavasagam, N. Thajuddin, K. Premkumar, and A. Ganapathi, Biogenic Silver Nanoparticles for Cancer Treatment: An Experimental Report, *Colloids and Surfaces B: Biointerfaces*, 2013, **106**, 86-92

103. P. Swain, S. K. Nayak, A. Sasmal, T. Behera, S. K. Barik, S. K. Swain, S. S. Mishra, A. K. Sen, J. K. Das, and P. Jayasankar, Antimicrobial Activity of Metal Based Nanoparticles Against Microbes Associated with Diseases in Aquaculture, *World J. Microbiol. Biotechnol*., 2014, **30**, 2491-2502J.

104. L. Lu, R. W. Sun, R. Chen, C. K. Hui, C. M. Ho, J. M. Luk, G. K. Lau, and C. M. Che, Silver Nanoparticles Inhibit Hepatitis B Virus Replication, *Antivir. Ther.*, 2008, **13**, 253-262

105. I. Papp, C. Sieben, K. Ludwig, M. Roskamp, C. Böttcher, S. Schlecht, A. Herrmann, and R. Haag, Inhibition of Influenza Virus Infection by Multivalent Sialic-Acid-Functionalized Gold Nanoparticles, *Small*, 2010, **6**, 2900-2906

106. J. L. Elechiguerra, J. L. Burt, J. R. Morones, A. Camacho-Bragado, X. Gao, H. H. Lara, and M. J. Yacaman, Interaction of Silver Nanoparticles with HIV-1, *J. Nanobiotechnol.*, 2005, **29**, 3–6.

107. H. H. Lara, N. V. Ayala-Nuñez, L. Ixtepan-Turrent, and C. Rodriguez-Padilla, Mode of Antiviral Action of Silver Nanoparticles Against HIV-1, *J. Nanobiotechnol*., 2010, **8**, 1-10

108. S. W. Kim, J. H. Jung, K. Lamsal, Y. S. Kim, J. S. Min, and Y. S. Lee, Antifungal Effects of Silver Nanoparticles (AgNPs) against Various Plant Pathogenic Fungi, *Mycobiology*, 2012, **40**, 53-58

109. G. A. Martínez-Castañón, N. Niño-Martínez, F. Martínez-Gutierrez, J. R. Martínez-Mendoza, F. Ruiz, Synthesis and Antibacterial Activity of Silver Nanoparticles with Different Sizes, *J. Nanopart. Res*., 2008, **10**, 1343-1348

110. J. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri, J. T. Ramírez and M. J. Yacaman, The Bactericidal Effect of Silver Nanoparticles, *Nanotechnology*, 2005, **16**, 2346–2353

111. A. M. Fayaz, K. Balaji, M. Girilal, R. Yadav, P. T. Kalaichelvan and R. Venketesan, Biogenic Synthesis of Silver Nanoparticles and Their Synergistic Effect with Antibiotics: A Study Against Gram-Positive and Gram-Negative Bacteria, *Nanomedicine: NBM*, 2010, **6**, 103–109

112. A. R. Shahverdi, A. Fakhimi, H. R. Shahverdi and S. Minaian, Synthesis and Effect of Silver Nanoparticles on the Antibacterial Activity of Different Antibiotics Against Staphylococcus Aureus and Escherichia Coli, *Nanomedicine: NBM*, 2007, **3**, 168-171

113. A. N. Brown, K. Smith, T. A. Samuels, J. Lu, S. O. Obare, and M. E. Scott, Nanoparticles Functionalized with Ampicillin Destroy Multiple-Antibiotic-Resistant Isolates of Pseudomonas Aeruginosa and Enterobacter Aerogenes and Methicillin-Resistant Staphylococcus Aureus, *Environ. Microbiol*. 2012, **78**, 2768–2774

114. G. Chen, J. Lu, C. Lam and Y. Yu, A Novel Green Synthesis Approach for Polymer Nanocomposites Decorated with Silver Nanoparticles and their Antibacterial Activity, *Analyst*, 2014, **139**, 5795-5799

115. S. Shankar, L. F. Wang and J. W. Rhim, Preparations and Characterization of Alginate/Silver Composite Films: Effect of Types of Silver Particles, *Carbohydr. Polym.*, 2016, **146**, 208-216

116. C. Acharya, C. R. Panda, P. K. Bhaskara, A. Sasmal, S. Shekhar and A. K. Sen, Physicochemical and Antimicrobial Properties of Sodium Alginate/Gelatin-Based Silver Nanoformulations, *Polym. Bull.*, 2017, **74**, 689-706

117. H. Kong and J. Jang, Antibacterial Properties of Novel Poly(methyl methacrylate) Nanofiber Containing Silver Nanoparticles, *Langmuir*, 2008, **24**, 2051-2056

118. A. Manzur, A. Oluwasamni, D. Moss, A. Curtis, C. Hoskins, Nanotechnologies in Pancreatic Cancer, *Pharmaceutics*, 2017, **9**, 39

119. M. Au, T.I. Emeto, J. Power, V.N. Vangaveti, H.C. Lai, Emerging Therapeutic Potential of Nanoparticles in Pancreatic Cancer: A Systematic Review of Clinical Trials, *Biomedicines*, 2016, **4**, 20

120. J. Zhou, J. Wang, Q. Xu, S. Xu, J. Wen, Z. Yu, D. Yang, Folate-Chitosan-Gemcitabine Core-Shell Nanoparticles Targeted to Pancreatic Cancer, *Chin. J. Can. Res.*, 2013, **25**, 527-535

121. S. Ji, J. Xu, B. Zhang, W. Yao, W. Xu, W. Wun, Y. Xu, H. Wang, Q. Ni, H. Hou, X. Yu, RGD-Conjugated Albumin Nanoparticles as a Novel Delivery Vehicle in Pancreatic Cancer therapy, *Cancer Biol. Ther.*, 2012, **13**, 206-215

122. S-t. Wu, A.J. Fowler, C.B. Garmon, A.B. Fessler, J.D. Ogle, K.R. Grover, B.C. Allen, C.D. Williams, R. Zhou, M. Yazdanifar, C.A. Ogle, P. Mukherjee, Treatment of Pancreatic Ductal Adenocarcinoma with Tumor Antigen Specific-Targeted Delivery of Paclitaxel Loaded PLGA Nanoparticles, *BMC Cancer*, 2018, **18**, 457

123. M. Malekigorji, M. Alfahad, P. Kong Thoo Lin, S.M. Jones, A. Curtis, C. Hoskins, Thermally Triggered Theranostics for Pancreatic Cancer Therapy, Nanoscale, 2017, **9**, 12735-12745.

124. A. Oluwasanmi, W. Al-Shakarchi, A. Manzur, M.H. Aldebasi, R.S. Elsini, M.K. Albusair, K.J. Haxton, A.D.M. Curtis, C. Hoskins, Diels Alder- Mediated Release of Gemcitabine from Hybrid Nanoparticles for Enhanced Pancreatic Cancer Therapy, *J. Control. Release*, 2017, **266**, 355-364

125. S.C. Coelho, D.P. Reis, M.C. Pereira, M.A.N. Coelho, Gold Nanoparticles for Targeting Varlitnib to Human Pancreatic Cancer Cells, *Pharmaceutics*, 2018, **10**, 91

126. Y. Wang, B.B. Newell, J. Irudayaraj, Folic Acid Protected Silver Nanocarrieris for Targeted Drug Delivery, *J. Biomed. Nanotechnol.* 2012, **8**, 751-759

127. H. Padinjarathil, M. M. Joseph, B. S. Unnikrishnan, G. U. Preethi, R. Shiji, M. G. Archana, S. Maya, H. P. Syama and T. T. Sreelekha, Galactomannan Endowed Biogenic Silver Nanoparticles Exposed Enhanced Cancer Cytotoxicity with Excellent Biocompatibility, *Int. J. Biol. Macromol*., 2018, **118**, 1174-1182

128. A. R. Zlotta *et al*., Percutaneous Transperineal Radiofrequency Ablation of Prostate Tumor: Safety, Feasibility and Pathological Effects on Human Prostate Cancer, British J. Urology, 1998, **81**, 265-275

129. A. Gillams, Tumor Ablation: Current Role in the Kidney, Lung and Bone, *Cancer Imaging*, 2009, **9**, S68-S70

130. R. Girelli, I. Frigerio, R. Salvia, E. Barbi, P. Tinazzi Martini and C. Bassi, Feasibility and Safety of Radiofrequency Ablation for Locally Advanced Pancreatic Cancer, *British Journal of Surgery*, 2010, **97**, 220-225

131. M. S. Sabel, M. A. Nehs, G. Su, K. P. Lowler, J. L. Ferrara and A. E. Chang, Immunologic Response to Cryoablation of Breast Cancer, *Breast Cancer Res Treat*., 2005, **90**, 97-104

132. A. Oto, I. Sethi, G. Karczmar, R. McNichols, M. K. Ivancevic, W. M. Stadler, S. Watson and S. Eggener, MR Imaging-Guided Focal Laser Ablation for Prostate Cancer: Phase I Trial, *Radiology*, 2013, **267**, 932-940

133. A. Shagan, T. Croitoru-Sadger, E. Crem-Salkmon and B. Mizrahi, Near-Infrared Light Induced Phase Transition of Biodegradable Composites for On-Demand Healing and Drug Release, *ACS Appl. Mater. Interfaces*, 2018, **10**, 4131-4139

134. D. B. Chithrani, S. Jelveh, F. Jalali, M. van Prooijen, C. Allen, R. G. Bristow, R. P. Hill and D. A. Jaffray, Gold Nanoparticles as Radiation Sensitisers in Cancer Therapy, *Radiation Research*, 2010, **173**, 719-728

135. N. Chattopadhyay, Z. Cai, Y. L. Kwon, E. Lechtman, J. P. Pignol and R. M. Reilly, Molecularly Targeted Gold Nanoparticles Enhance the Radiation Response of Breast Cancer Cells and Tumor Xenografts to X-radiation, Breast Cancer Res. Treat., 2013, **137**, 81-91

136. J. Swanner, J. Mims, D.L. Carroll, S.A. Akman, C.M. Furdui, S.V. Torti, R.N. Sigh Differential Cytotoxic and Radiosensitizing Effects of Silver Nanoparticles on Triple-Negative Breast Cancer and Non-Triple Negative Cells, *Int. J. Nanomed.*, 2015, **10**, 3937-3953.

137. R. Lu, D. Yang, D. Cui, Z. Wang, L. Guo, Egg White-Mediated Green Synthesis of Silver Nanoparticles with Excellent Biocompatibility and Enhanced Radiation Effects on Cancer Cells, *Int. J. Nanomed.* 2012, **7**, 2101-2107

138. P. Liu, H. Jin, Z. Guo, J. Ma, J, Ma, D. Li, H. Wu, N. Gu, Silver Nanoparticles Outperform Gold Nanoparticles in Radiosensitizing U251 Cells *In Vitro* and in an Intracranial Mouse Model of Glioma, *Int. J. Nanomed.*, 2016, **11**, 5003-5014

139. M. Skonieczna and D. Hudy, Biological activity of Silver Nanoparticles and their applications in Anticancer Therapy, Silver Nanoparticles 2018.

140. P. V. AshaRani, G. L. K. Mun, M. P. Hande and S. Valiyaveettil, Cytotoxicity and Genotoxicity of Silver Nanoparticles in Human Cells, ACS Nano, 2009, 3, 279-290

Figure legends

Figure 1. Chemical structure of gemcitabine.

Figure 2. Pancreatic cancer microenvironment. The tumor microenvironment consists of numerous cell types and extracellular matrix, which collectively affect drug delivery. Pancreatic cancer is notably characterized by fibrosis separating cancer cells from blood vessels. The dotted arrow shows the path than an i.v. given nanoparticle must travel to reach cancer cells and achieve its effects [40].

Figure 3. Antibiotics enhance the anticancer activity of gemcitabine in a mouse model of colon carcinoma. (A) A subcutaneous model of colon carcinoma (MC-26 cells) was established in immunocompetent BALB/c mice. Bacteria expressing luciferase were injected intravenously and selectively detected in the tumors with IVIS. (B and C) E. coli Nissle 1917 (5×10^6 bacteria) were injected into the tail vein of mice with MC-26 tumors. The antibiotic ciprofloxacin (Cipro) (150 mg/kg) was administered intraperitoneally (every 12 hours), and gemcitabine (Gem) (150 mg/kg) was administered on days 0 and 4. The antibiotic prevented bacterial growth (B) and increased the efficacy of the chemotherapy [(C) Left panel, no significant difference; right panel, ***$P < 0.001$ by two-way analysis of variance (ANOVA) with a Bonferroni adjustment]. N = 16 to 18 mice (groups without Cipro) and 9 to 13 mice (groups with Cipro). Tumor size was normalized to the tumor size on day 0. Bars represent standard error of the mean. (D) WT E. coli or CDD-deficient E. coli (DCDD) were injected into the tail vein of mice with MC-26 tumors. Gemcitabine was

administered intraperitoneally (150 mg/kg) on days 0 and 4. Gemcitabine significantly inhibited tumor growth when DCDD bacteria rather than WT bacteria were administered (*P < 0.05 by two-way ANOVA with a Bonferroni adjustment). N = 15 mice (WT – Gem), 6 to 8 mice (WT + Gem), 10 mice (DCDD – Gem), and 4 to 6 mice (DCDD + Gem). Tumor size was normalized to the tumor size on day 0. Bars represent standard error of the mean. (E) Devices for local intratumor release of drug microdoses were used to release gemcitabine and antibiotics, alone and in combination, directly into the microenvironment of bacteria-colonized tumors to assess in vivo efficacy (13). Histological staining by cleaved caspase 3 shows significantly more apoptosis when gemcitabine is released in combination with antibiotics (ii and iv) but less apoptosis when gemcitabine (i) or ampicillin (iii) is administrated alone. Scale bars, 200 mm [29].

Figure 4. PANC-1 cells treated with 2.6 nm AgNPs. AgNPs triggered both apoptotic and non-apoptotic cell death at 2.5 μg/ mL (A-C) and 3.5 μg/mL (D) concentration. The evidence of both apoptotic (arrow) and necrotic/necroptotic (black arrow head) cell morphology have been found (a and b). Apoptotic cell with typical nuclear condensation (N), blebbing (asterisk) (b). The necroptotic cell death was preceded by intensive cytoplasm vacuolization (v), nuclear membrane dilatation (white arrow heads) and disrupted cellular membrane ad cytoplasmic swelling (a, c and d). Final stage of cellular death, degradation of cell organelles and cytoplasm (d). Loss of cell membrane integrity, chromatin condensation of nucleus (N). The representative cell is shown, presenting final stage of cellular death, degradation of cell organelles and cytoplasm, total loss of cell membrane integrity. However, the nuclear membrane integrity was maintained, the nucleus (N) was condensed, with peripheral clumps of heterochromatin. At this level of cell damage it was impossible to specify the cell death pathway. Magnifications: a ×2000; b ×4000; c ×5000; d ×6000 [91].

Figure 5. Schematic representation of mechanisms of action of silver nanoparticle antimicrobial effect.

Figure 6. Schematic representation of active transport of nanoparticles into cells.

Figure 7. *In vivo* evaluation on BxPC-3 xenograft models in Nu/Nu female mice (4-6 weeks old) dosed once a week at 3 mgKg-1 for 4 weeks. A) Comparison of tumor volume over study duration∞ Study stopped before completion due to tumor volume approaching maximum humane volume (0.9 cm3). B) Comparison of tumors after excision: 1) control, 2) control with laser irradiation, 3) HNP, 4) HNP with laser irradiation, 5) GEM, 6) GEM with laser irradiation, 7) HNP-L-GEM, 8) HNP-L-GEM with laser irradiation. Where laser irradiation was required this was carried out 24 h after dosing under anaesthetic. The tumor was irradiated at 1064 nm as for 20 sec using a ML-LASER-YB5 Q-switched Nd:YAG Laser Treatment System. Pulse width: 10 ns, pulse repetition frequency: 6 Hz, laser spot diameter: 3 mm, cooling system: water cooled with airflow cooling. The beam was collimated through concave lenses to a 1 mm diameter. C) Comparison of tumor weight after excision * denotes significance compared to controls, ** denotes significance compared to GEM, *** denotes significance compared to HNP-L-GEM without laser irradiation ($p<0.01$) (n=5, ±SE) [124].

Author(s)	Date	Treatment	Clinical Trial Phase(s)	Change in PFS	Overall survival	Ref.
Reni *et al*	2005	Gemcitabine, 5-FU, cisplatin, epirubicin vs. gemcitabine	III	4 months: 60% vs 28%	2 year: 11.5% vs 2.1%	[30]
Cunningham *et al*	2009	Gemcitabine, capecitabine vs. gemcitabine	II/III	5.3 vs 3.8 months	Median: 7.1 vs 6.2 months	[31]
Conroy *et al*	2011	Folinic acid, irinotecan, oxaliplatin vs. gemcitabine	II/III	6.4 vs. 3.3 months	11.1 vs 6.8 months	[33]
Von Hoff *et al*	2013	Gemcitabine, nab-paclitaxel vs. gemcitabine	III	5.5 vs 3.7 months	8.5 vs 6.7 months	[34]
Reni *et al*	2016	Gemcitabine-nab-paclitaxel, cisplatin, capecitabine vs nab-paclitaxel-gemcitabine	II	12.5 vs 9.9 months	20.7 vs. 19.1 months	[35]

Tables

Table 1. A summary of the clinical trials comparing new treatment strategies to gemcitabine, or other gemcitabine-based therapeutics.

Table 2. A summary of different silver nanoparticle-based treatments, tested on either cell culture or in a xenografted mouse model.

Author(s)	Date	Treatment(s)	Cancer Type	Mouse model	Cell lines	Results	Ref.
Zielinska *et al.*	2018	AgNPs, gemcitabine	Pancreatic	No	PANC-1, hTERT	Greater cytotoxicity against cancer cell line than non-cancerous. AgNPs had a better effect than gemcitabine during testing	[91]
Guo *et al.*	2013	AgNPs	Acute myeloid leukaemia	No	DAMI, HEL, HL-60, NB4, SHI-1, THP-1	Dose-dependent reduction in cell viability, with THP-1 cells being most sensitive and SHI-1 the least sensitive to AgNPs.	[87]
Lim *et al.*	2017	AgNPs	-	No	U937	Size-dependent effect of AgNPs on toxicity, smaller NPs had greater cytotoxic effects at lower concentrations. Apoptosis and DNA damage.	[92]
Arora *et al.*	2008	AgNPs	Human skin carcinoma, human fibrosarcoma	No	A431, HT-1080	Both cell types showed dose-dependent cytotoxicity. Apoptosis induced at different concentration ranges. Fucntional damage caused.	[93]
Lim, Gurung and Hande	2017	AgNPs	Glioblastoma and Breast Cancer	No	U257 MCF-7, MDA-MB-231	Cytoskeletal deformities in cell lines. Shape changes in the cancer cells. Reduction of cell proliferation in cancer	[86]

						cells, caused DNA damage, cell death and reduced c-Myc expression.	
Sriram *et al.*	2010	AgNPs	Dalton's Lymphoma	Yes	DLA	Tumour volume decrease of 68.5% using AgNP treatment	[94]
Barcińska *et al.*	2018	AgNPs	Pancreatic	No	PANC-1, hTERT	Increased nitric oxide concentration present in PANC-1 cell line.	[95]
Yuan, Peng and Gurunathan	2017	AgNP + gemcitabine	Ovarian	No	A2780	A strong synergistic effect was seen, with lower concentrations of AgNPs required to successfully cause cytotoxicity to the cell line	[96]
Dziedzic *et al.*	2016	AgNP + berberine	Human squamous carcinoma cells	No	SCC-23 (ATCC CRL-1628)	Dose and time dependent effect over 24 and 48 hours. Berberine had a more inhibitory effect on the actions of AgNPs.	[97]

Table of contents